Deciphering Decomposition and the Effects of Disturbance in Forest Soil Microbial Communities with Metagenomics and Stable Isotope Probing

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Abstract

Forest industries are expected to bolster the renewable resource economy, but must contend with ecological challenges in maintaining the long-term fertility of forest plantation soils, and technological challenges in converting forest biomass into industrially relevant sources of carbon and energy. This thesis advances research related to both, first by describing the broad changes in soil microbial communities in the decades following timber harvesting, their implications for soil processes and the influence of biomass retention for mitigation (Chapter 3) and, second, by conducting the first comprehensive culture-independent survey of lignocellulolytic organisms in forest soils to expand knowledge of their diversity and catabolic capabilities (Chapter 4).

Analysis of over 1,300 bacterial (16S rRNA gene) and fungal (ITS region) pyrotag libraries demonstrated consistent changes in microbial communities at harvested sites across North America, such as i) the increase of desiccation- and heat-tolerant organisms, ii) the general decline of ectomycorrhizal (EM) fungi with a rise of select EM genera (*Suillus* and *Thelephora*), iii) the moderation of population shifts by organic matter retention and iv) changes in the functional character of harvested soils, including reduced methanotrophic populations and cellulolytic activity. Biogeographical differences in community structure revealed the potential for variation in the impacts of harvesting. Overall, a number of taxonomic groups were identified that may be important indicators for assessing the long-term impact of timber harvesting.

Stable isotope probing revealed the degradation of model hemicellulose, cellulose and lignin substrates by specialized taxa, active on a sole substrate, and groups capable of degrading all three plant polymers, such as members of *Burkholderiales* and *Caulobacteraceae*. Bacterial lignin-degraders were more active than fungi in soil microcosms, represented by taxa with characterized lignolytic capability (*Sphingobacteriaceae* and *Sphingomonadaceae*) and novel taxa, such as members of *Elusimicrobia* and *Acidobacteria*. Differences in lignocellulolytic populations were observed among ecozones and soil layers. Mineral soils harboured a greater proportion of poorly characterized functional taxa and represent reservoirs of unexplored catabolic diversity. Metagenome assembly was ~3 to 20-fold higher as a result of SIP, providing a trove of sequence data containing carbohydrate- and lignin-active enzymes from lignolytic and cellulolytic taxa for future characterization.

Résumé

On s'attend des industries forestières qu'elles agissent comme un pilier de l'économie des ressources renouvelables. Pour y arriver, elles doivent d'abord adresser des défis écologiques en terme de maintien à long terme de la fertilité des sols des forêts de plantation, ainsi que des défis technologiques en terme de conversion de biomasse forestière comme source industrielle significative de carbone et d'énergie. La présente thèse avance la recherche sur ces deux défis : d'abord, en décrivant les changements généraux de communautés microbiennes durant les décennies suivant la récolte du bois, leur implication sur les processus de sol, et leur influence sur la rétention de biomasse à mitiger les changements (chapitre 3), ensuite, en menant la première campagne d'évaluation détaillée d'organismes lignicellulolytique de sols forestiers afin d'élargir les connaissances sur la diversité de taxa non-cultivés et sur les enzymes catalytiques qu'ils possèdent (chapitre 4).

Des analyses de plus de 1300 banques pyrotags bactériennes (ARNr 16S) et fongiques (région ITS) ont démontré des changements réguliers des communautés microbiennes aux sites échantillonnés à travers l'Amérique du Nord, dont i) l'expansion des organismes tolérants à la sécheresse et la chaleur, ii) la chute accrue de champignons ectomycorhizes (EM) avec une augmentation de champignons spécifiques EM (*Suillus* et *Thelephora*), iii) la régulation de changements de population par la rétention de matière organique et iv) des changements dans les caractéristiques fonctionnelles des sols récoltés, incluant une réduction des populations métanotrophes et de l'activité cellulolytique. Des différences biogéographiques dans les structures de communautés ont révélé le potentiel de variation des impacts de récolte. En somme, une variété de groupes taxonomiques pouvant servir d'importants indicateurs pour évaluer l'impact à long terme de la récolte forestière fut identifiée.

L'utilisation de marquage isotopique stable (SIP) révéla que la dégradation d'hémicellulose modèle, de cellulose, et de substrats ligneux est entreprise par des taxa spécialisés, actifs sur un seul substrat, mais aussi par des groupes capables de dégrader les trois polymères végétaux, dont des membres des *Burkholderiales* et *Caulobacteraceae*. Les décomposeurs bactériens de lignine furent plus actifs que les champignons, représentés par des taxa possédant des capacités lignolytiques caractérisées (*Sphingobacteriaceae* et *Sphingomonadaceae*) ainsi que de nouveaux taxa, comme *Elusimicrobia* et *Acidobacteria*. Des différences de populations lignicellulotytiques furent observées parmi les zones

biogeoclimatiques et couches de sol, avec les sols minéraux abritant une plus grande proportion de taxa de fonction peu caractérisée. L'utilisation de SIP mena à un assemblage métagénomique ~ trois à vingt fois plus élevé, fournissant une mine de données de séquences contenant des enzymes actives de carbohydrate et lignine provenant d'une variété de taxa putativement lignicellulolytiques.

Preface

The course of my thesis research was planned in consultation with Dr. William W. Mohn

Material from a published paper (R., Wilhelm, A., Szeitz, T.L., Klassen, and W.W. Mohn. 2014. *Appl. Environ. Microbiol*. **80**:7206-7211,) has been adapted and incorporated into the methods section of this thesis. With respect to my role in this work, I identified the need for a method to quantify ^{13}C-enrichment of nucleic acids, devised the methodology in consultation with Auclair *et al.*, (2011; *Can. J. Microbiol.*, **58**: 287-292), prepared and analyzed samples and wrote the paper. A. Szeitz operated the mass spectrometer, designed the UHPLC protocol and offered invaluable advice on how to improve sensitivity and accuracy. Klassen and Mohn provided comments on the final draft prior to publication.

Other contributors to the work presented in this thesis include Lionel D. Jensen, who performed the respiration assays included in Section 3.3.1; Hilary Leung, whose SIP-hemicellulose bacterial pyrotag libraries were re-analyzed in Chapter 4 and who processed approximately one third of soil samples; Larissa McNeil processed the other third of soils and the majority of DNA extractions and PCR for samples described in Section 2.2.1; Dr. Erick Cardenas performed the first batch of draft genome binning, provided hidden Markov models for a number of lignin-modifying gene families and provided guidance for bioinformatic analyses throughout; Dr. Pedro Dimitriu provided guidance for statistical analyses and Dr. Kendra Maas facilitated the bulk sequencing of samples and collation of metadata used for the analyses in Section3.2. The custom synthesis of DHP-lignin by Dr. Rahul Singh was critical to research presented in Chapter 4.

I am responsible for all other experimental design, data collection, analysis, research and writing presented in this thesis.

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List of Abbreviations

|	symbol for "or" or "nor" as in 'neither OM1|OM2 showed significant differences'
^{12}C	most abundant (~98.9%) stable isotope of carbon
^{13}C	a heavier stable isotope of carbon (~1.1% natural abundance)
AA	'Auxiliary Activity,' the category for lignin-modifying genes in the CAZy database
BC	British Columbia
BS	'Black Spruce' ecozone with sites near Thunder Bay, Ontario
CAZy	a carbohydrate-active enzyme (also CAZyme)
C:N	ratio of total carbon to total nitrogen, 'C:N ratio'
DHP	dehydrogenatively polymerized
DNA	deoxyribonucleic acid
enrOTU	OTUs that have 'enriched' abundance in ^{13}C- relative to ^{12}C-sequencing libraries
GH	glycosyl hydrolase
IDF	'Interior Douglas Fir' ecozone with sites near Kamloops, B.C.
JP	'Jack Pine' ecozone with sites near Sault Ste. Marie, Ontario
LCA	lowest common ancestor – a classification method based on consensus of top BLAST hits
LP	'Loblolley Pine' ecozone with sites near Kurth, Texas
LPMO	lytic polysaccharide monooxygenase
MS	mass spectrometry
NCBI	National Center for Biotechnology Information (United States of America)
NMS | NMDS	non-parametric multidimensional scaling
OM	organic matter, typically used in referring to organic matter removal or retention
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PCA	principle components analysis
PP	'Ponderosa Pine' ecozone in the Sierra Nevada Mountains of California
SBS	'Sub-Boreal Spruce' ecozone in northern B.C.
SIP	stable isotope probing
UHPLC	ultra-high performance chromatography

Acknowledgements

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This work could not have been done without the intellectual oversight and magnanimity of my supervisor Dr. William Mohn. Dr. Mohn leads a diverse research group and has built a community of committed researchers in microbial ecology. He is an adroit teacher from whom I have gained valuable insights. I thank him for the opportunity to conduct my research as well as for giving me access to current research trends by supporting my attendance at conferences.

The success of this research is indebted to the forward thinking scientists who devised and implemented the Long-term Soil Productivity Study and those who have sustained its operations. All soil samples used in this thesis were collected by collaborators in the Canadian Forestry Services (Dr. Paul Hazlett), the Ontario Ministry of Natural Resources (Dr. David Morris), and the U.S. Forest Service (Dr. Matt Busse and Dr. Andy Scott). These individuals also provided valuable data on soil conditions in the decade and half since harvesting.

I thank Hilary Leung for his company at the bench and his readiness to discuss or assist in trouble-shooting all things lab-related; Andras Seitz for his valuable expertise in mass spectroscopy; Dr. Linoj Kumar for providing lignocellulosic substrates used in respiration experiments described in Section 3.3.1; Dr. Thomas Beatty for lending his vertical rotor for use in density gradient ultracentrifugation; Dr. Marie-Claude Fortin for effectively managing our project and ensuring my research received its budgeted funds; Gordon Stewart for being a dependable, well-organized and knowledgeable lab manager and Dr. Josh Neufeld for his valuable early work in SIP and for providing *G. xylinus* for producing bacterial cellulose.

I would like to acknowledge the work of individuals whose passion for programming languages gave me the impetus to become a practitioner. I thank Dr. Rita McNamara, Dr. Keith Loese and Dr. Benjamin Purzycki, for facilitating an R group, Dr. David Williams-King for instruction on scripting, Dr. Haddock and Dr. Dunn for writing “Practical Computing for Biologists,” and Dr. Jenny Bryan for allowing me to audit her incredibly useful courses (STAT540 and STAT545).

I would like to acknowledge the biologists, bioinformaticians and scientists around the world upon whose creative intelligence and hard work this research depends. In particular, the vision of public repositories and analytic tools such as those provided by NCBI, Argonne National Lab (MG-RAST), Schloss *et al.* (Mothur) and Joint Genome Institute (IMG-ER).

This research was enabled by support from WestGrid (www.westgrid.ca) and Compute Canada Calcul Canada (www.computecanada.ca). In particular, the patient assistance of Dr. Doug Phillips, at the University of Calgary, in trouble-shooting the effective multi-threaded use of Ray-meta for metagenome assembly.

Thanks to Dr. Guillaume Lamarche-Gagnon who translated my abstract into French under field conditions en route to Svalbard.

Dedication

I dedicate this work to my parents, Diane Bielicki and James Wilhelm, who gave so much and who cared deeply about me *and* about living an examined life.

To my brother whose own curiousity and pluck enriched my life.

To my family and the memory of my grandmothers, who patiently encouraged me as I focused on building a career in microbiology.

To my companions over the past five years, including Jessie Saunders, Dr. Nikolaus Fortelny, Hilary Leung, Dr. Erick Cardenas, Dr. Yaseen Mottiar, Pauline Morand, Dr. Jackie Goordial, Patrick Conroy, Dr. Greg and Emma Gervalis, Dr. Tim Höllering, Camilo Cortes Aguirre, Dr. Thomas Hauch-Fenger, Cheryn Wong, Magdalena Grömer, Dr. Dan Brox, Dr. Stephen Hay, Mark Schutzbank and Tim Shaw, Dr. Alex Martin, Stacey Auld, Dr. Nagissa Mahmoudi, Christina Toms, Dr. Christina Turner, Dr. Ben Sylvester, Dr. Irwin Chang, Dr. Natalie Ceperely, Dr. Don Beith, Inti Dewey, Dr. Meike Singer, Eric and Linds Cole, Dr. Kim and Noel Ma-krakel, Greg Uchitel, Brandon Miliate, Ste. Anne's Franchises everywhere, and Lucas at the Bike Kitchen *et al.*

To Green College of UBC, where there exists a culture of learning and sharing that sets precedent for what the university experience should be.

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To those who endeavor to understand the complexity of soil microbial ecology and who will undertake research to answer questions posed here. And, to the revitalization and continuing legacy of forestry in B.C. and Canada of which future generations can be proud.

To the journalists at the Canadian Broadcasting Corporation, in particular Anna-Maria Tremonti and Bob MacDonald, the Globe and Mail and the British Broadcasting Corporation and to other cultural icons who kept me engaged and entertained during my commute and long hours of lab work, in particular Gord Downie, Dr. Thomas King, Geoff Berner, and Rae Spoon.

To Rita Laszlo, whose mutualism adds joy to my content.

Chapter 1: Introduction

1.1 Above and Belowground Perspectives on Timber Harvesting, Soil Microbial Communities and the Long-term Sustainability of Forest Plantations

1.1.1 Current Context of the B.C. Forest Industry

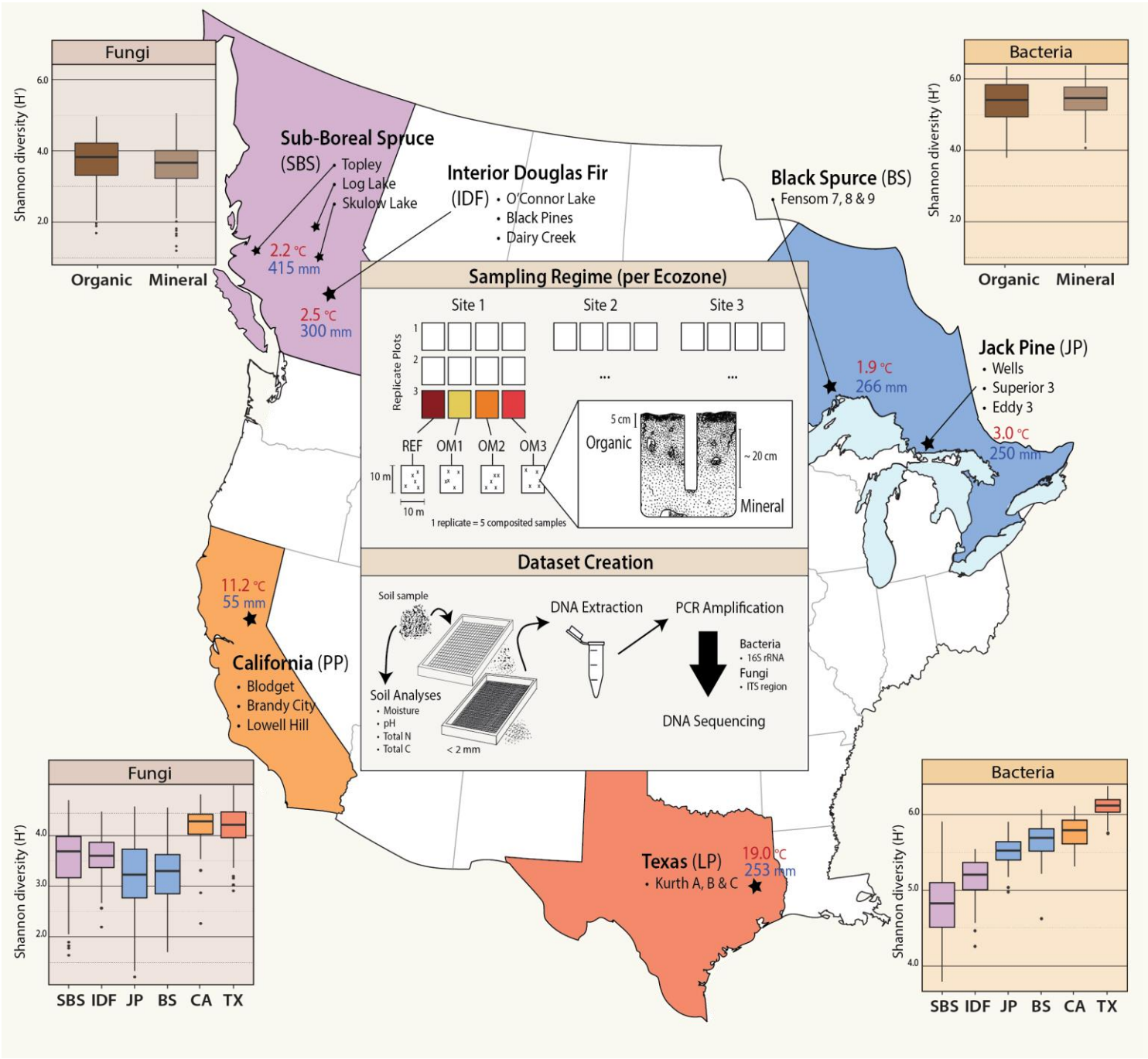
The forest industry remains a vital sector in British Columbia's economy that sustains large manufacturers, thousands of small businesses and whole frontier towns. In 2011, wood products accounted for 22% of all goods sold in British Columbia (by volume) and a total of 5.5% of the GDP (B.C. Ministry of Forests, 2012). Canada-wide, the forest sector comprises 1.25% of Canada's GDP (~$20 billion) also driven by industries in Ontario and Quebec. Yet, since the turn of the millennia, the B.C. forestry sector has seen job losses in the tens of thousands, the closure of mills and mass emigration from once thriving communities (B.C. Government and Service Employees Union Report, 2011). In 2012, the B.C. auditor general issued a report revealing large areas damaged by wildfire and mountain pine beetle had not been replanted and concluded that the future vitality of the industry lay in jeopardy (Office of the B.C. Auditor General, 2012). Both the Auditor General and public sector union stress the need to generate more value from forest products and commit to improved stewardship of the land. As stakeholders from business, government and local communities plan future development, there are still many unanswered questions about the basic nature of long-term forest management, such as how to maintain soil fertility under intensified land use. This research examines the belowground impacts of harvesting on microbial communities and processes related to forest ecology and soil fertility, providing a basis to develop principles of long-term stewardship (Chapter 3). At the same time, this research addresses the need to generate more value from forestry products, by surveying lignocellulolytic organisms in a search for industrially relevant biocatalysts (Chapter 4).

1.1.2 An Introduction to Forest Management and the Long-term Soil Productivity Study

Logging rights in B.C. were first granted by the Crown in the 1800s, and logging practices were eventually regulated by B.C.'s first *Forest Act* in 1912 (Marchak, 1983). Despite periods of rapid growth of resource extraction, by the late 1990's the amount of managed forested land in Canada and the United States had largely stabilized by reforestation and decades of improved forest management (Powers, 2006). Without the capacity to expand further, demands for shorter crop cycles, densified plantations and the harvesting of additional sources of woody biomass has increased (Fox, 2000; Allmer *et al.*, 2009). Yet, the long-term sustainability of such land-use intensification is uncertain, since the consequences of harvesting (i.e. the removal of substantial amounts of organic nutrients) on soil fertility and forest regeneration remains poorly understood. Given the slow rate of forest regeneration, on average between 50-70 years (~ 25 years in more southern latitudes) before subsequent harvest, the science examining the effects of multiple crop cycles on forest soil fertility is a young science (Nambiar, 1996). Yet, there is a governmental mandate in both the U.S. (*National Forest Management Act*, 1976) and in Canada (*Forestry Act*, 1985 and *Timber Regulations*, 1993) to steward forested lands, though Canadian legislation does not explicitly provision for impacts on soil. Foreseeing the need to evaluate the long-term effects of biomass removal and other associated impacts of harvesting, such as compaction, the U.S. Forestry Service initiated the Long-term Soil Productivity Study in 1989, which was joined by Canadian counterparts soon after. The LTSP network includes over one hundred experimental sites across North America which have all implemented a similar experimental design assessing three intensities of organic matter removal and, in many cases, three intensities of soil compaction. The work detailed in this thesis was based on samples taken from LTSP sites in British Columbia, California, Ontario and Texas (Figure 1.1).

Figure 1.1. Overview of six North American ecozones and LTSP sites where soil sampling occurred. Data on mean annual temperature and precipitation along with the Shannon diversity (H') estimates for fungal and bacterial communities from data described in this thesis are provided. Each ecozone contained three sampling sites and, at each site, multiple experimental plots for each harvesting treatment as illustrated by the center panel providing an overview of the experimental design for Section 3.2.

Note: the term 'ecozone' is not used in accordance with the classification system provided by Environment Canada (Ecological Stratification Working Group, 1996), but as a general descriptor of differences in local assemblages of organisms and in climatic factors.

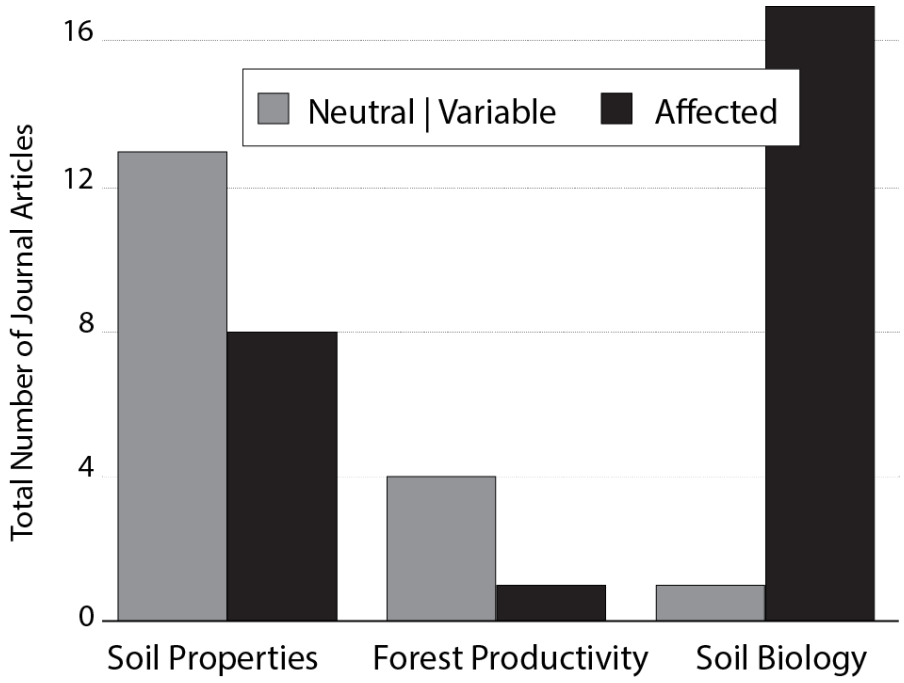


1.1.3 Soil Ecology and Long-term Soil Productivity

The long-term sustainment of any industry based on plant-based biomass, whether forest or agricultural, is contingent on prudent soil management. Nutrient depletion from biomass removal is one of many concerns for long-term soil fertility of forest plantations, others include: soil compaction, erosion, heating, drying, and changes in soil chemistry and prevailing microbial processes, including those related to carbon sequestration and greenhouse gases (Schoenholtz *et al.,* 2000; Powers *et al.,* 2005; Schulze *et al.,* 2012; Merilä *et al.,* 2014). Assessments of the ecological impact of timber harvesting and organic matter removal on soil communities near unanimously report impacts, while, in contrast, most studies on physicochemical properties of soil or forest productivity report neutral, variable and temporal responses (Figure 1.2; Table E.1). These findings suggest that forest regeneration may be broadly insensitive to variation in soil microbial communities, or that the community and concomitant biological processes are themselves variable or have forthcoming impacts. These suppositions remain largely untested due to historical overgeneralizations of microbial community structure and challenges overcoming the substantial diversity and variability of soil communities. Without sufficiently detailed knowledge of i) the prominent soil community members; ii) differences among forest soil communities; iii) the function of microbial processes in ecosystem maintenance and iv) how communities respond to long-term management practices, questions surrounding the impacts of harvesting on soil microbial processes remain in the realm of conjecture.

To date, timber harvesting reportedly alters long-term ecological processes related to mycorrhizal symbioses and soil gas fluxes. Populations of type-II methanotrophs were found to decline in the decades following harvesting, resulting in the decreased uptake of methane by soils.

Figure 1.2. A census of the conclusions from studies of the impact of timber harvesting on forest regeneration years and decades post-harvest. Studies were categorized by whether they were studying physicochemical properties of soil, the forest biomass ('productivity') or biological features of soil. Studies which showed variable responses among study sites were categorized with neutral findings due to typically small effect sizes even where effects were observed. Table E.1 contains information on every study consulted.



The time until recovery to pre-harvest rates of methane consumption in pine plantations was estimated to be 47 years (Nazaries *et al.*, 2011). Shifts in the composition of ectomycorrhizal fungi (EM), symbionts of *Pinaceae*, persist in the decades following harvest (Hartmann *et al.* 2009; Hartmann *et al.* 2012) and reportedly upwards of 50 years (McGuire *et al.* 2014). EM fungi dramatically improve host fitness, providing surplus water and scavenge inaccessible nutrients from mineral rock, such as phosphorus, copper, and iron, or nitrogen from organic matter (Cairney and Chambers, 1999). Shifts in EM populations have significant potential to impact forest regeneration, but the long-term impacts are not clear due to the poorly understood natural succession of EM fungi (Visser *et al.*, 1995; Twieg *et al.*, 2007).

In a comprehensive meta-analysis of timber harvesting impacts on microbial communities, Holden *et al.,* (2013a) concluded that harvesting broadly reduced microbial biomass and heterotrophic activity. These effects did not significantly differ between unharvested and partially logged forests, only in clear-cut sites. This raises questions about whether differences in timber harvesting strategies, such as the machinery used, degree of plant biomass removal and site-preparation for replanting, may mitigate changes in soil microbial community structure. The underlying cause of reduced heterotrophic activity and microbial biomass is not clearly understood, yet, recent studies have implicated the possible importance of organic matter removal. In a recent landmark LTSP-based study, the first to utilize high-throughput sequencing, the degree of organic matter removal had a greater impact than soil compaction, but both were less than differences in community composition among soil layers and geography in the 'ecozones' surveyed (Hartmann *et al.,* 2012). The retention of some degree of organic matter onsite resulted in microbial communities that differed, albeit slightly, from both unharvested and intensely harvested sites. This was not altogether surprising, given that coarse woody debris selects for organisms with specialized capabilities, illustrated by the natural succession of decomposers as forest litter matures (Voriskova and Baldrian, 2013). In one instance, the retention of coarse woody debris following harvesting increased the diversity of wood-inhabiting fungi above that of unharvested sites (Brazee *et al.,* 2014). Not only does the retention of woody debris shift the quality and quantity of organic matter input to soils, it mitigates other physical changes to the soil environment such changes in pH, aridity and temperature (Entry, 1986; Bååth *et al.,* 1995).

During the intervening years between harvesting and full canopy closure of reforested land, harvested soils experience substantial changes in physical conditions which include higher temperature and moisture extrema, diurnal fluctuation as well as higher average temperatures (Kranabetter *et al.,* 1999; Kulmala *et al.,* 2014) and lower average moisture availability throughout

the soil column (Childs and Flint, 1987; Paz, 2001; Redding *et al.,* 2003; Tan *et al.,* 2005). Soil moisture can, however, increase over the short-term following harvesting (Adams *et al.,* 1991) and occur only in near-surface layers (Fleming *et al.,* 1997), reflecting the influence in the loss of transpiration from trees in deeper soils. Warmer, arid soil conditions are expected to be major factors influencing the composition of post-harvest communities, though there is a paucity of information about how soil communities respond to these changes. Long-term studies of regenerating forests destroyed by wildfire experience similar shifts in environmental conditions (described in Section 1.1.7). The retention of organic matter can affect these abiotic changes, mitigating increases in aridity and soil temperature (Paz, 2001) that, over the long-term, may influence microbial succession. Assessing the relative importance of organic matter retention as a nutritional substrate or in terms of moderating physical changes motivated this thesis research.

The scope and scale of the effect of organic matter retention on soil microbial communities following harvesting may signal its value as a mitigation strategy against long-term disturbance. Questions about the impact of organic matter removal are of increasing importance as the production of biofuel, wood pellets and other materials incentivize the removal of more forest biomass, previously left onsite. Allmer *et al.,* (2009) report that 38% of all fine woody debris is now being claimed for commercial use in Sweden, raising concerns about how to manage woody debris without depleting nutrient capital, altering soil processes or substantial habitat loss (Harmon, 2001). This thesis addresses such questions by contrasting long-term changes in microbial community structure at three intensities of biomass removal implemented in the LTSP Study experiment.

1.1.4 Harvesting Impacts on Lignocellulose-degrading Populations

The decomposition of lignocellulosic biomass shapes soil structure, pH, carbon and nitrogen content and other properties which govern fertility. The composition and abundance of

soil organic matter, particularly in the upper 'organic' horizon, affects the bioavailability of nutrients and energy flux through the heterotrophic food-web and also the sequestration of carbon (Merilä *et al.,* 2010; Fontaine *et al.,* 2011; Ge *et al.,* 2013). The decomposition of lignocellulose is a rate-limiting step in the decomposition of plant biomass, in particular in coniferous forests, where carbon accumulates as soil detritus and humic compounds. Partially decomposed woody biomass accounts for an estimated 50% of all terrestrial carbon (Myneni *et al.,* 2001), building a case for studying the conditions that govern the net storage of carbon in these environments, at the forefront of which are physiological and ecological traits of decomposers (Singh *et al.,* 2010).

Timber harvesting results in the loss of tree hosts and increase in belowground necrotic root tissue that generally shifts soil fungal communities from mycorrhiza-dominated to decomposition-dominated systems (Hartmann *et al.,* 2012). Despite the shift towards saprobic populations, the consensus is that timber harvesting slows decomposition (Whitford *et al.,* 1981; Yin *et al.,* 1989; Prescott *et al.,* 2000). Reduced microbial respiration has been observed upwards of 15 years after reforestation (Webster *et al.,* 2016), suggesting that not only early-stage abiotic constraints, such as reduced water availability, but late-stage developments, likely relating to biological changes, affect the decline of heterotrophic activity. Long-term shifts in the composition of forest soil decomposers at harvested sites have been linked with a decreased potential to degrade complex carbohydrates like lignocellulose (Cardenas *et al.,* 2015). Therefore, the decline in heterotrophic activity may be driven by both changes in quality of organic litter and the capacity of organisms that thrive in post-harvest conditions to decompose it. This is evidenced by the fact that not all populations of saprobes fare equally well following harvesting. *Basidiomycota* populations, such as brown and white-rot species, decline, while faster growing ascomycotal populations expand (Bader *et al.,* 1995; Hartmann *et al.,* 2012; Šťursová *et al.,* 2014; McGuire *et al.,* 2014). Certain actinobacterial decomposers, such as *Streptomycetaceae*, decline in the face of

an overall greater predominance of fungi (Hartmann *et al.,* 2009; Hartmann *et al.,* 2012; Lewandowski *et al.,* 2015). Greater fluctuations in soil temperature and moisture, found at harvested soils, are also expected to select for hardier taxa such as those observed in arid environments, like yeasts and dark-septate fungi (Gallo *et al.,* 2009) and bacterial phyla well-regarded for their tolerance to harsh conditions, such as *Armatimonadetes, Firmicutes, Chloroflexi*, *Deinococcus-Thermus* and *Actinobacteria* (Rastogi *et al.,* 2009; Gabani *et al.,* 2012; Soares *et al.,* 2012).

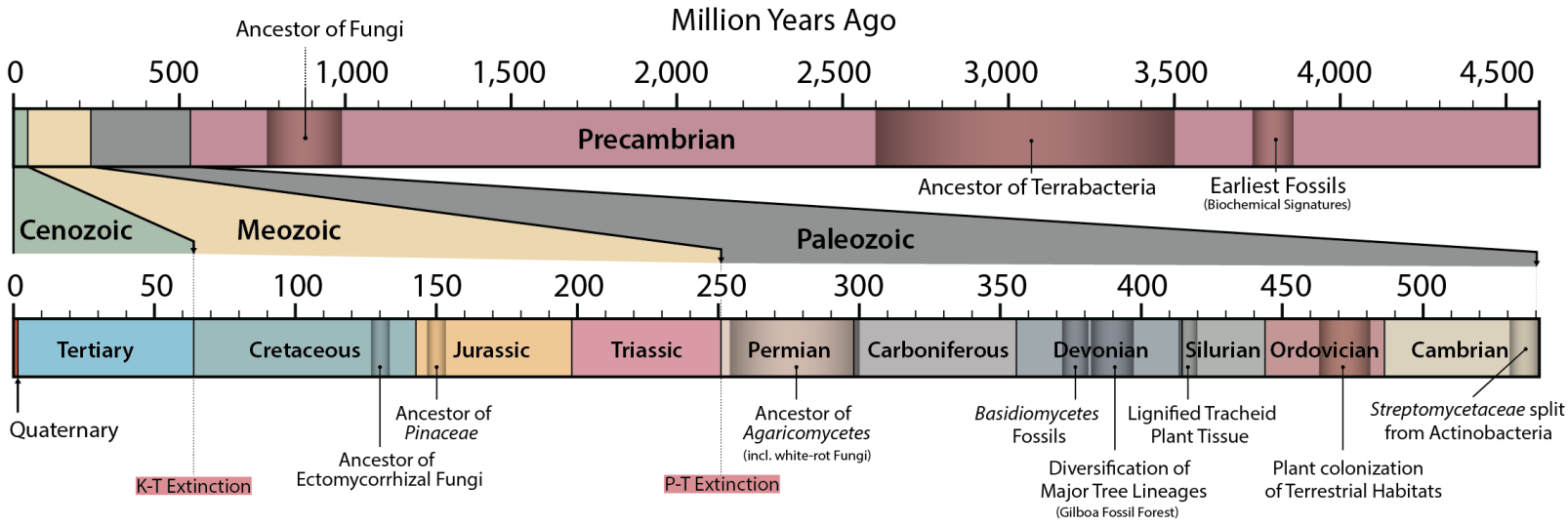
This thesis research is the first attempt to directly link the changes in the rate of decomposition, brought about from timber harvesting, with changes in cellulolytic community by means of stable isotope probing (SIP). SIP enables the concomitant measurement of the rate of cellulose decomposition along with in-depth, sequencing-based assessment of the cellulolytic community structure. This thesis examines whether long-term changes in temperature, aridity or decreased organic matter, brought about by timber harvesting, significantly alter cellulolytic community structure, diversity and activity (Section 3.3). One way in which these expectations may prove off the mark is that forest soil communities are adapted to disturbances and cope with changes in environmental conditions post-harvest and during maturation. The following sections describe the potential resiliency of forest soil communities and the potential similarity between the long-term impacts of timber harvesting and natural disturbances like wildfire.

1.1.5 Forests: A History of Disturbance

A discussion of the impacts of timber harvesting would be incomplete without consideration of the role disturbance has played in the natural history of forest ecosystems. One of the central aims of forestry science is to design timber harvesting methods that emulate natural disturbance, based on evidence that many plant species, including many of the very trees we harvest, have evolved to punctuated large-scale disturbances (Bond and Keeley 2005).

The earliest fossil preserve of a forest ecosystem is the Gilboa Fossil Forest in central New York State, dating to ~390 Mya (Stein *et al.*, 2012). Softwood, conifer-like forests were the dominant forest type during the Mesozoic Era, surviving both Permian-Triassic and Cretaceous-Tertiary (K-T) extinctions (Thomas, 2014; Figure 1.3). Their prominence has been attributed to an ability to resist severe drought and colonize immature mineral-rich soils likely with the help of mycorrhizal fungi (Thomas, 2014). The evolutionary divergence of *Pinaceae* has been dated to ~150 Mya and the symbiotic partnership of *Pinaceae* with ectomycorrhizal fungi has been dated to ~130 Mya, suggesting the development of *Pinaceae*, and their hardiness, has been shaped by symbiosis (Berbee and Taylor, 1992; LePage *et al.*, 1997; Wang *et al.*, 2000). By the end of the Cretaceous period, hardwood species increasingly infringed on the territory of conifers owing to a warmer, moister global climate (Thomas, 2014).

Figure 1.3. Natural history of major forest species relating to topics covered in this thesis. Molecular clock estimations for the original ancestral split for major lineages are shown with error estimates spanning the shaded area. The top plot presents the complete geological history of Earth. The bottom plot presents a more detailed account of the most recent 500 million years. All references appear in text.



Of equal importance to the historical development of forests would be the natural history of soil, known as ‘paleopedology’ (Retallack 2008). The biosphere of ancient soils is not well understood, and is beyond the scope of this thesis. However, it is clear that early colonizers of mineral-rich terrestrial soils possessed extreme tolerances to radiation and desiccation as evidenced by the recent phylogenetic delineation of the major bacterial lineage: ‘Terrabacteria.’ The ancestors of Terrabacteria possessed extreme tolerances to desiccation, radiation and heat believed to have been necessary for the expansion and diversification of bacterial life on land approximately 3.5 – 2.6 Gya (Battistuzi *et al.,* 2009). Members of this superphylum are, to this day, commonly abundant in soils, such as *Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes* and *Deinococcus-Thermus*, and many taxa from these groups have the capacity to degrade cellulose and other plant polymers. It is, therefore, possible that endemic forest soil microorganisms have the capacity to adapt and endure a range of extremes and continue to fulfil functional roles important for ecosystem regeneration in the decades following harvesting.

Mimicking natural disturbance has been a guiding principle of conventional forestry operations since the 1990s. These principles are founded on the observation that forested land are subject to continual disturbances and that numerous species that comprise forests have evolved to take advantage of these disturbances, such as pyrophilous or ruderal species. Management practices emulate disturbances through prescribed burns and ‘retention forestry,’ which aims to maintain biological refugia by retaining a greater number of living trees and plant detritus, such as coarse woody debris and snag trees (Gustafsson *et al.,* 2012). Both burning and retention have been shown to mitigate a variety of harvesting impacts on macro-fauna, maintaining diversity and higher order trophic interactions, such as predator-prey relationships for beetle populations (Heikkala *et al.,* 2016). How well these forestry principles extend to the belowground biosphere has not been studied, though the LTSP and others, such as the Ecosystem Management Emulating

Natural Disturbance study (EMEND) are beginning to include belowground surveys in their data collection (Hannam *et al.,* 2006; Hartmann *et al.,* 2012). Overall, a better understanding of the similarities and differences of how soil communities are impacted by forestry and natural disturbance is needed before the full merits or shortfalls of such mimicry can be known.

1.1.6 Contrasting Timber Harvesting with Natural Disturbances

For the most part, natural disturbances affect small areas of forest (<1 ha) producing a mosaic of forest with varying legacies resulting from the senescence of even-aged stands, physical damage by wind, localized fire or biological damage from pathogen or insect outbreaks. Minor disturbances are estimated to occur on an average cycle of 50 - 200 years at a given plot of land. In contrast, large-scale disturbances causing the destruction of forests on the order of ~10 to 100 ha occur on an average cycle of 800 to 10,000 years, typically resulting from wildfire, glaciation, hurricanes and insect or pathogen epidemics (Seymour *et al.,* 2002). In comparison, the typical plot size of a managed forest plantation is between one and ten hectares and is harvested on a 50 to 70-year cycle. In terms of both frequency and scale, timber harvesting surpasses the magnitude of major natural disturbances in any one managed area of land, though not in terms of total land affected (see caveat below).

A shortened interval between disturbances has the potential to create a long-term imbalance in the regeneration time for forest species in favour of ruderal, early colonizing taxa that thrive in the years immediately following disturbance (Roberts *et al.,* 2016). For example, shorter intervals between prescribed burning of forested land increased the total effect size of changes in EM communities (Oliver *et al.,* 2015). Shorter intervals between regeneration also increase the net time soil communities are exposed to warmer and drier ‘canopy-free’ conditions relative to natural disturbances. Further, under natural disturbance regimes, forests and forest soils would have hundreds of years to regenerate, making the accumulation of organic matter another major point of

difference to timber harvesting regimes. These points of difference between managed and natural forest disturbances have the clear potential to broadly affect the long-term ecology of forest plantations and soil microbial processes.

Conventional timber harvesting differs in other ways from large-scale natural disturbances that may have other long-term homogenizing effects on forest ecosystems. Soil compaction is unique to harvesting disturbance, resulting from the use of heavy machinery during harvesting, though it has been found to have a minor impact on soil communities (Hartmann *et al.,* 2012). There are far reaching concerns that reforestation efforts have a homogenizing effect on genetic diversity, despite concerted efforts in breeding, on the genetic composition of mixed-aged stands (Friedman and Foster, 1997). Homogenization can lead to increased susceptibility to forms of disturbance (Fettig *et al.,* 2014; Klapwijk *et al.,* 2016) and will likely affect belowground organisms such as ectomycorrhizal fungi (EM), which demonstrate host-preference and even differences according to stand maturity (Visser, 1995; Twieg *et al.,* 2007). Management strategies offers the possibility of mitigating undesirable changes in soil communities. Yet, the current lack of baseline and long-term monitoring data on the effects of repeated forest disturbance limit our understanding of the full extent of impacts. Studying similar canopy-removing disturbances, like wildfire, may augment the lack of long-term data.

One caveat to aforementioned differences between harvesting and natural disturbances is that the total area of land affected by natural disturbance far surpasses the total land affected by the forest industry. In 2013 alone, Canadian forests were affected by insect and wildfire at a scale of 20 and 4.5 million hectares, respectively, dwarfing that of forestry activity (~0.75 million hectares) (Stats Canada Forest Inventory, 2013). However, natural disturbances do not match the frequency of disturbance at a given location under management.

1.1.7 Long-term Impacts of Forest Fire on Soil Communities

Wildfire can remove a comparable amount of aboveground biomass to timber harvesting, but typically leaves subsurface soil organic matter intact. The complete loss of upper organic layer soils can occur in high-severity forest fires, though the occurrence is rare (Jurgensen *et al.,* 1997). The initial combustion of both living and dead organic matter and extreme temperature alters subsurface communities and reduces microbial biomass and rates of decomposition (Dooley and Treseder, 2012; Holden *et al.,* 2015). During forest renewal, soil inhabitants are exposed to a new baseline of environmental conditions which are analogous to those following harvesting, such as increased dryness, diurnal fluctuations and increase soil temperatures. Oliver *et al.,* (2015) report that the diversity and richness of fungal communities had not declined eleven years on, but that the overall community structure had shifted towards heat-tolerant fungi, such pyrophilous taxa.

Estimates for the return of microbial communities to pre-harvest or pre-fire composition are on the order of decades (Holden *et al.,* 2013a; Holden *et al.,* 2013b). Holden *et al.,* (2013b) found the greatest differences in fungal composition between burned and unburned sites occurred in the first 10 to 20 years following fire, roughly the time till canopy closure, after which affected sites had begun to resemble unaffected sites. Oliver *et al.,* (2015) found that disparate fire-affected soils shared similar compositional changes in fungi in the decade following disturbance, demonstrating a consistent selection pressure following disturbance. The decline of *Basidiomycota* at the expense of increased ascomycotal populations has been observed in decades following fire (Buscardo *et al.,* 2015; Holden *et al.,* 2013b), similar to what has been reported post-harvesting (Hartmann *et al.,* 2012). An explanation for these differences is not clear, but both fire and harvesting can damage the fine hyphal networks of *Basidiomycota*, which typically have slower growth rates than *Ascomycota* (Strickland and Rousk, 2010; Holden *et al.,* 2015). The fact that many *Ascomycota* are thermo-tolerant or that EM fungi (*Basidiomycota*) are sensitive to fire

disturbance (Holden *et al.,* 2013b) are also likely factors. Yet, some species of EM fungi are heat-tolerant and prosper in the years following fire, such as *Rhizopogon*, *Suillus*, *Thelephoraceae*, *Tomentella* and *Wilcoxina* among others (Buscardo *et al.,* 2015; Glassman *et al.,* 2015; Oliver *et al.,* 2015). Arbuscular mycorrhiza (*Glomeromycota*) are resilient to fire disturbance, likely due to their dependence on understory vegetation which recovers rapidly post-fire (Xiang *et al.,* 2015).

Shifts in the bacterial community following fire are also pronounced and, similar to fungi, select for stress-tolerant taxa. Weber *et al.,* (2014) studied bacterial communities at burned and unburned sites three-months after a fire and reported that 'the presence of specific taxa may be more important in predicting community compositional shifts after exposure to high burn severity than overall community composition or physical and chemical parameters of unburned soils.' They reported no significant differences in bulk soil community, suggesting disturbance did not turnover, but rather community structure was reorganized. The following taxa were identified as responding positively post-disturbance: *Oxalobacteraceae* (*Oxalicibacterium*, *Naxibacter* & *Massilia*), *Bacteroidetes* (*Flavisolibacter*, *Pedobacter* & *Adhaeribacter*), and *Actinobacteria* (*Arthrobacter*), while some taxa were more abundant in unburned forest soils: *Verrucomicrobia* (*Spartobacteria* & *Opitutus*), *Acidobacteria* (*Granulicella* and Groups 1,3 6, 7 and 16), *Gemmatimonadetes*, *Planctomycetes* (*Zavarzinella*), and *Bacteroidetes* (*Mucilaginibacter*). The report by Tas *et al.*, (2014) on longer-term (7 years later) effects of fire on bacterial communities in tundra soil found similar responses at the phylum level, where *Planctomycetes* and *Verrucomicrobia* populations declined. However, the major group of bacteria that rose to prominence following fire were members of candidate phylum *AD3*, with virtually nothing known about these organisms. Bacterial diversity did not significantly differ between burned and unburned sites, following trends reported for fungi. From these studies, it is clear that a subset of organisms, likely stress-tolerators and ruderal species (Grime, 1977), are able to take advantage of

the relatively harsher soil conditions in the period following disturbance, resulting in nuanced overall structural change without substantial effects on diversity.

1.1.8 Research Objectives for Assessment of Harvesting Impacts (Chapter 3)

The research detailed in Chapter 3 seeks to improve understanding of the impacts of timber harvesting, and relative importance of organic matter retention, in shaping the long-term diversity and structure of microbial communities in general (Section 3.2), and specifically cellulolytic populations (Section 3.3). The LTSP framework afforded the opportunity to compare and contrast the effects of harvesting at three intensities of organic matter removal ('OM removal') among different biogeographic and climatic zones ('ecozones') in North America (Figure 1.1). Approximately 680 phylogenetic gene marker libraries for both 16S rRNA genes (bacteria) and ITS region (fungi) were made from a highly replicated experimental design which accounted for heterogeneity of soils within plots, and within ecozones. Multi-faceted sequencing and biochemical data were collected from microcosm experiments with ^{13}C-cellulose to quantify changes in activity and identify corresponding composition changes in cellulolytic populations.

Timber harvesting is expected to select for organisms adapted to changes in organic matter quality as well as the new post-harvest regime of warmer, drier soil conditions. The changes will likely feature a shift towards more generalist, stress-tolerant species, resulting in no net changes to microbial diversity. Cellulolytic members of *Basidiomycota* are expected to be negatively impacted, given reports of their decline post-harvest (Hartmann *et al.*, 2012), while harsher conditions may favour bacterial degraders. Alternatively, cellulolytic fungi may predominate where coarse woody debris is retained, resulting from the selection of specialized wood-degrading fungi. While the impact of harvesting on the alpha-diversity of cellulolytic taxa has not been assessed, repeated prescribed burnings were shown to reduce the alpha-diversity of cellulolytic

fungi (Bastias *et al.,* 2009), suggesting that cellulolytic populations may possess fewer stress-tolerant taxa that succeed following disturbance.

In accordance with the goals of the LTSP to 'develop indices of soil quality practicable in monitoring' (Powers, 2006), this research sought to document new phenomena of interest and identify specific taxonomic groups which may be relevant to monitoring efforts. The research was oriented to examine fine-scale differences occurring among all ecozones ('globally') and phenomena which are ecozone-specific ('locally') in order to contribute to a better understanding of whether variation in forest regeneration among LTSP sites may be attributed to differences in microbiota. A systematic assessment was made of the previous phenomena related to ectomycorrhizal, methanotroph and saprotroph populations, testing these observations across broadly different geographic zones, forests and soil types. These assessments, along with gauging the mitigating influence of varying amounts of organic matter retention, were made to help inform long-term forest management practices. Comparisons to the long-term effects of forest fire were included to relate features of anthropogenic and more natural forms of large-scale disturbance.

Beyond the examination of harvesting impacts, there are few examples of comprehensive, molecular-based study comparing microbial communities from different coniferous forests at the scale and degree of replication as the research presented in Chapter 3. As such, this research also provides a general characterization of forest soil communities among different forest types and ecozones across North America. Similarly, in gauging the impacts of timber harvesting on the cellulolytic community using SIP, Chapter 3 attributes function to previously unknown and uncultured cellulolytic taxa. In this aspect, parts of Chapter 3 complement the aims of the research presented in Chapter 4. Section 3.3 provides the first part of a survey of cellulolytic taxa from organic and mineral soils and an assessment of the cellulose-degrading potential therein.

Descriptions of Cellulolytic and Lignolytic Populations of Forest Soils: their Natural History and History of Industrial Use

Chapter 4 focuses on a largely separate research topic than the effects of timber harvesting, though section 4.2.7 revisits the data collected for Chapter 3 and assesses harvesting impacts on taxa designated as putatively lignocellulolytic.

1.2.1 Valorization of Lignocellulosic Biomass

The earliest studies on lignin and cellulose decomposition were carried out to understand threats posed to textile and wood-products by plant-degrading organisms. Commercial ethanol production from wood was attempted in 1898 by Simonsen using a non-biological method, and experiments with fermentative processes soon followed (Moore, 1914). As early as 1935, attempts were made to gasify lignin for energy (methane) and, at the same time, recover pure cellulose for ethanol fermentation (Levine *et al.,* 1935). Following the outbreak of WWII, and subsequent wars like Vietnam, northern nations operated in tropical regions where the decay of plant fibre-based materials became a significant economic driver for research in both cellulose (Siu, 1951) and lignin-degrading (Gottlieb, 1951) organisms. The manufacture of vanillin was perhaps the earliest example of the successful valorization of lignin by Kürschner, who laid the foundations for commercial production of vanillin in 1928. In 1948, an editorial published in Scientific American stated the case for valorization of lignin waste, highlighting its potential as nature's most abundant source of aromatic precursors (Gesinger, 1948).

The steady growth in industrial processing of wood biomass since Gesinger's time has prompted widespread attempts to valorize the correspondingly sizeable waste streams. In 2012, lignocellulosic biomass wastes were estimated at approximately 2×10^{11} t/year worldwide (Tuck *et al.,* 2012). Cellulosic ethanol production is now a commercially viable enterprise exemplified by

the Beta Renewables plant in Crescentino, Italy, generating 75 million L $year^{-1}$. Given their structural role in plant tissues, is not surprising that both lignin and cellulose are being used to strengthen materials such as concrete (Ataie *et al.,* 2014) and in the production of composite plastics (Chung *et al.,* 2013; Kalia *et al.,* 2011), foam (Li and Ragauskas, 2012) and carbon nanofibers (Dumanli *et al.,* 2012; Li *et al.,* 2015). Cellulose has the potential use in a wide variety of fermentative processes, however, as a fibre, cellulose has been functionalized for specific applications such as research in molecular biology (Araujo *et al.,* 2012), medicine (Brinchini *et al.,* 2013) and tissue culturing (Bodin *et al.,* 2007). The number of applications of functionalized cellulose fibre are enormous given recent advancements in carbohydrate chemistry and widespread interest in carbohydrate-active enzymes (Xu *et al.,* 2012). As nature's most abundant aromatic compound, lignin is a promising renewable source of aromatic precursors for a myriad of synthetic processes. It is expected to compete with a number of petrochemical products such as resins, lubricant and fuel additives, such as benzene, toluene, and xylene (Linger *et al.,* 2014; Ragauskas *et al.,* 2014). Recent applications include the anodic material for lithium ion batteries (Zhang *et al.,* 2015) and air filters and respirators (Chang *et al.,* 2016). The old adage that ‘you can make anything from lignin… but money’ is being tested under the current climate for renewable resources and a near 100-year history of research.

1.2.2 Origins of Research into Cellulolytic and Lignolytic Organisms and Enzymes

The science of wood decay originated in 1833 with Hartig’s study of the fungal plant pathogen, *Phellinus pini*, commonly known as "red ring rot." It remained the domain of biology, mostly mycology, until the latter half of the 19th century when organic chemists began disassembling the chemical components of wood and analyzing the bi-products of wood rot. The structure of cellulose was first described by Payen in 1838, who also coined the name ‘cellulose,’ basing his observations on the remnants plant matter after treatment with strong acids. The first

insight into the chemical composition of lignin was provided by Klason who described it, in 1896, as a coniferyl alcohol-based compound and later published a method for measuring lignin content that is commonly used today (Klason, 1910). The enzyme-mediated degradation of cellulose was first demonstrated by Sachs, in 1862, during his study of germinating grass seeds. The earliest studies to demonstrate the biologically-mediated degradation of lignin involved white-rot fungi (Bayliss, 1908; Zeller, 1916). Then, as now, the central challenge in characterizing catabolic capabilities hinged on how the cellulose or lignin was prepared, casting doubt, especially in the case of lignin, on the true nature of the microbial activity (early criticism by Gottlieb, 1951). By 1927, the activity of brown and white rot fungi had been delineated with a growing complement of knowledge on cellulase and phenol-oxidase enzymes (Falck, 1927). Yet, the definition of white-versus-brown rot has been eroded in the age of genomics, as canonical degradative capabilities have been found in the genomes of non-canonical members of each category (Riley *et al.*, 2014; Floudas *et al.*, 2015). This break from a near century old convention exemplifies the possibilities afforded by phylogenetics and comparative genomics to characterize how cellulolytic and lignolytic organisms, and the catabolic pathways they possess, have evolved.

1.2.3 Natural History of Lignocellulose Synthesis and Degradation

The origins of cellulose and lignin biosynthesis predate terrestrial life, leaving no traces of its earliest development in today's fossil record. The cellulose synthase genes common to plants are believed to originate from *Cyanobacteria* (photosynthetic bacteria), yet we find cellulose synthesis spread across a variety of bacterial phyla, including a number of *Proteobacteria* (Nobles *et al.*, 2001). The closest common ancestor to land plants are marine Aerophyte green algae, which possess many features of the cellulose structure and overall carbohydrate complexity of modern forms of plant cell walls, though without any lignin (Mikkelsen *et al.*, 2014; Domozych *et al.*, 2012). The growth in lignin content in plant cell walls followed the colonization of land, which

occurred around 470 million years ago and resulted in the diversification of plant cell wall architecture (consult Figure 1.3 for overview of natural history).

The evolution of lignin biosynthesis began in early land plants with the shunting of phenylalanine pathway to synthesize hydroxylated aromatics for UV radiation protection (Weng and Chapple, 2010). This newly evolved form of phenylpropanoid synthesis led to the synthesis of lignin precursors, hydroxyphenyl and guaiacyl lignin. By the late Silurian period (416 Mya), vascular plants had evolved lignified tracheid tissue for conducting water and, by the end of the Middle Devonian (398 to 382 Mya), trees had evolved independently in several major groups, and an abundance of chemically stable, lignified plant matter began to accumulate (Robinson, 1990). The rate of accumulation reached a maximum during the Carboniferous and Permian, resulting in the formation of vast coal deposits derived primarily from lignin (Berner *et al.*, 2003). Coal deposits from the Permian and Carboniferous period outweigh all other periods and were responsible for fueling the industrialization of modern civilization. Similarly, at the time of the Carboniferous, the accumulated mass of lignified plant matter was a rich resource to any organisms that developed the capability of exploiting it.

The earliest ancestors of terrestrial decomposers of cellulose and aromatic polymers predate the colonization of land. The origin of terrestrial decomposers was believed to result from Earth's changing climate, leading to shifting ocean water levels and the accumulation of ocean biomass on land. Our ability to reconstruct the ecology of this ancestral habitat is limited and, so too is our understanding of the early evolution of terrestrial decomposers (for a review see: Raven, 1997). Our understandings of the natural history of fungal lignocellulose decomposers has been aided by the recovery of fossils from filamentous fungi. The earliest *Basidiomycetes* have a fossil record that dates back to the Upper Devonian (382 – 372 Mya; Stubblefield and Taylor, 1986), which contain the class *Agaricomycetes*, widely considered the most successful degraders of

lignocellulose. Both white and brown rot fungi are members of *Agaricomycetes*, whose earliest ancestor has been dated to the Permian period (298 - 252 Mya) from deposits in Antarctica and in tree trunks found in North America (Taylor and Osborn, 1996). The sharp decline in coal deposition by the end of the Permo-Carboniferous (~300 Mya) matches molecular clock estimates of when multiple gene duplications of catalase-peroxidases occurred in white-rot *Agaricomycetes*. These duplications are believed to underlie effective lignin decomposition, leading to the hypothesis that the early ancestors of white-rot fungi were the first to benefit from the growing surplus of lignified plant matter (Floudas *et al.*, 2012).

While prosperity may have been assured to any organism capable of modifying lignin, the catalase-peroxidases utilized by white-rot fungi evolved primarily to cope with oxidative stress (Benzie, 2000). During the late Carboniferous period, atmospheric oxygen concentrations reached the highest levels planet Earth had seen (~35%; Graham, 1995). Peroxidase activity became critical to detoxify oxidative compounds and the adaptation to utilize increasingly abundant lignin as reductant has bene hypothesized to be the indirect outcome (Morgenstern *et al.*, 2008). Given that the coupling of catalase-peroxidase activity to lignin was a recent development, the peroxidase superfamily to which these catabolic enzymes belong (Class II) contains two other major classes of intracellular protective enzymes (Class I) and lignin biosynthetic enzymes (Class III), which likely predate the Class II catabolic homologs (Zámocký *et al.*, 2015). As such, homology-based searches of lignin-degrading peroxidases requires careful phylogenetic discrimination between classes as well as supporting evidence through assays of lignin-modifying activity.

White-rot fungi are highly successful lignin-degraders as a result of multiple gene duplications of Class II catalase-peroxidases, but also due to the physical ability of hyphae to penetrate through plant walls (Selosse and Tacon, 1998; James *et al.*, 2006). Similarly, the majority of actinobacterial families exhibit mycelia-like growth, providing an example of

convergent evolution believed to result from spatial complexity in the colonization of soils, though this physiology has not been directly correlated with growth on lignin (Ventura *et al.,* 2007). Perhaps unsurprisingly, many of the best known bacterial lignocellulose decomposers are Actinobacteria. *Streptomyces viridisporus* T7A, was the first bacterium characterized to decompose lignin (Pasti, 1990). Notably, the split between *Streptomycetaceae* and other major actinobacterial families occurred at much the same period in time as the appearance of filamentous fungi, approximately 500 Mya (Embley and Stackebrandt, 1994). Substantial rates of lignin degradation have really only been observed by *Basidiomycota*, though a wide variety of fungi demonstrate less well-characterized degradation, like 'soft rot' or 'brown rot' (Worral *et al.,* 1997; Riley *et al.,* 2014; Floudas *et al.,* 2015). The major focus of Chapter 4 is to expand the known diversity of lignin-degrading organisms; to assess the co-evolution of lignolytic with cellulolytic or hemicellulolytic traits, and to characterize the relative role of bacterial and fungal degraders.

1.2.4 Lignocellulose-degrading Niches

What we know of the ecology and metabolic diversity of lignolytic and cellulolytic taxa is largely based on a number of model organisms studied *in vitro*. Collectively these organisms exhibit aerobic, facultative and anaerobic lifestyles for both cellulose and lignin decomposition and inhabit a broad range of environments such as marine, insect, ruminant, plant (pathogen), sediment, soil and compost (*cellulose*: Hanson *et al.,* 2008; Scharf *et al.,* 2008; Distel *et al.,* 2002; Izquierdo *et al.,* 2010; Hess *et al.,* 2011; Dougherty *et al.,* 2012 and *lignin*: Geib *et al.,* 2008; Thevenot *et al.,* 2010; DeAngelis *et al.,* 2011). Co-culturing experiments demonstrate that decomposer communities exhibit high levels of syntrophic growth on lignocellulosic substrates (Leschine *et al.,* 1995), and environmentally-derived cell slurries show higher rates of decomposition of lignocellulose sourced from co-endemic plants (Ayres *et al.,* 2009; Prescott, 2010; Freschet *et al.,* 2012). The critical role of lignolytic and cellulolytic organisms is supported

by models of decomposition that show enzymes produced by small sub-populations drive overall decomposition in forest litter (Allison *et al.,* 2012; Goldfarb *et al.,* 2011). With modern tools in molecular biology, microbiologists can characterize the full variety of niches without the need for enrichment cultures, enabling a more accurate description of environment-specific taxa and catabolic mechanisms. For instance, a gut environment would select for organisms adapted to degrade physically altered, pre-digested substrates, whereas soil organisms likely possess a greater range of capabilities for gaining access (adhesion and penetration) to unaltered plant material, as well as a greater range of tolerances to environmental conditions and even interesting redox coupling. Ko *et al.*, (2009) demonstrated lignin and cellulose co-metabolism coupled with sulfate reduction using inoculum sourced from landfill soil. Targeted characterization of specific niches and the consortia using molecular techniques, such as SIP, will drive future discoveries of novel lignocellulose-degrading consortia and catabolic machinery.

1.2.5 Lignin-degrading Fungi and Bacteria

Wood-degrading fungi demonstrate a preference for the degradation of either cellulose, producing brown coloured wood rot, or lignin, producing a whitish colour of decaying wood. Both phenotypes share a common ancestor within the order *Agaricomycetes*. Specialization in lignin decomposition correlates with increased numbers of glycosyl hydrolase (GH) genes relative to non-specialized taxa and multiple gene duplications in GH and ‘auxiliary activity’ (AA), largely peroxidase, enzyme families (Floudas *et al.,* 2012). Brown rot species modify lignin to access cellulose and have incurred substantial GH and AA gene loss, having only retained the capacity to abundantly express a reduced set of cellulose-degrading enzymes (Eastwood *et al.,* 2011). Similar adaptive loss was reported in closely related ectomycorrhizal fungal genomes, demonstrating the phylogenomic basis for specialization and niche partitioning in the forest soil community (Martin and Selosse, 2008). However, mycorrhiza may partake in lignin and cellulose decomposition,

albeit believed to be related to nitrogen and phosphorous scavenging (Baldrian *et al.,* 2009; Rineau *et al.,* 2012). Outside of well-studied *Basidiomycota*, some *Ascomycota* (*Xylaria* and *Daldinia* spp.) are also capable of lignin modification, generally known as soft-rot (Floudas *et al.,* 2012). This capability depends on laccase-like activity and more closely resembles brown-rot (Liers *et al.,* 2011).

The study of lignin-degrading bacteria is a relatively young field of research, largely pioneered in the late 1970's by Donald and Ronald Crawford. To date, the bacterial strains proposed to be capable of decomposing lignin polymers or lignin-related compounds occur in the following phyla (or sub-phyla): *Actinomycetes*, *Bacteroidetes*, *Firmicutes*, *Alpha-* *Beta-* and *Gammaproteobacteria* (see Table E.2 for a list of all known lignin and cellulose-degrading isolates). All of these organisms have been isolated and characterized *in vitro* through screening or enrichment culturing with polyaromatic compounds, kraft-lignin or lignin-related dimeric substrates. The most commonly cited organisms are *Streptomyces viridisporus* T7A (Pasti *et al.,* 1990; Davis *et al.,* 2013), *Sphingobium sp.* SYK-6 (Masai *et al.,* 2007), *Sphingobacterium sp.* T2 (Taylor *et al.,* 2012; Rashid *et al.,* 2015), *Enterobacter lignolyticus* SCF1 (DeAngelis *et al.,* 2011), *Pseudomonas spp.* (Zimmerman, 1990) and *Amycolatopsis sp.* 75iv2 (Brown *et al.,* 2012). These taxa, and others, are described in detail in a number of reviews (Vicuna, 1988; Bugg *et al.,* 2010; Zimmerman, 1990; Brown and Chang 2014; Tian *et al.,* 2014). Consistent with the idea that lignocellulose degradation is performed by a specialized sub-set of microorganisms, many of the lignin-modifying genera also contain well-known cellulose-degraders, such as *Streptomyces*, *Enterobacter*, *Sphingobacterium*, *Pseudomonas* and *Acinetobacter* (Štursová *et al.,* 2012; Pourramezan *et al.,* 2012; Medie *et al.,* 2012). The limited number of experimentally-verified, bacterially-produced lignin-modifying enzymes is insufficient to make generalizations about functional diversity. However, bacterial lignin-modifying mechanisms appear to be based on

similar oxidative enzymes as fungi, such as peroxidase (DypAB) and laccase (bacterial laccase) enzymes. A unique set of glutathione-dependent enzymes that reductively cleave β-aryl bonds, termed the 'Lig operon,' was described by Masai *et al.,* (1993). These bonds constituted the majority of inter-molecular bonds within lignin.

Despite the culturing of bacteria with lignin-degrading capabilities, there have been few surveys describing their diversity within environmental lignin-degrading niches. There have been surveys of compost, deciduous forest (Pold *et al.,* 2015) and tropical forest soil (DeAngelis *et al.,* 2011; Woo *et al.,* 2014) all originating from the same research group (see Section 1.2.7 for more details). The gut microbiome of wood-boring insects and termites (Harazono *et al.,* 2003) have also been surveyed for lignin-degrading bacteria, implicating many familiar taxa (*Streptomyces*, *Burkholderia* and *Pseudomonas*) and some unique ones (*Citrobacter*, *Escherichia*, *Paracoccus*, *Elusimicrobia* and *Yersinia*). Studies of wood-ingesting mammalian gut microbiomes are underway for the beaver and moose (Dr. Keith Mewis and Dr. Kelly Wrighton, personal communication). Anaerobic lignin degradation has been reported in tropical forest soils, where lignolytic activity actually increased under fluctuating oxic conditions (Hall *et al.,* 2015). The phenomenon reflected the relatively high catabolic rate of oligomeric forms of lignin under anaerobic conditions (coupled to iron reduction), combined with presumably necessary oxidative conditions to fuel oxidative catabolism of polymerized lignin (Hall *et al.,* 2015). Anaerobic lignin degradation is supported by the recent description of *Enterobacter lignolyticus*, a facultative anaerobe capable of degrading model lignin compounds (DeAngelis *et al.,* 2011). Lignin-degradation has also been observed in anaerobic or sub-oxic marine environments (Benner *et al.,* 1984) based on the activity of *Ascomycetes* (Bucher *et al.,* 2004), *Streptomyces* (Buraimoh *et al.,* 2015), and *Sagittula stellata* (Gonzalez *et al.,* 1997). Given the relatively new reconception of bacteria as contributors to lignin-degradation and the recent development of metagenomic-based

research, environmental surveys of lignin-degrading bacteria are few and far between. One of the major goals of this research was to determine whether previously characterized bacterial lignin-degraders are active *in situ* in forest soils and expand our knowledge of their diversity.

1.2.6 Cellulose-degrading Fungi and Bacteria

Few microorganisms possess the enzymes necessary to depolymerize cellulose and these are largely fungal and bacterial in origin, though invertebrates and higher eukaryotes possess some cellulase activity (Lo *et al.*, 2003; Wilson *et al.*, 2011). Bacteria that have evolved a specialization for growth on crystalline cellulose, present in plant cell walls (Figure 1.4), possess an overall greater number of CAZymes than their generalist counterparts (Berlemont and Martigny, 2013). They also possess a full complement of glycosyl hydrolase (GH) enzymes (endo- and exo- acting; Berlemont and Martigny, 2013) and often lytic polysaccharide monooxygenases (LPMO). These hardcore cellulose degraders are broadly distributed across 15 bacterial phyla, although closely related organisms (≤ 98% similar) do not necessarily, or even commonly, share the same capacity for cellulose degradation (Berlemont and Martigny, 2013). These characteristics suggest a significant role for horizontal gene transfer and convergent evolution in cellulose degradation, reflecting the diversity and abundance of cellulose-rich niches on Earth. Five phyla contain 89% of known bacterial cellulose-degraders (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, *CFB* and *Chlorobi*), including model degraders like *Cytophaga hutchinsonii* and *Clostridium cellulyticum*. Decomposition of crystalline cellulose is relatively common across fungal saprophytes (i.e. non-parasitic fungi), which includes taxa from *Basidiomycota*, *Ascomycota*, *Neocallismatigomycota* (anaerobic fungi) and even the most ancestral lineage in fungi, *Chytridiomycota* (Gleason *et al.*, 2011; Schneider *et al.*, 2012; Štursová *et al.*, 2012). A complete list of validated and predicted cellulose-degraders can be found in Table E.2. Unlike lignolytic populations, a number of

environmental surveys of cellulolytic populations have been conducted in forests and in other environments.

1.2.7 Lignocellulolytic Bacteria and Fungi in Forest and Other Soil Environments

Forest soils are generally more acidic than other commonly studied soil types and pH exerts a strong influence over microbial community composition (Lauber *et al.,* 2008). The groups of cellulolytic taxa identified in agricultural soils were notably absent in the most comprehensive surveys of forest soil cellulose-degraders to date (Štursová *et al.,* 2012; Eichorst and Kuske, 2012), such as *Cellulomonas*, *Kitasatospora*, *Micrococcus* and *Streptomyces* (*Actinobacteria*), *Mesorhizobium* and *Sphingomonas* (*Alphaproteobacteria*), and *Firmicutes*, such as *Bacillus* and *Paenibacillus* (El Zahar Haichar *et al.,* 2007; Ulrich *et al.,* 2008; Schellenberger *et al.,* 2010). One shared characteristic between forest and agricultural soils in these studies was the predominance of *Betaproteobacteria* (*Burkholderia*) and *CFB* (*Mucilaginibacter*, *Cytophaga*, and *Pedobacter*). In contrast, Štursová *et al.* (2012) as well as Eichorst and Kuske (2012) identified cellulolytic populations of acidobacterial subdivision I, and cultured a representative cellulolytic isolate (Lladó *et al.,* 2016), implicating members of this ubiquitous phylum as cellulolytic for the first time. The aforementioned surveys of forest soils also identified cellulolytic members of the following groups: *Myxococcales*, *Asticcacaulis* (*Alphaproteobacteria*), *Cellvibrio* (*Gammaproteobacteria*), *Cohnella* (*Firmicutes*), *Armatimonadetes* (formerly *OP10*) and *Verrucomicrobia*. The single forest soil sample considered in Wang *et al.,* (2015) was dominated by *Caulobacter*, followed by *Beta-*, *Delta-* and *Gammaproteobacteria* and *Bacteroidetes*. Verastegui *et al.,* (2014) found that *Myxococcales* were indicators of cellulose-decomposition in forest soils, while members of *Caulobacterales* (*Phenylobacterium*), and *Actinomycetales* were common cellulolytic taxa found in tundra, agricultural soil and forest.

In fungi, members of *Ascomycota* predominate cellulose decomposition in soil. In proteomic data from forest litter, *Agaricomycetes* produced the fewest cellulases relative to ascomycotal groups, such *Leotiomycetes*, *Eurotiomycetes* and *Sordariomycetes* (Schneider *et al.*, 2012). *Agaricomycetes*, and *Basidiomycota* in general, are more common wood-inhabiting organisms, though some cellulolytic yeast-like groups, such as *Trichosporon* and *Cryptococcus*, occur in soils (Štursová *et al.*, 2012). In a survey of cellulolytic bacteria and fungi among five soil types, Eichorst and Kuske (2012) found cellulolytic chytrids and dinoflagellates along with ascomycotal groups, such as *Arthrobotrys, Cladophialophora, Chaetomium, Dactylaria, Hypocrea* and *Trichocladium.* Hemicellulolytic populations of forest soils were predominantly *Burkholderia* and *Pseudomonas*, with lesser populations of *Duganella*, *Variovorax*, *Mucilaginibacter* and *Paenibacillus* (Leung *et al.*, 2016).

A survey of lignin-degrading communities in temperate forest soil, using *in situ* enrichment and sequencing, identified putative activity in a number *in vitro* bacterial lignin degraders, such as *Pseudomonas sp.*, *Sphingobacterium sp.*, *Rhodococcus sp.*, *Sphingobium sp.* and *Ochrobactrum sp.*, as well as other taxa known to degrade lignocellulose, not previously implicated in lignin catabolism: *Cytophaga sp.*, *Paenibacillus sp.* and *Agrobacterium sp.* (Pold *et al.*, 2015). A similar study examining lignin-degrading bacteria in Puerto Rican rain forest soil identified lignolytic members of *Alphaproteobacteria*, *Betaproteobacteria*, *Acidobacteria* and *Chloroflexi* (DeAngelis *et al.*, 2011). Surprisingly, in both environmental surveys by DeAngelis *et al.*, canonical lignin-degraders from *Streptomycetaceae* were absent. These reports provide insight into the native, *in situ* communities actively decomposing lignocellulose in forest soils. Yet, of these studies, only Leung *et al.* (2016) and Eichorst and Kuske (2012) considered deeper mineral forest soil communities, the latter studying the 'root zones.' An oft overlook soil type, in favour of plant-matter rich upper layers, mineral soils represent a potential source for novel lignocellulolytic taxa

and catabolism. It has been suggested that mineral soils may favour bacterial decomposers over fungal ones, given few wood-degrading fungi have been found in mineral soils (Hall *et al.,* 2015) and all are aerobic, thus not well suited to deeper, anaerobic soils. Mineral soils were targeted in this thesis in order to capture a broader diversity of lignocellulolytic populations.

1.2.8 Symbioses of Lignocellulolytic Fungi and Bacterial

General physiological differences exist between bacterial and fungal lignocellulose-degraders which impart advantages during different stages of decomposition and under varying conditions in forest soils. Fungi predominate in the upper organic and litter horizons, due to a greater capacity for dispersal, colonization and penetration of fresh plant matter; substantially higher metabolic rates, and more powerful oxidative enzymes (Moore-Kucera *et al.,* 2008; Voriskova and Baldrian, 2013). In one proteomic study of forest litter, the main producers of extracellular hydrolytic enzymes were fungi with no bacterial hydrolases detected (Schneider *et al.,* 2012). Bacteria tend to predominate in later stages of litter decomposition as they possess greater metabolic diversity; adaptation to low oxygen environments and resilience to broad environmental conditions (Berg *et al.,* 2010). Yet, despite these predispositions, decomposition is a stochastic process performed by diverse populations of bacteria and fungi and a range of both antagonistic and mutualistic interactions have been reported (de Boer *et al.,* 2005; Rousk *et al.,* 2008). Fungal colonization of wood was shown to reduce bacterial abundance and diversity, but also consistently select for specific co-habiting bacteria, namely *Burkholderia spp.* and X*anthomonadas spp*. (Folman *et al.,* 2008). In a separate study, the rate of decomposition by mixed fungal and bacterial cultures demonstrated non-additive, synergistic effects (Romani *et al.,* 2006). A variety of mechanisms for mutualism between wood-degrading fungi and bacteria have been proposed, but none well characterized, such as the syntrophic removal of metabolic by-products, detoxification, provision of nitrogen and the production of growth promoting compounds

(Frey-Klett *et al.,* 2007; Effmert *et al.,* 2012; Martin *et al.,* 2012). Conversely, the magnitude of antagonistic interactions in forest soil communities was exemplified in the reported five-fold greater abundance of antibiotic production and resistance genes in forest soils compared to more oligotrophic soils (Fierer *et al.,* 2012). Antagonistic interactions include antibiotic production and resistance, cell-wall degradation (chitinase vs. peptidoglycanase), competition to sequester resources (ex. siderophore production), interference of growth strategies and the modification of quorum sensing molecules (Zhang and Dong, 2004). Tolonen *et al.,* (2014) reported the co-metabolism of cellulose and fungal cell walls by *Clostridium phytofermentans*, where chitinases were actually produced in greater abundance than cellulases, revealing the co-evolution of competitive strategies for cellulose decomposition. In this thesis, the contributions and interactions between these two domains of life is considered and their relative cellulolytic and lignolytic activity described.

1.2.9 SIP in Characterizing Lignocellulose-degrading Communities

Even as the culture-dependent constraints of early microbial ecology studies fade, high-throughput sequencing approaches are still challenged by the heterogeneity of soil and the rich diversity of microorganisms harboured therein. An indication of this is the poor metagenomic assembly from sequencing libraries derived from soil. In the case of one metagenomic study of forest soil, only 2% of reads could be assembled (Cardenas *et al.,* 2015) in contrast to the near 30% assembly from a marine environment (Iverson *et al.,* 2012). As a result, the diversity of lignocellulolytic groups has generally been underestimated and information on the ecological features of these communities is somewhat scarce. SIP offers the possibility of studying these populations by enriching for those organisms which assimilated carbon from a given substrate, thereby reducing the complexity of soil-derived sequencing data. However, even among studies which have successfully applied SIP to study cellulose decomposition, there are other pitfalls, such

as the selection of substrate and the choice of incubation conditions, which makes SIP a hybrid between *in vitro* and *in situ* approaches. For example, there have been a number of recent SIP-cellulose studies (Bastias *et al.* 2009; Schellenberger *et al.* 2010; Štursová *et al.* 2012, Koranda *et al.* 2014; Torres *et al.,* 2014) that made use of the commercially available ^{13}C-cellulose manufactured by IsoLife which was found, here, to be unsuitably pure to target the exclusive activity of cellulolytic taxa (Section 2.2.3.1). The research described in this thesis represents a conservative SIP approach, which included the development of methods to optimize experimental conditions, to validate ^{13}C-enrichment of DNA and assay the quality of substrate utilized.

Recent '-omic' and functional-based screening methods have provided a steady discovery of novel organisms and enzyme families possessing activity on lignin and cellulose (Bugg *et al.,* 2010; DeAngelis *et al.,* 2010; Hess *et al.,* 2011; Levasseur *et al.,* 2013). On one hand, these discoveries reflect our underestimation of the biochemical complexity required for decomposing lignocellulose and diversity of adaptations at the organismal level. On the other hand, many of the enzymes of interest catalyze a broad range of substrates and are involved in other cellular processes, exemplified by the various classes of catalase-peroxidases. One should expect that a major proportion of novel discoveries based on functional screening or other *in vitro* testing may be attributed to non-specific activity and may not translate into ecological significance, especially for those displaying low activity. SIP methods offer a unique solution to this problem by enabling functionally-oriented studies of microbial communities directly in an environmental and ecological context. SIP methods have a significant likelihood of novel discovery, having been successful in the targeted recovery of desired functional traits (Dumont *et al.,* 2006; Pinnell *et al.,* 2014) and the discovery of novel functional taxa (Buckley *et al.,* 2007; Chen *et al.,* 2008; Eichorst and Kuske, 2012).

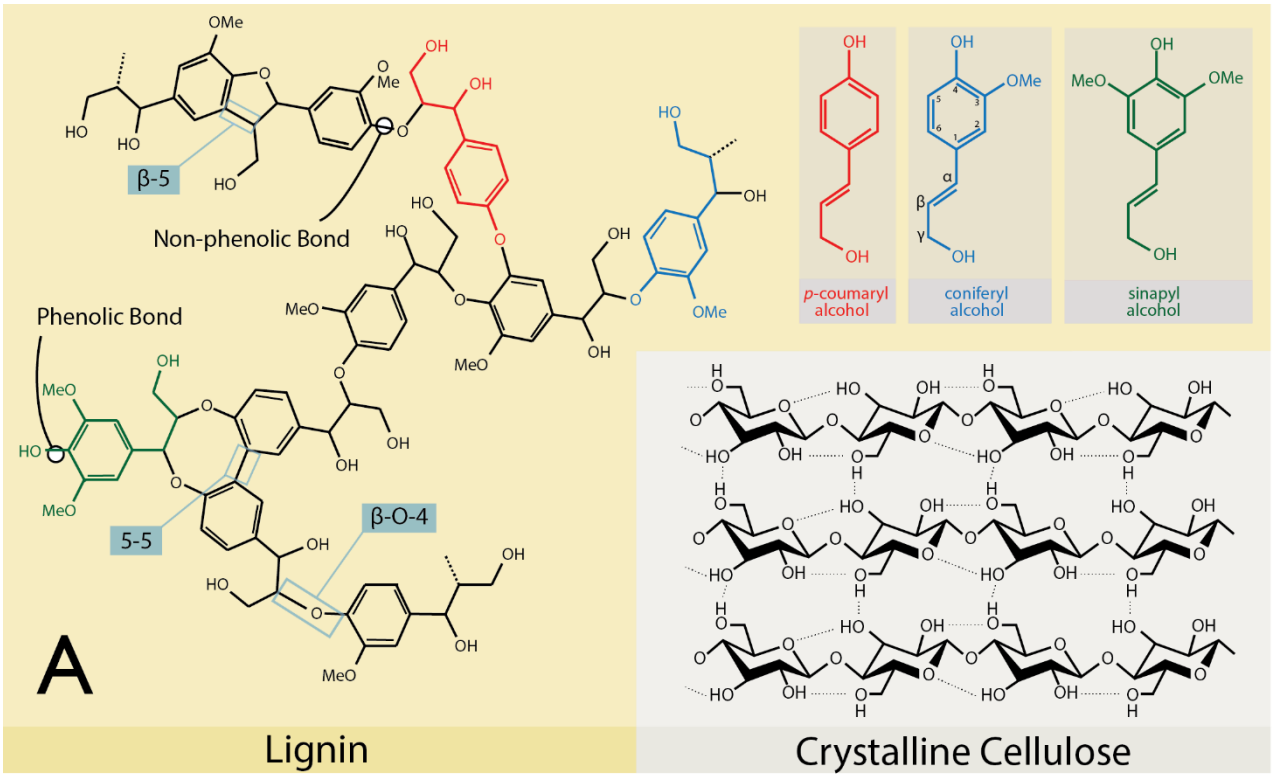
1.2.10 Overview of Classes of Ligninase and Cellulase Enzymes

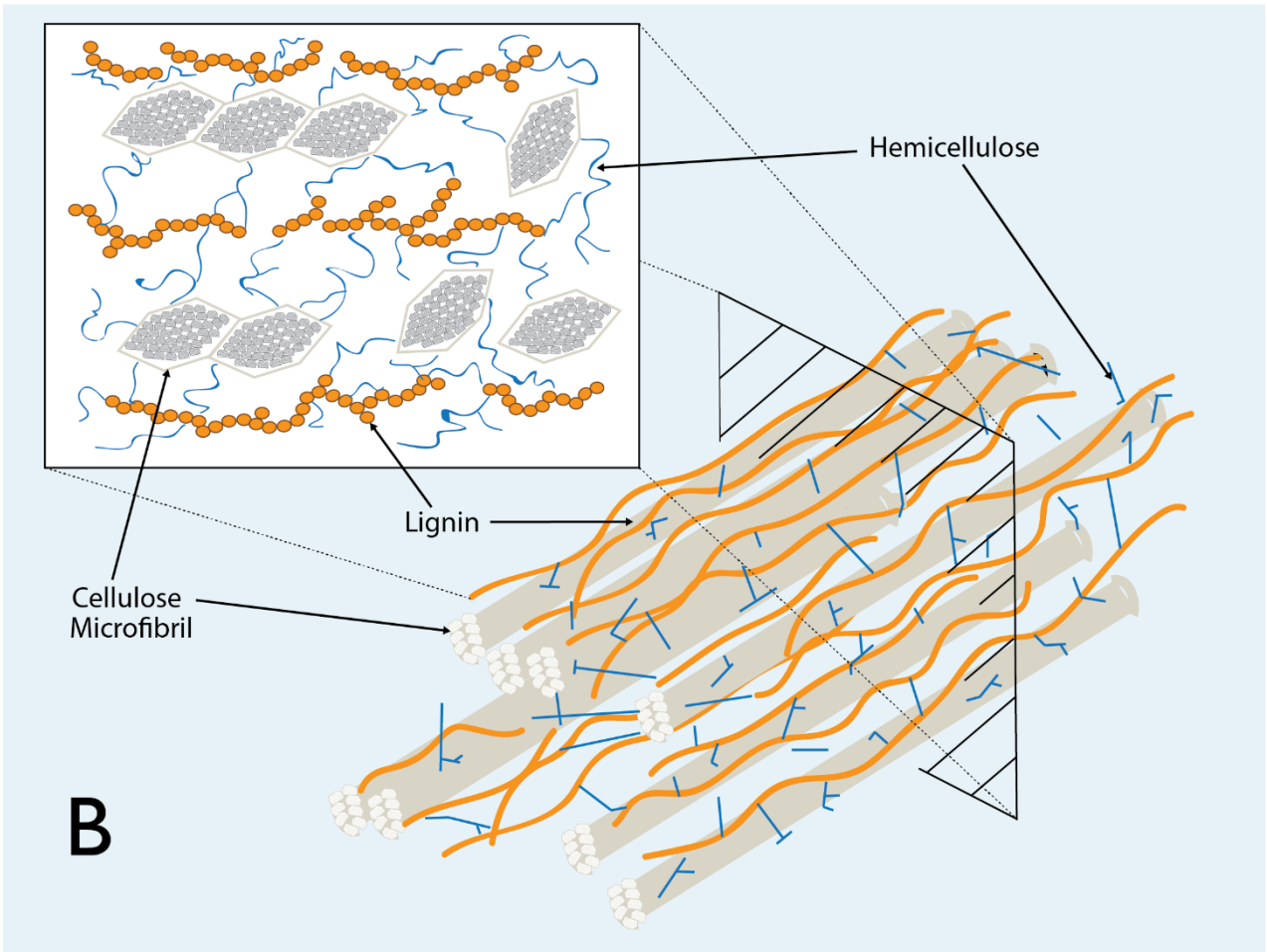
The breakdown of lignocellulose is slow and energetically unfavourable because of chemical properties selected by plant evolution, such as the facilitation of transpiration (hydrophobic), the ability to withstand high pressures and support heavy structures like branches (rigid) and the ability to resist microbial attack (random, stable and racemic). The synthesis of lignocellulose requires over 1,000 genes (Somerville *et al.*, 2004) and results in a complex, interwoven matrix of four major polymeric compounds: lignin, cellulose, hemicellulose and pectin. Of the four general components, lignin and cellulose are the most chemically inert and, due to the complexation of lignocellulose (Figure 1.4), impede overall decomposition (Ding *et al.*, 2012). For example, the half-life of cellulose was estimated to be on the order of millions of years in the absence of biological activity (Wilson, 2011). The following sections summarize current knowledge about the enzymes and pathways utilized to breakdown cellulose and lignin.

1.2.11 Cellulases and Accessory Enzymes

For many decades, the central paradigm for cellulose degradation revolved around the production of extracellular, 'free' (unbound) hydrolases that synergistically cleave within the crystalline inner stretches of polymer (endoglucanases) or at the termini (exoglucanase) producing oligosaccharides which are eventually cleaved into glucose by β-glucosidases. Many of the most prolific and best-characterized cellulolytic organisms employ this strategy (*T. reesei* and *C. hutchinsonii*). Exoglucanases are fast-acting, processive enzymes, unlike endoglucanases which adsorb and desorb at a much slower rate (Maurer *et al.*, 2012). Exoglucanases also act in a unidirectional manner, either from the non-reducing (EC 3.2.1.91) or the reducing (EC 3.2.1.176) ends of cellulose polymers. Endoglucanases acting in a processive manner, though rare, have been reported (Wilson and Kostylev, 2012). Some cellulases exhibit both endo- and exocellulase activity, which is not too surprising given the differences between exo- and endo-activity can

Figure 1.4. Representations of the structure of (A) lignin, crystalline cellulose and (B) their complexation with hemicellulose to form lignocellulose, the structural component of plant cell walls. The structural diagram for lignin was modified from Zakzeski *et al.* (2010) and the representation of lignocellulose was modified from Zeng *et al.* (2014).





differ by slight structural changes near the active site (Oliviera *et al.,* 2013). Exo- and endoglucanases from the GH family 6, which possess near identical structure and catalytic residues, differ in activity based on the presence or absence of a tunnel or loop conformation at the active site (Damude *et al.,* 1996). Both endo- and exoglucanases show a rich diversity of structure and function that result from the paralogous expansions as well as convergent evolution (Lynd *et al.,* 2002; Cantrel *et al.,* 2012). This diversity is further multiplied by their modular associations with carbohydrate-binding modules (CBMs). The discovery of novel GH families has been aided by such modular associations, such as CBMs, conserved protein motifs (Busk and Lange, 2013) or signal peptides (Emanuelsson *et al.,* 2007; Warnecke *et al.,* 2007), that provide targets for homology searches.

In 1983, Lamed *et al.,* reported on a new paradigm in cellulose-degrading machinery, termed the 'cellulosome,' a self-assembling multi-enzyme scaffold capable of binding to lignocellulose substrates and catalyzing a number of catabolic reactions. The first cellulosome was characterized from the rumen-inhabiting anaerobe *Clostridium thermocellum*, and subsequently in other anaerobic bacteria and fungi (Bayer *et al.,* 1994). The cellulosome revealed the degree of specialization possessed by decomposers and how evolution has addressed the complexity of heteropolymeric carbohydrate substrates. A cellulosome is constructed from a number of functional enzymes possessing 'dockerin' domains, which bind to 'cohesin' domains located on a large 'scaffoldin' protein, which is tethered to an anchor in the cellulolytic organism's cell wall. These structural elements, namely cohesins, dockerins and scaffoldins, provide a target for metagenomics surveys of cellulosomes, termed "cellulosomics" (Bayer *et al.,* 2008). Cellulosomes are significant to the metabolism of many ruminants and have recently been found in the human gut (Cann *et al.,* 2016).

In 2000, Shipman *et al.,* described a multigene 'starch utilization system' (Sus operon), which was the first example of the 'polysaccharide utilization loci' (PUL) paradigm. A PUL refers to a syntactic gene cluster which encodes a set of proteins that bind a polysaccharide to the outer membrane, cleave it and transport oligosaccharides inwards. PULs were first described in *Bacteroidetes*, from which the best characterized examples originate. A number of PUL-like operons possess synergistic activity on both cellulose and a wide range of other polysaccharides, demonstrating the importance of the PUL-paradigm (Mackenzie *et al.,* 2012; Koropatkin *et al.,* 2012). The most current elaboration on cellulose-degrading machinery was the discovery, in 2013, of an unbound, extracellular enzyme that possessed multiple catalytic sites (both endo- and exo-glucanases) and multi-functional catalytic sites, acting on both xylose and cellulose (Brunecky *et al.,* 2013). These proteins, produced by a thermophilic bacterium, exemplify a hybrid between the multi-functionality of a cellulosome and the classic 'free' extracellular cellulase.

Hydrolysis may be the most common mechanism for the depolymerization of polysaccharides in nature and enzymes of this class, i.e. glycosyl hydrolases, comprise most of the best researched cellulolytic enzymes. Yet, there exists a growing number of non-hydrolytic enzyme classes which decompose polysaccharides or act synergistically to enhance breakdown. The discovery of lytic polysaccharide monooxygenases (LPMOs) represented a significant paradigm shift in understanding the decomposition of recalcitrant polymers like chitin and cellulose (Harris *et al.,* 2010; Vaaje-Kolstad *et al.,* 2010). LPMOs are believed to act synergistically to accelerate cellulose depolymerization, cleaving crystalline cellulose and providing hydrolytic enzymes access the substrate. LPMO redox reactions have been coupled to chlorophyll, yielding substantially elevated cellulose hydrolysis in an artificial thykaloid membrane containing LPMOs when exposed to light (Cannella *et al.,* 2016). There are other non-hydrolytic cellulase-enhancing factors, such as expansins, which can disrupt or loosen crystalline

cellulose and enhance substrate availability (Arantes and Saddler, 2010). Polysaccharide lyases are another class of non-hydrolytic enzymes, however they do not act on cellulose (Linhardt *et al.*, 1986). Carbohydrate esterases also do not cleave cellulose, but perform reactions that accelerate decomposition, such feruloyl esterases which deacetylating cellulose (Biely, 2012; Kroon *et al.*, 2000) and free carbohydrates bound to lignin (Blum *et al.*, 2000; Benoit *et al.*, 2006).

The diversity of cellulolytic machinery results, in part, from the structural heterogeneity of plant polymers which requires highly adapted enzyme-substrate adsorption chemistry and diverse catalytic activity. To aid in unraveling this complexity, polysaccharide degrading enzymes are being organized into families by their primary sequences and folding topologies (Henrissat, 1991) and, subsequently, categorized and annotated in the publicly available 'Carbohydrate-active Enzyme Database' (CAZy db; Cantarel *et al.*, 2009). This database is accompanied by 'CAZypedia,' which provides more detailed knowledge on the enzyme classes and families therein (http://www.cazypedia.org/). Most recently, the CAZy database released a tool for predicting PULs based on sequences from *Bacteroidetes*, which may prove useful in identifying PULs in the genomes of other species (http://www.cazy.org/PULDB/; Terrapon *et al.*, 2015). The 'dbCAN' offers a complementary service for annotating CAZymes using hidden Markov models (www.csbl.bmb.uga.edu/dbCAN/; Yin *et al.*, 2012). Despite spanning a full century of research, our understanding of the diversity of activity of cellulolytic enzymes continues steady growth.

1.2.12 Lignin-modifying and 'Auxiliary Activity' Enzymes

The discovery that lignin decomposition is a fundamentally oxidative process is attributed to Bavendamm, who, in 1927, differentiated between white and brown-rot fungi based on colorimetric changes from the oxidation of phenolic compounds. Bose and Sarkar were the first to characterize the oxidative enzyme as a 'laccase' from white-rot fungi in 1937. Laccases were one of the earliest enzymes to ever be characterized (Bertrand, 1894), while peroxidases were

described later (Reed, 1916). The work of Manskaya was the first to implicate free radical chemistry in lignin biosynthesis, by demonstrating the partial polymerization of coniferyl alcohol using a peroxidase (Manskaya, 1948).

The evolution of lignolytic peroxidases was driven by the heterogeneous, hydrophobic and electrochemically stable structure of lignin, which resists direct enzymatic attack (Figure 1.4). To overcome steric resistance, class II catalase-peroxidases evolved surface exposed catalytic residues exemplified by lignin peroxidases (LiPs; Ruiz-Dueñas *et al.,* 2009) or oxidized mediator compounds, like Mn^{2+} or veratryl alcohol, which could access and oxidize deeper structures within the lignin, exemplified by manganese peroxidases (MnP). Versatile peroxidases, as the name implies, are able to make use of both mechanisms (Camarero *et al.,* 1999). Collectively, the class II catalase-peroxidases possess some of the strongest oxidation potentials of any characterized enzymes, which is not surprising given the need to oxidize lignin's highly stable non-phenolic bonds. Both LiP (with a redox potential of +1500 mV) and horseradish peroxidase (+950 mV) can create phenoxy radicals, while laccases are reported to oxidize only phenolic bonds (< +800 mV) (Hayashi and Yamazaki 1979; Kersten *et al.,* 1990; Li *et al.,* 1999). Peroxidases and other lignin-modifying enzymes are categorized as 'Auxiliary Activity' enzymes in the CAZy database (Levasseur *et al.,* 2013). However, 'PeroxiBase' offers the capability to more accurately compare and delineate catalase-peroxidase families (Fawal *et al.,* 2013).

A second class of peroxidases, known as 'Dye-decolourizing peroxidases' (DyP), show lignin-modifying activity, but belong to the peroxidase–chlorite dismutase superfamily (Zámocký *et al.,* 2015). DyPs were first differentiated as novel peroxidases due to their ability to oxidize a range compounds which were poorly oxidized by other classes of peroxidase (Kim *et al.,* 1995). As of January 2014, the DyP superfamily was comprised of mainly bacterial enzymes (~97%), with the remainder found in Eukaryotes and a handful present in Archaea (Colpa *et al.,* 2014).

DyP-like enzymes have been isolated and characterized in lignin-degrading bacteria (Ahmad *et al.*, 2011; Brown *et al.,* 2012; Davis *et al.,* 2013). The best-characterized DyP enzymes, DypB and Dyp2, oxidize and decompose lignin and exhibit MnP-like activity by way of Mn^{2+} oxidation (Ahmad *et al.,* 2010; Brown *et al.,* 2012). The characterizations of DyP enzymes is relatively new and they have not been assigned an AA family in CAZy. However, a comprehensive phylogenetic analysis of DyPs was recently completed (Singh and Eltis, 2015), leading the way to better functional characterization and homology searches.

Laccases exhibit the capacity to oxidize a remarkably diverse set of phenolic substrates. Their lignolytic activity, in the absence of peroxidases, has been demonstrated in both fungal (Ander and Eriksson, 1976) and bacterial systems (Majumdar *et al.,* 2014). Laccases differ from peroxidases in their use of molecular oxygen to oxidize polyphenols (ex. tannins), but lack the oxidative power to readily cleave the predominantly non-phenolic bonds within lignin. Efficacious degradation of lignin has been demonstrated by coupling laccases with redox mediator compounds, similar to the activity of peroxidases (Munk *et al.,* 2015). Lignin-degrading laccases are also challenging targets for bioinformatics analyses because their diverse functional roles in cell chemistry. Laccases are multicopper oxidases, which can be involved in intra- and extracellular developmental processes, morphogenesis, pigmentation and pathogenesis (Ausec *et al.,* 2012). Their role is further complicated by their ability to act on a wide range of substrates and ambiguous coupling with redox mediators (Reiss *et al.,* 2013). Identifying secreted laccases also does little to distinguish putative lignin-degrading activity, given that over 70% of all bacterial laccases contain signal peptides (Ausec *et al.,* 2012). Laccases are deposited in the CAZy database under the AA family I, while HMMs for two- and three-domain laccases have been published (Ausec *et al.,* 2012).

The selection of bioinformatic approaches used to study ligninases and cellulases will differ according to what features are distinguishable at the sequence level. To date, lignin-degrading genes have not been found associated with other features which can, by proxy, suggest a functional role, unlike cellulases which can be identified by their association with PULs, cellulosomes, CBMs and in gene clusters (Bélaich *et al.*, 1997; Morita *et al.*, 2009; Dai *et al.*, 2012). Identifying lignin-degrading genes is further complicated by the fact related genes are involved in a variety of core functions such as oxidative stress, detoxification and biosynthesis and, thus, conserved features of previously characterized lignolytic enzymes must be carefully delineated or enzyme activity validated. As a result, bioinformatics predictions of cellulolytic activity have shown promise (Hess *et al.*, 2011), while most lignin-degrading enzymes have been discovered by functional assays. Bioinformatic approaches for identifying lignin-degrading genes have therefore gravitated to other 'guilt-by-association' methods, relying on organism-level traits which suggest adaptations to a lignin-degrading niche (Brown *et al.*, 2012; Eastwood *et al.*, 2011). One motivation for this thesis was to combine stable isotope probing with metagenomics provide an underlying functional basis for the bioinformatic identification of putative lignolytic enzymes.

1.2.13 Research Objectives in the Survey of Cellulolytic and Lignolytic Taxa from Forest Soils Across North America (Chapter 4)

The major goal of research described in Chapter 4 was to survey the biodiversity of lignocellulolytic populations in forest soils from across North America. Decomposers of all three major components of lignocellulose, namely hemicellulose, cellulose and lignin, were surveyed, pioneering the use of SIP-lignin. Shotgun metagenomics enabled the recovery of draft genomes from lignin- and cellulose-degrading taxa, permitting the identification of catabolic genes and aromatic degradation pathways to validate putative function. Studies have already used SIP to survey cellulolytic communities, but have focused on a relatively small number of samples from a

narrow range of sources given the cost and labour required for SIP experiments. Furthermore, a number of recent SIP-cellulose were compromised by the use of impure ^{13}C-labeled maize cellulose (Bastias *et al.* 2009; Schellenberger *et al.* 2010; Štursová *et al.* 2012, Koranda *et al.* 2014, Torres *et al.*, 2014). This research endeavoured to process a large number of samples in order to make broad, replicated comparisons among lignocellulolytic communities from a wide range of forest soils.

The extent to which lignocellulolytic organisms have evolved the capacity to decompose all three major components of lignocellulose was assessed in Chapter 4. The presumption was that these traits would co-occur, given the example of certain wood-degrading fungi that can degrade all three substrates (Eastwood *et al.*, 2011; Floudas *et al.*, 2015). Thus, the identification of species (OTUs) which exhibit enrichment in ^{13}C-libraries from all three substrates was attempted. SIP-hemicellulose data was included, but, as the data was not collected for this thesis and has been previously published (Leung *et al.*, 2016), it was not the focus of Chapter 4. The characterization of active, *in situ* lignin-degrading bacteria would be a significantly novel contribution to our understanding of the process of decomposition. Comparisons of the activity of bacterial and fungal lignin-degraders was made. A SIP-lignin experiment with the addition of fungicide was used to confirm the degree of bacterial assimilation of carbon from lignin. The expectation is that fungi will predominate in the degradation of both lignin and cellulose, at least in organic layer soils.

In addition to comparisons of taxa that degrade individual lignocellulosic polymers, the presented in this Chapter comprises a diversity of sample types from forests across North America and from upper organic and deeper mineral soil layers. The distribution and soil layer preference of lignocellulolytic taxa can inform our understanding of how differences in climate, soil-type and forest-type select for certain physiological traits. The relative abundances of putatively lignocellulolytic taxa in field samples (i.e. not microcosm based) was used as further evidence to

support biogeographical trends and to relative abundance of lignocellulolytic populations relative to the community at large. Lignocellulolytic populations were expected to be a minority (Allison *et al.*, 2012; Goldfarb *et al.*, 2011), though recent research has suggested that one of the most abundant bacterial taxa in forest soils could potentially degrade lignin by-products (VanInsberghe *et al.*, 2015). An assessment of whether putatively lignocellulolytic taxa were impacted by timber harvesting was made, given the overlap of samples used in Chapter 3, with the assumption that lignocellulolytic populations may be most abundant in OM1 where woody biomass was retained.

Finally, a link between community composition and rate of decomposition was explored. In SIP-data, community or functional gene composition can be used to explain variation in the quantitative estimates of ^{13}C-enrichment of DNA or PLFA. Chapter 4 investigated possible associations between the presence or absence of certain taxa and/or functional genes and higher or lower rates of lignolytic or cellulolytic activity. While informative, SIP microcosm- based estimations of activity may not reflect *in situ* rates, nor are culture-independent designation of lignocellulolytic activity without error. Thus, the strength of SIP evidence was weighed and caveats for the SIP method discussed.

Chapter 2: Materials and Methods

2.1 Sample Collection from Long-term Soil Productivity Study Sites

2.1.1 Site Descriptions

Soil samples were collected from reforested experimental plots within the Long-Term Soil Productivity (LTSP) Study from eighteen different sites spread across six prominent conifer-dominated North American 'ecozones' (Figure 1.1). The term 'ecozone' is a general descriptor that delineates differences in local assemblages of organisms and climatic factors. In this thesis, it is not used in accordance with the classification system provided by Environment Canada (Ecological Stratification Working Group, 1996). Ecozones were chosen to exemplify a broad range of climates and regions of North America where forestry is a major industry. Ecozones differed by a number of factors, including soil type, mean annual temperature and precipitation as well as predominant tree species reported in Table E.3. Ecozones also differed based on bulk soil chemistry, such as carbon and nitrogen content and pH (Figure 2.1). Each of the three sites within an ecozone contained triplicate plots for each of the four harvesting treatments: an unharvested reference plot, 'REF,' and three harvesting intensities resulting in varying degrees of organic matter removal: 'OM1,' where tree boles (stems) were debranched and woody debris left *in situ*; 'OM2,' where whole trees and branches were removed and, 'OM3,' where whole trees were removed and the upper organic layer of forest floor scraped away (Figure 2.2). Compaction was controlled at these sites and plots with minimum compaction were sampled. The age of forest varied from 11 to 17 years, with a majority over 15 years post-harvest.

2.1.2 Sample Collection

Samples from all three sites within an ecozone were collected within, at most, three months of each other between March and September. Sampling occurred between 2008 and 2012, depending on the ecozone (Table E3). Each site in BS_{ON} and JP_{ON} had triplicate plots from

which replicate samples were collected, while in the other four ecozones triplicate samples were collected from three separate transects in a single plot. In sampling, the litter layer was first removed and organic layer samples (~ 5 cm of the O-horizon) were collected with a trowel. Then, the top 20 cm of mineral soil (including the A and occasionally upper B-horizon) was collected using a Stoney auger (5 cm diameter). Sampling was performed to reflect consistent soil characteristics among treatments and sites. To account for heterogeneity at the plot level and ensure sufficient soil material, a single soil sample from a plot or transect was composited from between three to five sampling points. Samples were stored at 4°C during transport, sieved through 2-mm mesh and stored at -80°C until DNA was extracted within three months of sampling date.

2.2 Molecular Methods

2.2.1 Preparation of 16S rRNA gene and ITS region Pyrotag Libraries

DNA was extracted from 0.5 g of soil using the FastDNA™ Spin Kit for Soil (MPBio, Santa Ana, CA) according to the manufacturer's protocol. PCR amplification was performed on bacterial 16S rRNA gene (V1–V3) and fungal internal transcribed spacer region (ITS2) using barcoded primers according to methods described in Hartmann *et al.,* (2012). PCR amplifications were performed in triplicate and pooled prior to purification and quantification. All DNA was quantified using Pico-Green fluorescent dye (ThermoFisher, MA, USA). Samples were sequenced using the Roche 454 Titanium platform (GS FLX+) at the McGill University and Genome Québec Innovation Centre, yielding on an average > 5,000 bacterial and fungal (200 bp) reads per sample. Read libraries were quality filtered and processed according to the Schloss "454 SOP" (accessed October 2013; Schloss *et al.,* 2009). 16S rRNA gene libraries were clustered into operational taxonomic units (OTUs) at 1% dissimilarity to produce abundance

matrices. Fungal sequences were clustered at 5.5% dissimilarity using CrunchClust according to Hartmann *et al.,* (2012) due to the hypervariability of the ITS region. Taxonomic classification was performed using the RDP Classifier (Wang *et al.,* 2007) with the Greengenes database for 16S rRNA genes (database gg_13_8_99; August 2013) and the Mothur-formatted release of UNITE for ITS (sh_mothur_release_08.12.2013; August 2013). All OTU counts were normalized to total counts per thousand reads.

Figure 2.1. Bar plot of differences in total carbon, total nitrogen, soil moisture and pH among ecozones.

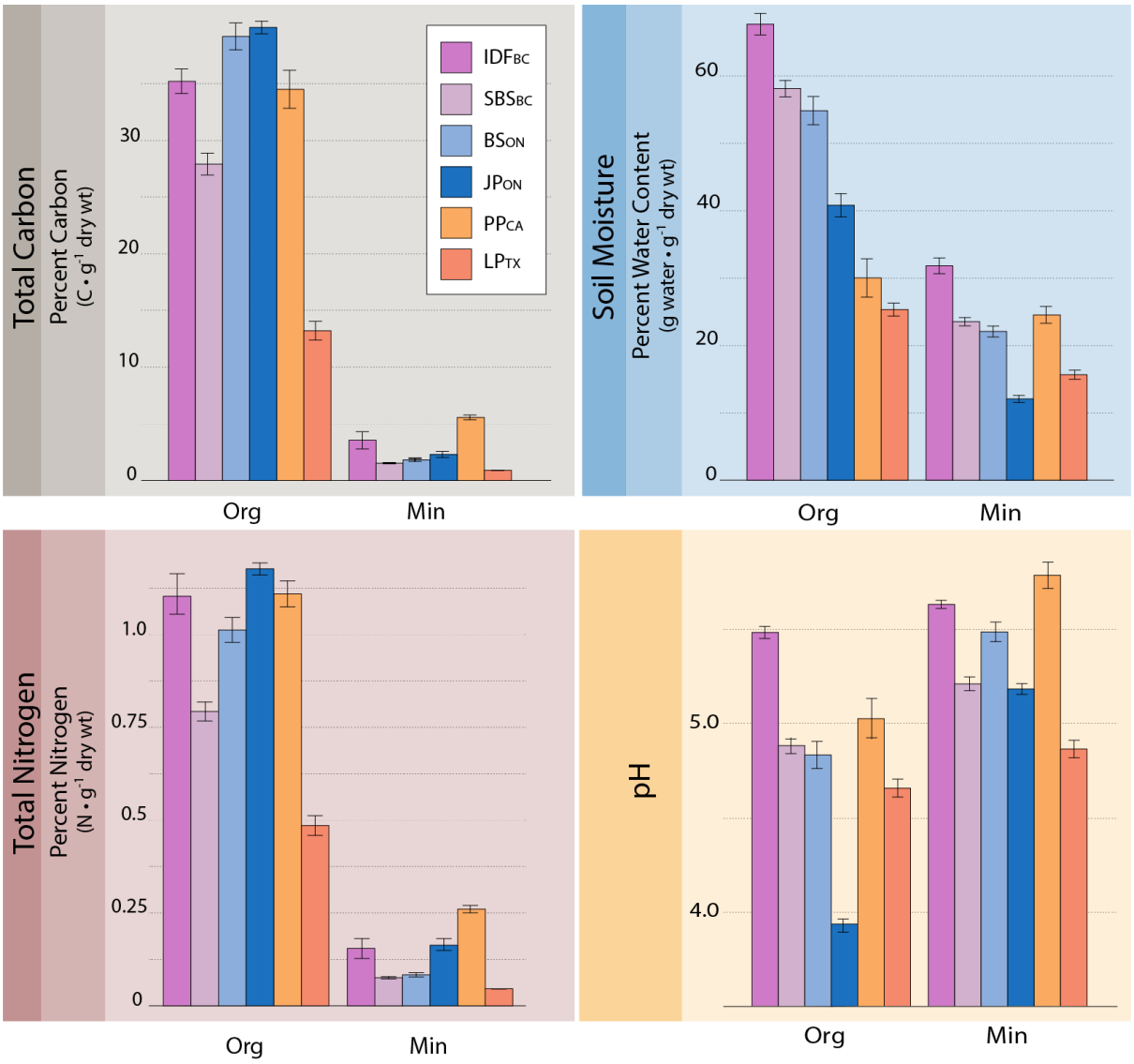


Figure 2.2. Photographs capturing the initial set-up of the harvested Californian LTSP sites (A-C) and the appearance approximately 15 years after reforestation when sampling was conducted (D). Image A illustrates harvesting treatment 'OM1' as well as the efforts to minimize soil compaction. Image B and C illustrate harvesting treatment 'OM2' and 'OM3', respectively. [Photo credit: Matt Busse; mbusse@fs.fed.us]

2.2.2 Preparation of and Sequencing of Shotgun Metagenome Libraries

All SIP-Cellulose metagenomic libraries were prepared from 40-50 ng of ^{13}C-enriched DNA using the Nextera DNA Sample Preparation Kit (Illumina Inc., CA, USA), while all SIP-Lignin metagenomic libraries were prepared from 1 ng of ^{13}C-enriched DNA using the Nextera XT DNA Sample Preparation Kit. The discrepancy was due to the difference in amount of ^{13}C-enriched DNA recovered from each incubation. Preliminary tests comparing different library preparation methods from identical samples concluded that the 1 ng (Nextera XT) approach comparable to the 50ng (Nextera), if not better due to the ability to pool fewer fractions to achieve the library prep kit requirements (see Section 4.2.1). Four sample libraries were multiplexed per lane for all sequencing libraries and sequencing was performed on an Illumina HiSeq 2500 (2 x 100-bp). Samples were combined for multiplexing based on average fragment size not sample origin or soil type.

Metagenomic data was collected in four major batches. The first batch of libraries were prepared for the SIP-cellulose investigation into treatment effects (Section 3.3.4) that included four libraries from ^{13}C-DNA recovered from PP_{CA} mineral soils corresponding to REF, OM1, OM3 and ^{12}C-DNA from REF plots. Sufficient ^{13}C-DNA was achieved by pooling all Californian sites, which was necessary due to the depletion of DNA stock from the creation of 16S rRNA gene and ITS pyrotag libraries. The second installment of libraries were prepared for the SIP biodiversity survey of cellulolytic taxa (Section 4.2) and included thirty-eight libraries from both organic and mineral soil for every REF plot for every site in all five ecozones: IDF_{BC}, BS_{ON}, JP_{ON}, PP_{CA} and LP_{TX}, including one ^{12}C-DNA control composited from comparable heavy factions of DNA from three sites from within each ecozone and for each layer (note: pyrotag libraries have fully paired controls, while metagenomic libraries have only one control per sample type). The third batch was for the SIP biodiversity survey of lignolytic taxa (Section 4.2)

and included forty-six libraries from organic and mineral soil (composites OM1 and OM2 due to shortage of soil) for each site in Ontario (BS_{ON} & JP_{ON}), PP_{CA} and LP_{TX}. Fewer ecozones were covered due to limited availability of substrate and the intention of ensuring sufficient replication. Due to poor recovery of DNA and soil inhibitors, numerous additional library preparations failed and therefore all forty-six libraries represent unbalanced pairing of ^{13}C- and ^{12}C-samples (out of a total of 72 samples prepared). Each sample type is represented by at least one ^{12}C-sample (for example: PP_{CA}-organic layer or LP_{TX}-mineral layer), with commonly more than one. The final batch of metagenomes were sequenced for the SIP-lignin antibiotics experiment (Section 2.2.5.1), where microcosms were incubated with or without fungi-specific antibiotics (Section 4.2.4), yielding a total of eight metagenomes corresponding to paired incubations of mineral soil (^{13}C-lignin + antibiotic versus ^{13}C-lignin w/o) for two sites in BS_{ON} (A7 & A8) and one each in PP_{CA} (BR) and LP_{TX} (TXA).

2.2.3 Stable Isotope Probing

2.2.3.1 Preparation and Properties of ^{13}C-labeled Cellulose

Bacterial cellulose was chosen as the substrate to target cellulolytic activity via SIP due to irremediable impurities in commercially available plant-derived ^{13}C-cellulose from IsoLife (58% glucose + 4.4% lignin + unknown percentage of sugars from hemicellulose; Appendix D) which was initially tested and ultimately abandoned. Bacterial cellulose is more comparable to plant-derived cellulose than other preparations, such as Avicell, Sigmacell or filter paper, which are typically low molecular weight and less crystalline and, most importantly, are also not free of oligosaccharide contaminants.

Plant cellulose is produced in the form of microfibrils which are fine, crystalline structures derived from the aggregation of 18 or 36 (still disputed) or more chains of β-(1,4)-D-glucose (Newman *et al.,* 2013). The crystalline nature of cellulose is a key determinant of the

rate of mineralization (amorphous regions decompose more rapidly) and type of enzymes required for hydrolysis, where specially evolved carbohydrate binding modules are necessary for effective hydrolysis of crystalline cellulose (Wilson, 2011). Bacterial cellulose is produced by homologous cellulose synthase proteins, but they are arranged in a linear array of single or multiple rows in contrast to plants which possess hexagonal conformations. As a result, bacterial cellulose microfibrils, termed "nanocellulose," are around 100 times thinner than the average plant microfibril (~30 nm). Despite these clear differences, bacterial cellulose fares well in a comparison of traits correlated to enzymatic degradation: it has comparable polymer length (3000 – 9000 units) and crystallinity (80-90% crystalline) and demonstrates similar mechanical properties to plant cell walls (Chanliaud *et al.,* 2002).

Bacterial cellulose was produced by growing *Gluconacetobacter xylinus* str. KCCM 10100 with ^{13}C-labeled glucose (99 atom % 13C, Cambridge Isotope Laboratories, MA, USA) in Yamanaka media under conditions outlined in Ruka *et al.,* (2012). To ensure media was highly oxygenated, a requirement for the production of cellulose, cultures were grown in a 200 mL volume in a much larger 2 L Erlenmeyer flask with sponge tops tightly covered with aluminum foil to retard evapouration. Cellulose was purified according to Dunford (2011) but with longer boiling times (4 hrs) and three repetitions of boiling in 1% sodium hydroxide. Following purification and soaking the cellulose in a mildly acidic solution, contamination from DNA and protein was not detectable by spectrophotometry (A_{260} and A_{280}). The carbohydrate composition of bacterial cellulose was assayed by HPLC after dissolving in strong sulfuring acid. The custom made bacterial cellulose was 100% glucose and nearly pure ^{13}C% (only impurities would be from protein added to culture broth).

2.2.3.2 Preparation and Properties of ^{13}C-labeled Lignin

Plant lignin is synthesized by the coupling of free-radical phenylpropanoids after activation by plant peroxidases. Synthetic dehydrogentively polymerized (DHP) lignin emulates the plant mechanism using horseradish peroxidase to generate radicals of *p*-hydroxyphenyl, guaicyl and syringyl lignins. The DHP lignin which most resembles extracted "milled wood" lignin is prepared from coniferyl alcohol via the "zutropfen" method (Saake *et al.,* 1996). DHP lignins typically have a lower molecular weight than plant lignin (~1600 – 9000 g · mol^{-1}, or 8 - 50 units) and possess different frequencies of inter-linkages. DHP contains more β-5, β-β bonds and fewer β-O-4 bonds (Figure 1.4), which predominate in softwood lignin (~80%) (Brunow and Lundquist, 1980). DHP lignin is a reasonable surrogate for softwood lignin given the recent report demonstrating that native softwood lignin is oligomeric and minimally branched contrary to prevailing convention (Crestini *et al.,* 2011). DHP lignin structurally resembles softwood lignin and has a long history of use as a model substrate for lignin-degrading activity.

^{13}C-labeled DHP lignin was synthesized from ring-labeled coniferyl alcohol using horseradish peroxidase as described Kirk and Brunow (1988). Ring-labeled coniferyl alcohol was synthesized from ^{13}C-ring labelled vanillin (Sigma Aldrich, CA) according to the procedure described in the Appendix. The resulting DHP lignin had a weight average molecular weight (M_w) of 2,624 g mol^{-1} which equates to polymer lengths of ~ 14 units. DHP lignin was washed once in distilled water to remove residual unpolymerized coniferyl alcohol and its purity verified by HPLC. The resulting labeled DHP lignin was 75% atom ^{13}C.

2.2.3.3 Microcosm Preparation

Microcosms were prepared by adding between 1-2 g dry wt organic or mineral soil to 30-mL serum vials, adjusting moisture content to 60% (mineral) and 125% (organic) (w/v).

Moisture content was selected based on preliminary CO_2 assays which identified maximal respiration rates for soil type (data not shown). Microcosms were pre-incubated for one week prior to the addition of substrate. Microcosms were amended with either 10% w/w of ^{13}C-cellulose or ^{13}C-lignin and paired with microcosms amended with identically prepared unlabeled, substrates (~1.1% atom ^{13}C). These ^{12}C-control microcosms were used to correct for natural, background ^{13}C-carbon content in SIP-phospholipid fatty acid (PLFA) work and to control for background populations of organisms with naturally higher genomic GC content (i.e. heavier DNA) in SIP-DNA work. Microcosms were then incubated at 20° C for either 9 days (organic soils + cellulose), 14 days (mineral soils + cellulose) or 60 days (both soil layers + lignin). The incubation length was optimized by preliminary time-course experiments described in results Section 3.3.2. Due to poor enrichment of microbial biomass in organic soils from DHP-lignin, organic soils were mixed 1:3 with double-autoclaved mineral soil from corresponding samples in order to dilute preexisting organic matter. Following incubation, soil samples were lyophilized and stored at -80° C until processing. All SIP-PLFA, SIP-pyrotag and SIP-metagenomic data for a given sample and substrate were derived from the same set of microcosms. In contrast, '*in situ*' sequencing data was derived from corresponding field samples that were not incubated. SIP-pyrotag libraries were taken from *Leung et al.,* (2016) who incubated with ^{13}C-hemicellulose in an identical fashion, except for a period of 48 hrs.

2.2.3.4 Analysis of SIP-Phospholipid Fatty Acids (PLFA)

PLFAs were extracted from 0.75 g (organic) or 1.0 g (mineral) dry wt soil according to Bligh and Dyer (1959) and ^{13}C-content was analyzed using ion ratio mass spectrometry (UBC Stable Isotope Facility) ported with gas chromatography as detailed in Churchland *et al.,* (2013) with the following exceptions: (i) methyl undecanoate (c11:0) was used for the internal standard,

and (ii) quantitation was based on an average of three serial dilutions of undecanoate, nonadecanoate (c19:0), and methyl cis-13-docosenoate (c22:1ω9). Peak identification was based on retention time compared against two reference standards: bacterial acid methyl-ester standard (47080-0; Sigma–Aldrich, St. Louis) and a 37-Component fatty acid methyl-ester mix (47885-U; Sigma–Aldrich, St. Louis). Unidentifiable enriched peaks, termed "unidentified fatty acids" (UFA), were included in analysis if they met the following conditions: i) detection in > 3 samples, ii) average $\delta\ ^{13}C > +50$ ‰ and iii) confirmed as long-chain alkane methyl esters by GC-MS using the identical instrumentation and method as in Leckie *et al.,* (2004). Taxonomic affiliations of specific PLFAs were assigned according to Högberg *et al.,* (2013), with c18:1ω9 and c18:3ω6 added as additional fungal PLFAs (Ruess and Chamberlain, 2010). All comparisons between taxonomic groups were performed using values normalized to total PLFA content. All SIP-PLFA raw data was processed identically in R using scripts that can be found at: https://github.com/roli-wilhelm.

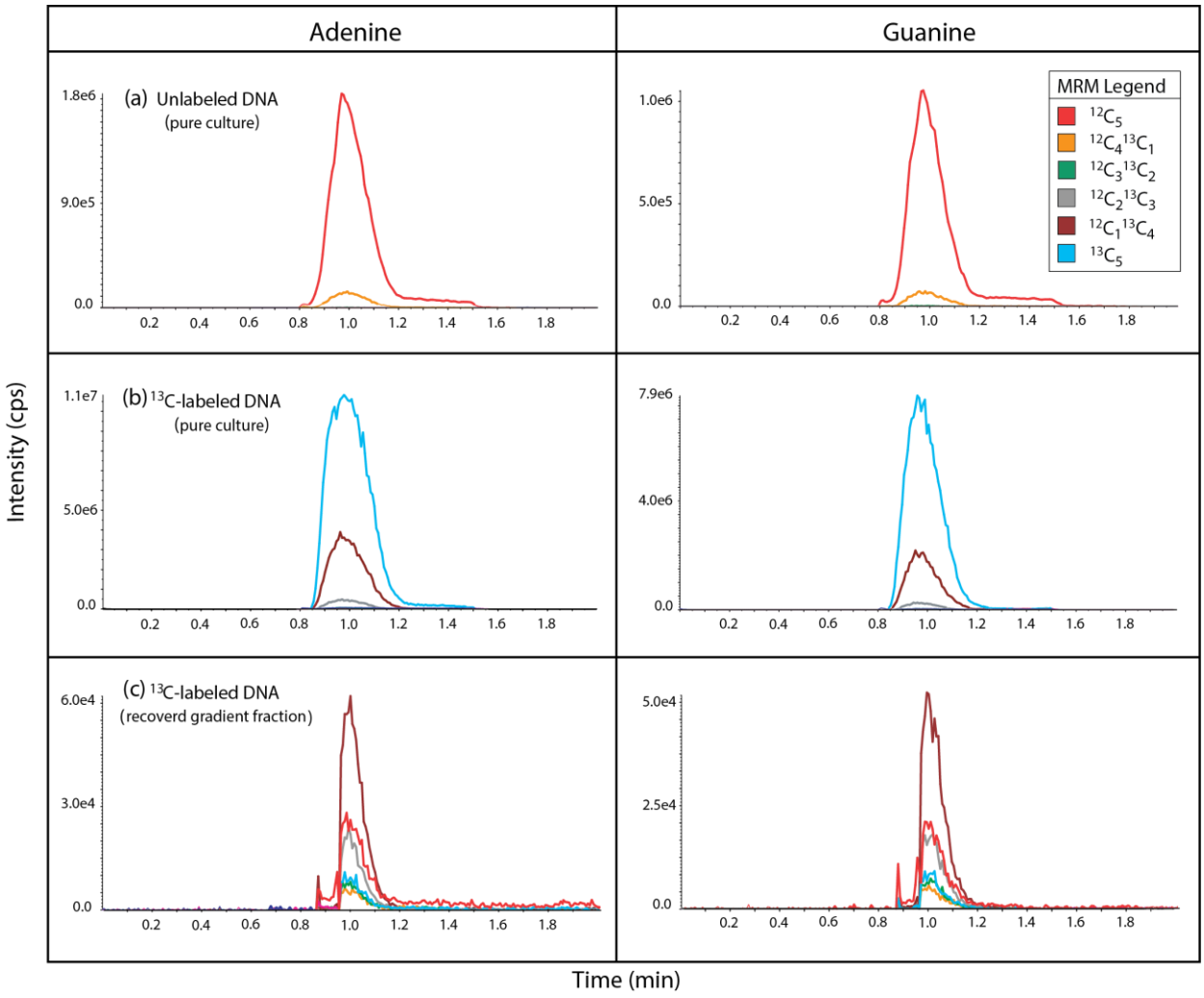
2.2.3.5 Quantitation of ^{13}C-enriched DNA

Quantitation of the total mass and atom % ^{13}C of DNA was measured with UHPLC-MS/MS. One nanogram of DNA was sufficient for quantitation, though typically 5 ng was used where possible to ensure a strong signal. DNA was diluted or concentrated to 5 µL and mixed with 95 µL of 88% formic acid in 250 µL PCR tubes and incubated at 70°C for 2 hrs to hydrolyze the DNA polymer. The acid solvent was completely evapourated using a Savant SpeedVac SC110A (Thermoscientific, U.S.) with a custom-made acid trap (a 500-mL air-tight container packed with soda lime) to prevent damage to equipment. The sample was suspended in 40 µL of UHPLC mobile phase (0.22 micron-filtered solution consisting of 98% solvent A,

water with 0.1% v/v formic acid, plus 2% solvent B, methanol with 0.1% v/v formic acid) and run according to the details described in Wilhelm *et al.,* (2014).

Atom % ^{13}C-carbon of DNA was calculated by summing the peak area for the various isotopic species of guanine and adenine. Both compounds contain five carbons, thus the incorporation of ^{13}C produced incrementally heavier species, for adenine *m/z* 136.0/119.0 to *m/z* 141.0/124.0, and for guanine *m/z* 152.1/135.1 to *m/z* 157.1/140.1 (Figure 2.3). The mass of unlabeled and labeled DNA was proportioned according to the following summations of peak area: i) All_{C12} + $5_{C12}1_{C13}$*0.077+ $3_{C12}2_{C13}$*0.0048; and ii) All_{C13} + $1_{C12}5_{C13}$ + $2_{C12}4_{C13}$ + $3_{C12}3_{C13}$*0.9952 + $4_{C12}2_{C13}$*0.923. According to these formula, we observed average natural abundances of 5.9% (+/- 0.2% SE) from lambda DNA and 7.7% (+/- 0.2% SE) from DNA extracted from unlabeled soil samples. Given the natural abundance of ^{13}C (1.11%), the theoretical amount of a six carbon compounds containing a single ^{13}C isotope in a mixture is 5.9%.

Figure 2.3. Examples of mass spectra for adenine and guanine for three samples: (a) unenriched DNA from a *Gluconacetobacter sp.* culture, (b) ^{13}C-enriched DNA from *Gluconacetobacter sp.* grown on pure ^{13}C-glucose, and (c) ^{13}C-enriched DNA recovered from a high density fraction following the CsCl gradient ultracentrifugation of DNA extracted from soil fed ^{13}C-cellulose.



2.2.3.6 Recovery of ^{13}C-enriched Nucleic Acids

^{13}C-enriched DNA was separated using cesium chloride density centrifugation according to the method outlined by Neufeld *et al.,* (2007) with the following modifications: i) the amount of DNA applied to the column was standardized according to the degree of ^{13}C-enrichment as determined by UHPLC, resulting in between 6 – 10 µg of DNA used per sample and ii) DNA was recovered from the cesium chloride solution (i.e. desalting) using Amicon Ultra-0.5 mL filters (EMD Millipore, MA, USA). Heavy fractions (1.727-1.735 g· mL^{-1}; typically F_1-F_7) were pooled and concentrated in the 500 µL filter columns by centrifugation for 3 min at 14,000 rcf. Samples were then washed three times with 450 µL of PCR-grade water. The modification in DNA recovery (shortened processing time and reduced labour) was necessary for higher sample throughput and also resulted in 2-fold higher yields. During fractionation, a constant flow rate was maintained by pumping coloured water into the top of centrifuge tubes with a syringe pump (Model R-E; Razel Scientific, U.S.) and fractions were collected every 30 seconds, resulting in 20 fractions of approximately 250 µL. Density was measured using a refractometer and converted to g CsCl · mL^{-1} using the following formula: density = (refractive index)*10.927 - 13.593. DNA was suspended in 30 µL of pure water and quantified using Pico-Green fluorescent dye or UHPLC. The ^{12}C-control DNA were treated identically, however typically insufficient DNA for PCR was recovered from heavy fractions, resulting in the need to pool additional fractions (~F_1-F_9). The successful recover of highly ^{13}C-enriched DNA was verified by the previously described method for quantitation (Section 2.2.3.5).

2.2.3.7 Estimating Effects of GC Content on DNA Recovery from Heavy Fractions

The efficacy of CsCl density gradient centrifugation at separating enriched DNA from high-GC bacteria was tested by artificially spiking in unlabeled genomic DNA from *Nocardioides sp.* (70% GC; IMG Taxon ID: 2519899648) in ^{13}C-enriched soil DNA extract. Genomic DNA was spiked at 5, 10, 15 and 20% (v/v) of total DNA applied to the CsCl gradient. After purification, PCR amplification and sequencing of 16S rRNA libraries, the heavy fractions of DNA yielded fewer than 3% reads corresponding to the *Nocardioides sp.*, though the number of reads that did match were roughly proportional to the amount of spiked DNA, 1.0%, 1.4%, 1.5% and 2.9%, respectively. Based on this data, we concluded that GC content would have marginal impact on recovering unlabeled DNA in heavier fractions.

2.2.4 Methodology Specific to Study of Treatment Effects

2.2.4.1 Soil Respiration Microcosms

Organic and mineral layer soil samples from Californian LTSP treatments REF, OM1 and OM3 were incubated with or without one of three milled lignocellulosic substrates derived from Douglas-fir: (i) "lignocellulose," from debarked, untreated Douglas-fir woodchips, (ii) "lignin + cellulose," from steam treated woodchips, where hemicellulose was solubilized and removed and (iii) "cellulose," from steam treated woodchips which were subsequently delignified (Kumar *et al.*, 2012). Microcosms were prepared by adding 4.5 g dry wt soil to 30-mL serum vials, adjusting moisture content to 60% (mineral) and 125% (organic) (w/v) and pre-incubated for one week. Substrate was then added (0.45 g) along with CO_2 traps, consisting of sterile glass vials containing 2 mL NaOH. Microcosms were incubated at 20 °C for an additional 14 days. Net respiration was determined by titration of the NaOH traps according to methods outlined by Haney *et al.* (2008).

2.2.5 Methodology Specific to Survey of Cellulolytic and Lignolytic Organisms

The biodiversity survey described in Chapter 4 was completed subsequent to the research on the effects of timber harvesting. Having founds ITS (fungal) pyrotag libraries to be relatively uninformative in identifying differentially abundant taxa in ^{13}C- vs. ^{12}C-libraries (see Section 3.3.4), only 16S rRNA libraries were use in subsequent analyses. This decision was made to conserve ^{13}C-enriched DNA, in particular for SIP-lignin experiments. Shotgun metagenomic data contained a much stronger signal for fungi and, therefore, was sufficient for the purposes of research. One caveat is that higher replication was achieved with pyrotag libraries and, while fungal reads were easily differentiated from bacterial reads by lowest common ancestor (LCA) analyses, the taxonomic resolution afforded by LCA was poor.

SIP-lignin was performed on a subset of ecozones given the desire to conclude with significant replication any observation and the scarcity of ^{13}C-enriched material. The synthesis of DHP-lignin was costly (~ $10,000 + labour) and yielded only ~ 800 mg of substrate. As a result, only LP_{TX}, BS_{ON} and PP_{CA} were selected for broadest coverage.

2.2.5.1 Fungicide Amended Microcosm Experiment

To demonstrate the degree of bacterial involvement in the degradation of DHP-lignin, a select number of soils were incubated with anti-fungal compounds. Cycloheximide and fungizone (amphotericin B) were selected based on reports on the efficacy of anti-fungal soil amendments by Ingham *et al.*, (1984). Mineral soil samples from two sites in BS_{ON} (A7 and A8) and one from both PP_{CA} and LP_{TX} were chosen based on their high levels of enrichment in prior SIP-lignin incubations. Soil microcosms were prepared as previously stated. Two parallel sets of microcosm were amended with ^{13}C-DHP lignin with one set dosed with antibiotic cocktail on a weekly basis at 1 mg g^{-1} soil (cylcoheximide) and 1.2 mg $\cdot$ g^{-1} soil (fungizone). A 10 mg $\cdot$ mL^{-1}

cylcoheximide solution was prepared in distilled, autoclaved water at pH 5.0 and a 30mg · mL^{-1} fungizone solution was prepared in DMSO. Both were stored at 4 °C and protected from light. Soil microcosms were dried out for 2 hrs in a flow-hood prior to the addition of antibiotics to prevent soils from becoming water-logged by the regular addition of moisture (140 µL per week). Samples were lyophilized, stored at -80 °C and analyzed for PLFA content and by shotgun metagenomic sequencing of ^{13}C-enriched DNA.

2.3 Bioinformatic Analyses

2.3.1 General Statistical Approaches

R (R Core Team, 2015; v. 3.1.0) was used for all statistical analyses with general reliance on the capabilities provided in the following packages: reshape2, ggplot2, plyr (Wickam, 2007; Wickam, 2009; Wickam, 2011), combinat (Chasalow, 2012), limma (Ritchie, 2015), Hmisc (Harrell and Dupont, 2015) and phyloseq (McMurdie and Holmes, 2014). Phyloseq proved highly useful in working with phylogenetic libraries as it kept OTU count matrices, taxonomic classifications and sample data as one easy-to-use object. Where necessary, P-values were adjusted according to the Benjamini & Hochberg (1995) false discovery rate (FDR) correction. All correlational testing was performed with 'rcorr' from the Hmisc package. The majority of graphs were made using ggplot2, saved as pdf and customized with vector graphics editing software. All raw data and R code used to process and analyze data was made available as Supplementary Data.

2.3.2 Phylogenetics, Taxonomic and Ecological Classifications

Phylogenetic trees were prepared either by maximum likelihood (bootstrapping, n=500) with MEGA6 (Tamura *et al.,* 2013) based on MUSCLE sequence alignment, trimmed to ensure complete alignment of all sequences (Chapter 3) or by parsimony in ARB (Westram *et al.,*

2011), adding directly to the pre-aligned and curated SILVA tree (Pruesse *et al.*, 2007; 'SSURef_NR99_123_SILVA_12_07_15'). As previously mentioned, classification was performed using the RDP Classifier (Wang *et al.,* 2007) using Greengenes for 16S rRNA genes (database gg_13_8_99; August 2013) and the Mothur-formatted release of UNITE for ITS (sh_mothur_release_08.12.2013; August 2013). Taxonomic classification of raw or assembled metagenomic data was based on lowest common ancestor analysis from MEGAN (Huson *et al.,* 2007; v. 5.10.1) using the output from DIAMOND blastx (Buchfink *et al.,* 2015; v. 0.7.9) against a local version of the 'nr' database from NCBI (downloaded October 2014). MEGAN classification was based on the consensus taxonomy of the top 10 valid hits to the 'nr' database. DIAMOND blastx searches were rapidly performed on the WestGrid system 'Breezy.'

The designation of functional guilds of fungi was based on FUNGuild (Nguyen *et al.,* 2015; v. 1.0). Radiation, desiccation, and heat-tolerance were assigned to a group if members were reportedly abundant in lithic or desert environments or if a cultured representative had been documented to have exceptional tolerance. The superphylum, 'Terrabacteria,' refers to the grouping of the following bacterial phyla: *Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria*, *Deinococcus-Thermus*, *Firmicutes* and *Tenericutes*. Ectomycorrhizal status was attributed to the following genera based on a broad literature review: *Alpova, Amanita, Amphinema, Boletus, Cadophora, Cantharellus, Cenococcum, Chroogomphus, Clavulina, Cortinarius, Craterellus, Elaphomyces, Endogone, Entoloma, Genabea, Genea, Gilkeya, Gomphus, Hebeloma, Helvella, Hydnum, Hygrocybe, Hygrophorus, Hysterangium, Inocybe, Laccaria, Lactarius, Leccinum, Lycoperdon, Lyophyllum, Melanogaster, Otidea, Paxillus, Phlebopus, Phylloporus, Piloderma, Pisolithus, Pseudotomentella, Pulvinula, Ramaria, Rhizopogon, Russula, Schizophyllum, Scleroderma, Sebacina, Sistotrema, Suillus, Thelephora,*

Tomentella, Tomentellopsis, Tricholoma, Truncocolumella, Tuber, Tylospora, Wilcoxina and Xerocomus.

2.3.3 Diversity, Rarefaction, Ordination and perMANOVA

Chao1 richness and Shannon diversity estimates were calculated on rarefied data using the 'plot_richness' function (phyloseq). Reported estimates represent the average of 500 random samplings. Simpson's measure of evenness was calculated using 'evenness' (RAM; Chen *et al.,* 2016) and was also calculated from the average of 100 rarefied random samples. Rarefaction curves were calculated using 'rarecurve' and permutational analysis of variance (perMANOVA) using 'adonis', both from the 'vegan' R-package (Oksanen *et al.,* 2015). Non-parametric multi-dimensional scaling ordination was performed on Bray-Curtis dissimilarity matrices using 'ordinate' (phyloseq).

2.3.4 Indicator Species Analysis

Indicator species analysis was performed using the R-package 'indicspecies' (De Cáceres and Legendre 2009). Two types of data were analyzed: OTU count data and count data binned by taxonomic classification. In the latter case, analysis was repeated at each taxonomic rank. In addition to running analysis on aggregated data, analysis was also performed on subsetted data according to individual ecozone and ecozone x soil layer. Results were compiled and de-replicated and can be found in the Supplementary Data.

2.3.5 Identifying Cellulolytic and Lignolytic Taxa

Due to the nature of incomplete separation of ^{13}C-enriched from unenriched DNA, particularly DNA with high GC content, a number of statistical methods were applied to identify OTUs and taxa differentially abundant between ^{13}C- and ^{12}C-libraries in both pyrotag and shotgun libraries. Two primary methods were used to identify differentially abundant OTUs or

taxonomic bins: "DESeq" (Anders and Huber 2010) and indicator species ('indicspecies), though two additional methods were used in characterizing SIP-cellulolytic communities in Chapter 3: "limma-voom" (Ritchie *et al.*, 2015) and a custom script for calculating relative abundance. DESeq and limma-voom were used due to their statistical correction for the high variance among low abundance OTUs. Overall, for an OTU or taxa to be deemed ^{13}C-enriched (enrOTU), and assigned a putative activity, it had to be on average 3-fold more abundant in ^{13}C libraries according to at least one method and in at least one ecozone. Due to insufficient replication of shotgun metagenomes, differential abundance in them was based only on indicator species analysis and on average relative abundances and, where possible, supported by pyrotag library data. The influence of community composition on the total amount of ^{13}C-enrichment in PLFAs was assessed using permutation-based regression tree analysis in the R package 'Boruta' (Kursa *et al.*, 2010).

2.3.6 Metagenome Assembly and Draft Genome Recovery

Shotgun metagenome libraries were preprocessed using Trimmomatic (Bolger *et al.*, 2014; v. 0.32), to trim sequencing primers and low quality ends, and FastX Toolkit (Gordon and Hannon, 2010; v. 0.7), to quality filter short or low quality read. Paired end and orphaned reads were included in metagenome assembly using the default setting of software 'Ray-meta' on WestGrid's high-memory node 'Breezy' (Boisvert *et al.*, 2012). In order to recover draft genome, super assemblies were constructed by compositing shotgun metagenomic libraries from the same ecozone and soil layer and assembling with the low-memory assembler MEGAHIT (Li *et al.*, 2015; v. 1.0.2). Two strategies were used to populate draft genome bins: i) Metawatt (Strous *et al.*, 2012; v. 2.1), was used to bin scaffolds derived from the super assembly based on tetranucleotide frequency; and ii) MetaBAT (Kang *et al.*, 2014; v. 0.18.6). MetaBAT required

that reads from each of the composited libraries be mapped back to the super assembly and indexed using Bowtie2 (Langmead and Salzberg, 2012). Indexed files were subsequently fed into MetaBAT which binned based on both tetranucleotide frequency and covariance of read abundance among composite libraries. MetaBAT was the more successful of the two binning methods and was used exclusively for the binning described in Chapter 4. The quality of draft genome bins was assessed by scanning for essential single-copy, house-keeping genes with the hidden Markov model provided by Albertsen *et al.,* (2013) and using CheckM (Parks *et al.,* 2015). The efficacy of recovering draft genomes was compared between compositing with or without corresponding ^{12}C libraries. Inclusion resulted in the recovery of the greatest number of draft genomes, likely due to the increase in sample number and increase variation in read abundances between ^{12}C- and ^{13}C libraries. Read mapping with Bowtie2 was also used to calculate the relative abundance of these draft bins across harvesting treatments in Chapter 3.

2.3.7 Characterization of Carbohydrate-Active Enzyme (CAZy) Content

The complete CAZy database (Cantarel *et al.,* 2008; www.cazy.org) was downloaded and formatted into a DIAMOND blastx searchable database (accessed 2014-05-06, for Chapter 3, and again on 2015-08-19 for Chapter 4). The database was downloaded with a script implemented in Metapathways 2.5 (Konwar *et al.,* 2015) which is publicly available at https://github.com/nielshanson/CAZy_utils. Metagenomic reads were annotated by CAZy family based on the top hit of blastx searches with minimum e-value of 10^{-5}, as described in Cardenas *et al.,* (2015). The following glycosyl hydrolase (GH) families contain enzymes with endoglucanase activity: GH5, 6, 7, 8, 9, 12, 26, 44, 45, 48, 51, 61, 74, 81 and 131 (Yagüe *et al.,* 1990; Hasper et al., 2002; Petersen *et al.,* 2009; Harris *et al.,* 2010; Vlasenko *et al.,* 2010; Warner *et al.,* 2011; Lafond et al., 2012; Boyce *et al.,* 2015; Brumm *et al.,* 2015). A custom

python script was used to identification of clusters of CAZymes which first identified putative CAZyme sequences by a) blasting against the CAZy database, b) using ‘hmmscan’ (HMMER v. 3.1b1; Eddy, 2011) using hidden Markov models for CAZymes provided by dbCAN (Yin *et al.,* 2012) and custom hidden Markov models for aryl and vanillyl alcohol oxidases, laccases, dye decolorizing peroxidases (Dyp2), and versatile peroxidases (Erick Cardenas, unpublished). The script then identifies groups of three or more CAZymes on assembled scaffolds and recovered these sequences. This script implemented gene-predicting software Prodigal (Hyatt *et al.,* 2010; v. 2.6.2).

Chapter 3: Long-term Impacts of Timber Harvesting on Soil Communities

3.1 Rationale

To date, studies of harvesting impacts on soil communities report that edaphic and geographic differences among samples can obscure the effects of disturbance. Heterogeneity accorded to those factors accounted for between 4 and 14-fold more variation in community structure than harvesting (Hartmann *et al.,* 2012; Cardenas *et al.,* 2015; Leung *et al.,* 2016). Yet, the majority of studies on harvesting disturbance report persistent, long-term shifts in microbial community composition resulting from timber harvesting, upwards of 50 years post-harvest (McGuire *et al.,* 2014). While the list of potential impacts is relatively long, and likely to grow in coming years (see Section 1.1.3), there has yet to be an assessment of how robust and generalized these trends are across different geographic zones, forests and soil types. Further, in the face of growing incentive to harvest woody debris previously left onsite, the effect of organic matter removal on soil community structure needs to be assessed. This research provides a comprehensive study of the differences in bacterial and fungal communities from soils at reforested sites across North America where varying amounts of organic matter have been removed (or retained).

This research used the highly replicated LTSP experimental design and both field samples (‘*in situ*’) and SIP-cellulose microcosms to assess impacts on community structure and function. Multiple datatypes were considered, including shotgun metagenomic and phylogenetic gene marker libraries (16S rRNA gene and ITS), respiration assays, and quantitative PLFA analysis. In accordance with the goals of the LTSP Study to ‘develop indices of soil quality practicable in monitoring’ (Powers, 2006), specific taxonomic groups were identified which may be relevant to monitoring efforts.

3.2 Community Responses to Timber Harvesting

All raw sequencing data described in Section 3.2 can be retrieved from the European Nucleotide Archive under the study accession PRJEB12501 (all ITS + B.C. 16S rRNA gene libraries) and PRJEB8599 (majority of 16S rRNA gene libraries). All additional data, including sample metadata and representative FASTA sequences for indicator taxa can be found in the Supplementary Data. Figure 1.1 describes the overall experimental design and datasets utilized in Section 3.2. A total of 685 bacterial 16S rRNA gene and 696 fungal ITS region samples were analyzed (average library size = ~ 5,000 reads). A complete breakdown of sample totals according to ecozone, soil layer and treatment can be found in Table E.4. Additional samples were processed from LP_{TX} and JP_{ON} ecozones due to the inclusion of plots that had been exposed to herbicide at the time of replanting. No differences in community composition were attributed to herbicide application (data not shown) and samples were included in analysis without special treatment. The following samples were removed due to insufficient sequencing depth (< 1,000 reads): TXA035 (ITS libraries) & JS061 (16S rRNA libraries).

3.2.1 Overview of Community Composition Across Ecozones

The majority of variation in both bacterial and fungal community structure was attributable to biogeography and soil layer, while a minor, though significant ($p < 0.01$), portion was due to degree of OM removal (Figure 3.1). Of the total variation explained by experimental factors (i.e. excluding residual variation), ecozone accounted for 64% and 67% of variation, bacterial and fungal, respectively, followed by site (15 and 21%), soil layer (18 and 7%) and OM removal (3 and 5%). Despite the large variation in community between ecozones, the most abundant bacterial OTUs among all ecozones were cosmopolitan genera abundant at every site and in both soil layers. The core OTUs shared among all ecozones (~15% of total OTUs) accounted for 72% of all bacterial reads while a similar, but less pronounced, trend was observed

for fungi, 5.5% and 37%, respectively (Figure 3.2). Variation in both fungal and bacterial communities among ecozones was driven by differences in composition of middle and lesser abundant taxa. Alpha-diversity significantly differed between ecozones (Figure 1.1), though community composition was more similar between proximal ecozones. The sampling depth obtained (~ 10 K per sampling site) did not exhaust either bacterial or fungal species richness according to rarefaction curves (Figure 3.3). While diversity was substantial, the rarity of certain taxa may be attributed to the fact sequencing libraries represent relative abundances. As such, cosmopolitan taxa occupy a large proportion of reads, causing other taxa to be overshadowed and creating the appearance of a long tail of relatively ‘rare’ taxa.

Figure 3.1. Bar plots of the percent variance (R^2) explained by each experimental variable based on perMANOVA (n_{perm}=1000) using Bray-Curtis dissimilarities for either 16S rRNA gene (bacteria) or ITS gene (fungal) libraries. Only statistically significant ($p < 0.01$) factors were included. Panel A shows the variability in each library type according to soil layer, while panel B shows variability within each ecozone with both layers combined. Consult Figure 1.1 for ecozone codes.

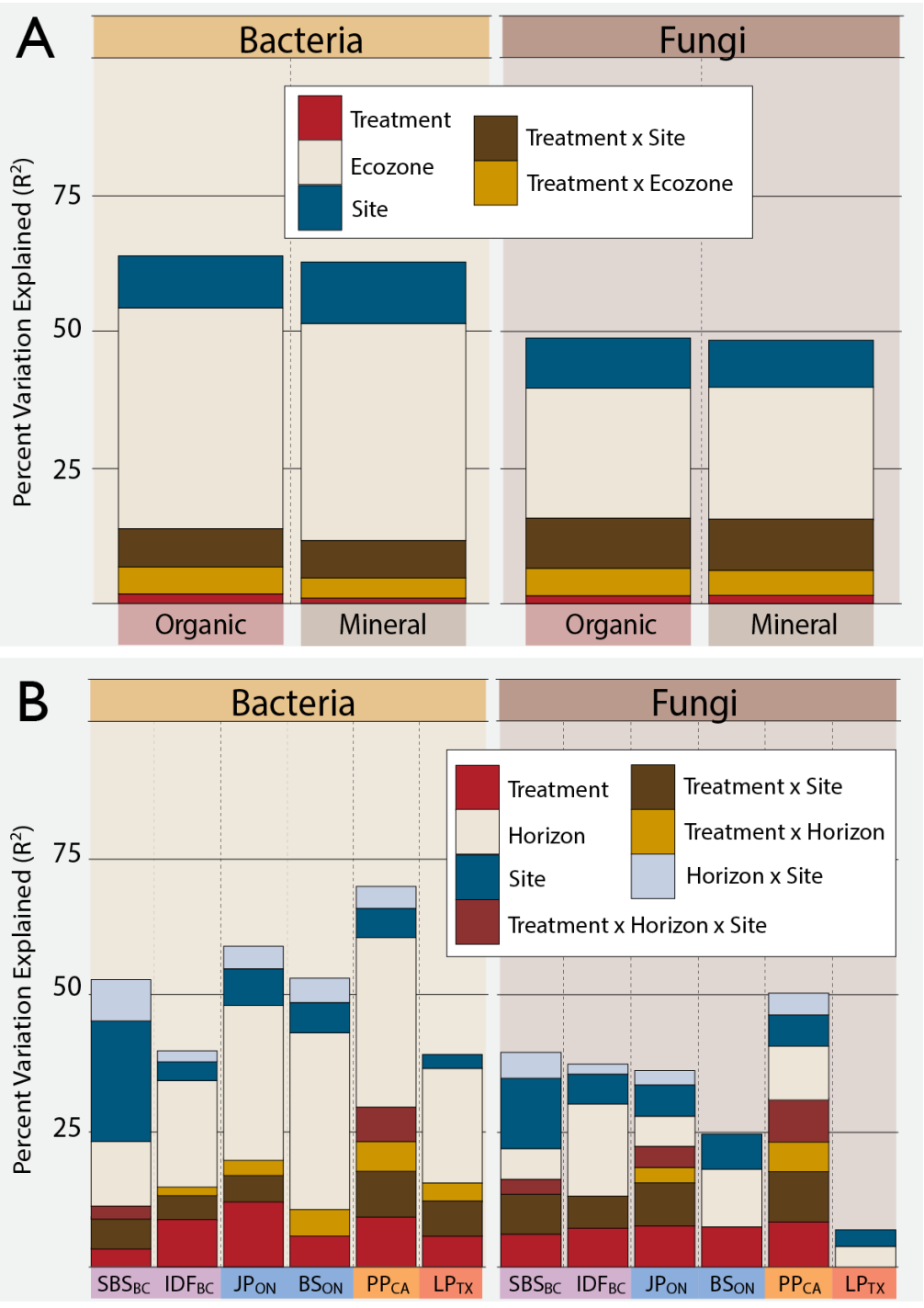


Figure 3.2. Venn diagrams displaying the number of overlapping OTUs overlap among ecozones and harvesting treatments for bacterial and fungal pyrotag libraries. The total number of OTUs is given followed by the percent abundance of all OTUs in brackets.

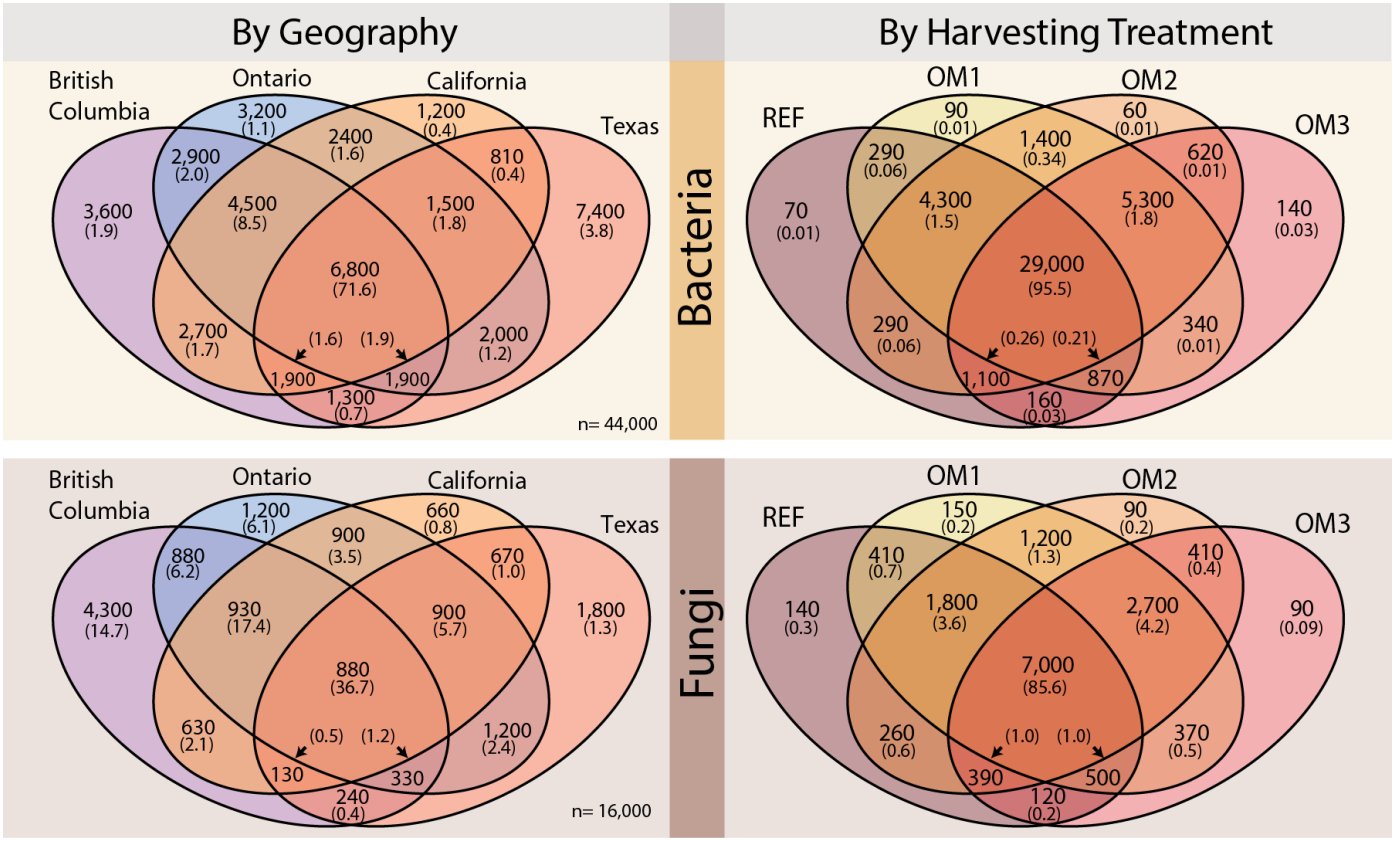
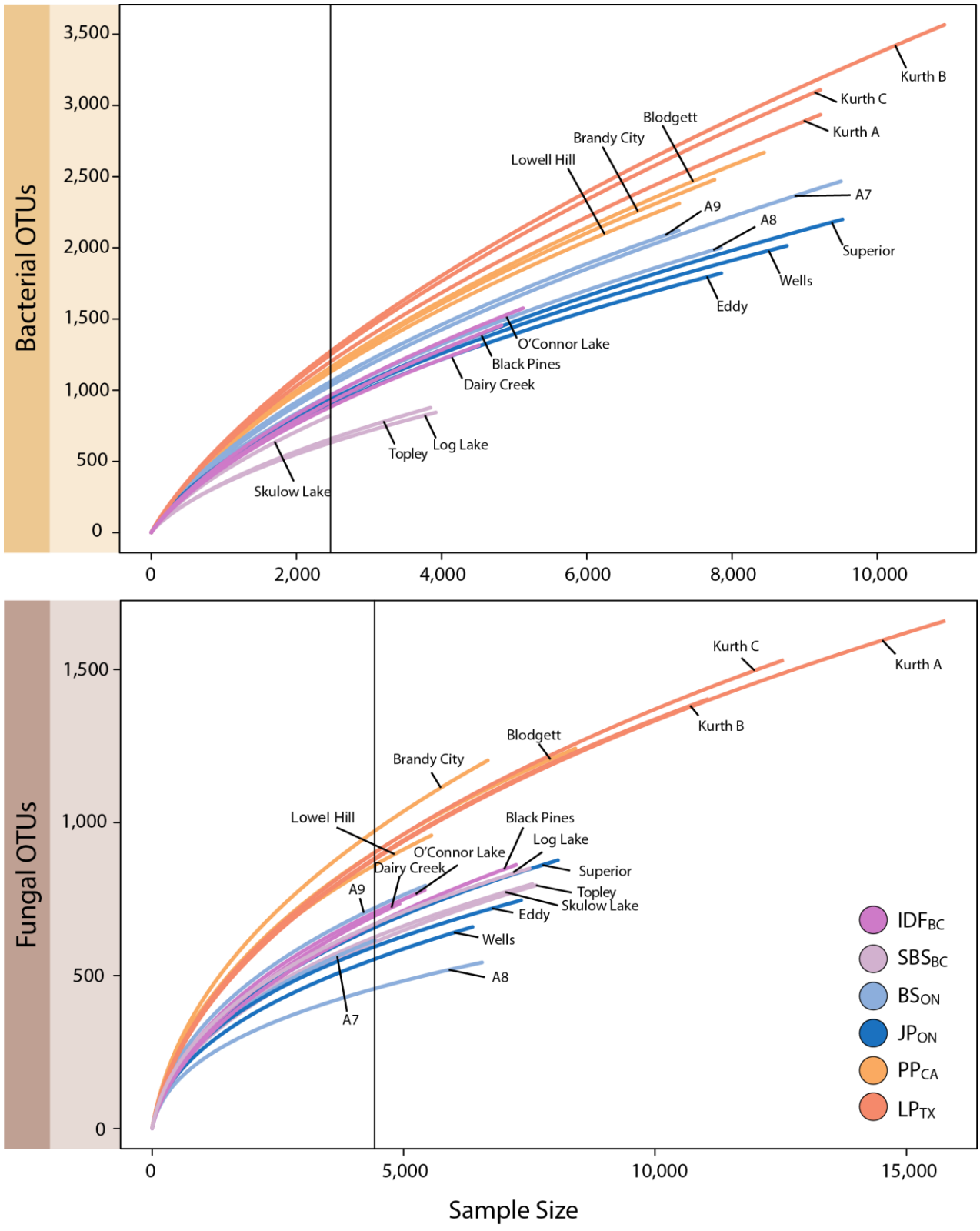


Figure 3.3. Rarefaction curves showing the depth of sampling obtained at each site also grouped by Ecozone (colour).



Cosmopolitan bacterial groups included *Rhodoplanes* (on average 5-7% of total proportion of library reads), *Mycobacterium* (0.4-4%), *Burkholderia* (0.5-3%), *Reyranella* (0.4-2%), and candidate acidobacterial genera: *Koribacter* (0.2-1.5%) and *Solibacter* (0.2-1%). Members of *Bradyrhizobiaceae* were by far the most abundant taxa in both soil layers, with the greatest abundance in western ecozones: IDF_{BC} and SBS_{BC} (~25% of all reads) and PP_{CA} (~13%), and to a lesser extent in JP_{ON}, BS_{ON} and LP_{TX} (~8%). Cosmopolitan fungal families included *Atheliaceae* (3 – 24%), *Russulaceae* (3.5 – 18%) and *Suillaceae* (3 – 9%) occurring at high abundances in most ecozones, while PP_{CA} was dominated by *Trichocomaceae* (8-14%) and LP_{TX} by *Mortierellaceae* (~15%). Broad differences between soil layers were observed in bacterial communities at higher taxonomic ranks with *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes* and *Actinobacteria* all more abundant in organic soil, while candidate phylum *AD3*, *Chloroflexi*, *Betaproteobacteria*, *Deltaproteobacteria*, *Firmicutes* and *Acidobacteria* more abundant in mineral soils. No significant differences between soil layers occurred for fungi at rank phylum, which was consistent with the lesser degree of variation explained in perMANOVA testing (Figure 3.1).

3.2.2 Harvesting Impacts on the Soil Properties

The harvesting gradient achieved by the removal of between 40-70% of aboveground biomass in OM1, 70-90% in OM2 and nearly 100% in OM3 treatment plots (Powers *et al.*, 2006), resulted in a clear gradation of soil organic matter at the time of sampling. Total carbon and nitrogen content per gram of soil was diminished according to the gradient in the organic layer of soil of all ecozones, but not in the mineral layer (Figure 3.4; full details in Table E.5). At time of sampling, organic layer soil development reflected the OM removal gradient with the average depth of organic soils in the BS_{ON} sites: 0.4 cm (OM3), 3.2 cm (OM2), 4.0 cm (OM1) and 4.8 (REF). Accurate data from other sites was missing, yet a similar depth gradient likely

existed, exemplified by the lack of an organic layer in OM3 sites in JP_{ON} and both B.C. ecozones. Soil pH was slightly increased in harvested plots in northern ecozones, but remained similar to unharvested plots or decreased in southern ecozones (Table E.5).

Mean daily soil temperature was significantly higher in harvested plots and increased with OM removal (Figure 3.5). Five years post-harvest at PP_{CA} sites, soil temperatures were consistently warmer in OM1 (+1.5 °C) and OM3 (+6 °C) than unharvested plots (Figure 3.5B). At Ontario sites, where long-term soil temperature data was available, there were marked differences between OM2 and OM3 (Figure 3.5 A and C). After 5 years, OM3 soils were ~ 4 °C warmer during summer months than OM2 soils at 5 cm belowground. This difference was no longer observed ten to thirteen years after harvesting in BS_{ON}, and had diminished to ~ 2 °C in JP_{ON}. The diminishing differences in soil temperature over time illustrate the influence of ground cover and canopy development on soil conditions (Figure 3.5A). During summertime, the variation in temperature was on average ~ 60% greater in OM3 than in OM2 ($t = -3.8$; $p < 0.001$), which, for example, amounted to differences in temperature extrema by an additional ± 1.8°C during the month of July. This increased variation in soil temperature did not diminish over time. Unfortunately, comparable long-term data for REF (unharvested reference plots) and OM1 was not collected. Soil moisture also reflected differences according to the OM removal gradient (Figure 3.4). While the data represents a single time-point, it is still informative that decreased moisture retention was found with increased OM-removal. These differences likely relate to the compounding effects of OM loss (absorptive material) and warmer soil temperatures (higher evapourative flux).

Figure 3.4. Bar plot illustrating differences in total carbon, total nitrogen, soil moisture and pH among treatments in all ecozones. REF corresponds to unharvested reference plots. Error bars correspond to one standard error of the mean.

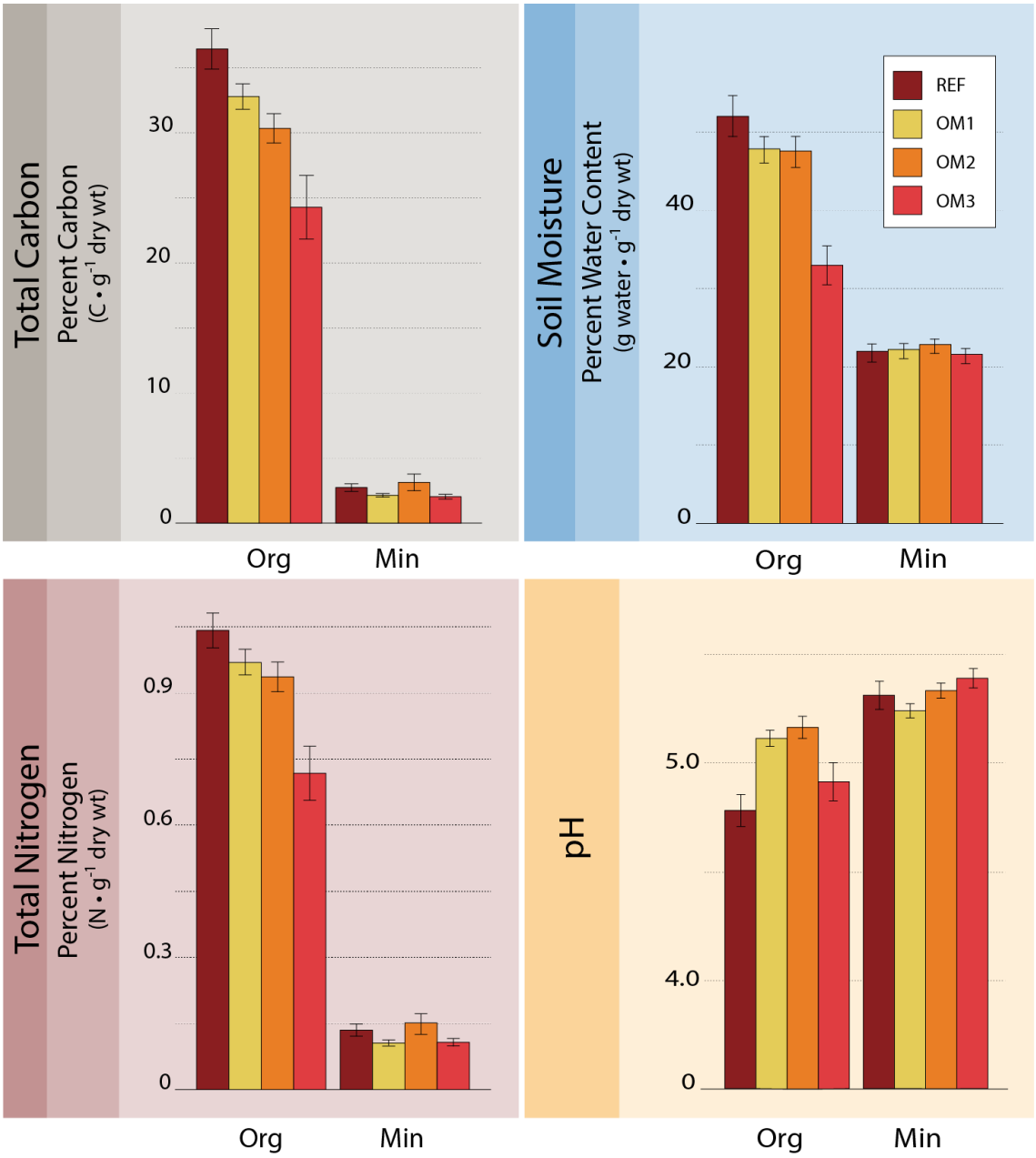
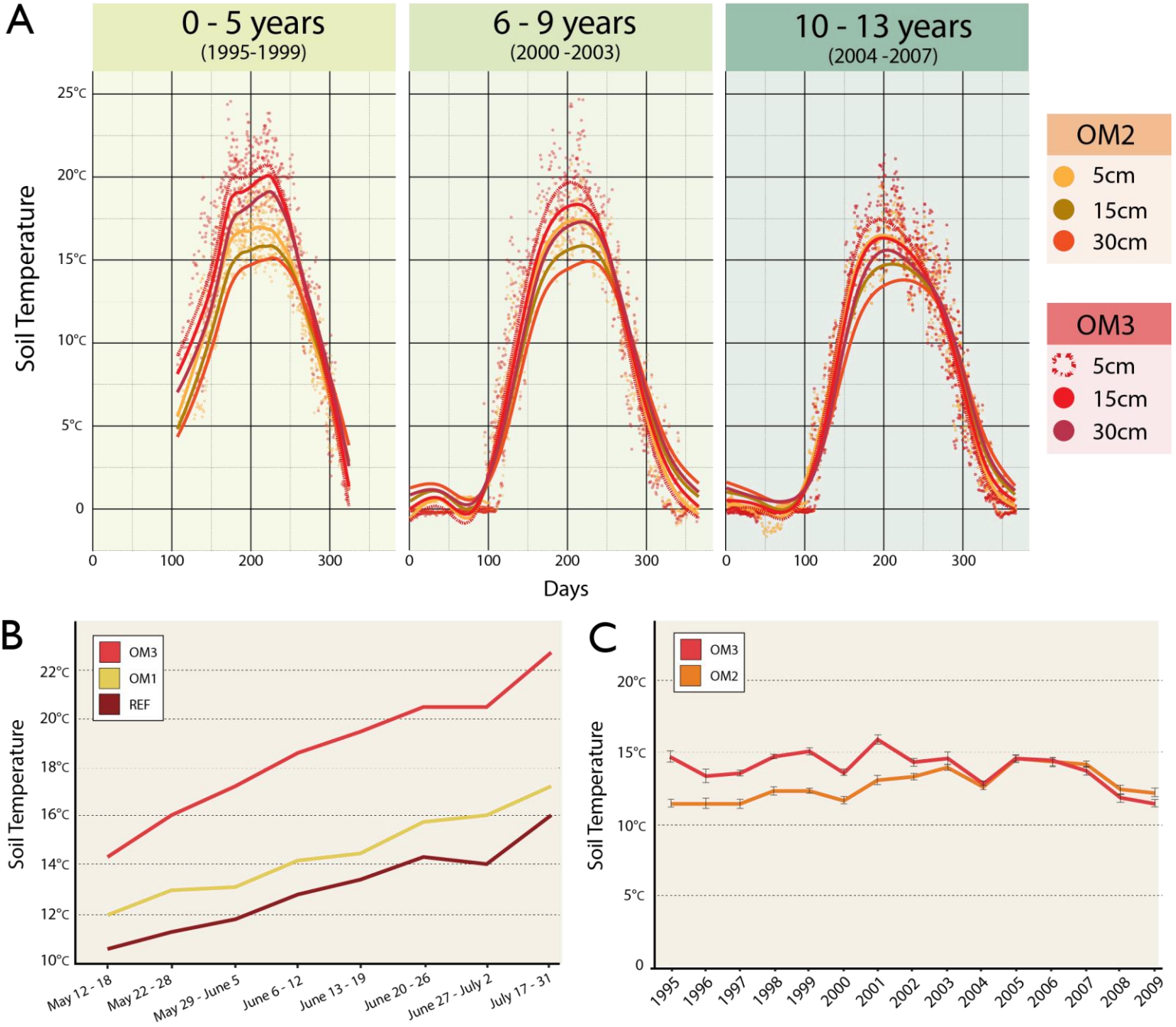


Figure 3.5. Mean daily soil temperature data for JP_{ON} (A), PP_{CA} (B) and BS_{ON} (C) ecozones. Panel A contains long-term temperature data at varying depths along the soil profile for the two highest intensities of OM removal. Multiple years were grouped and averaged over a 4 to 5-year window. Transparent dots correspond to all daily measurements at 5 cm for OM2 (yellow) and OM3 (red). Panel B depicts soil temperatures averaged across the entire soil profile for REF, OM1 and OM3 plots in PP_{CA} (sourced from Paz 2001 and reprinted with permission from Dr. Lucas Paz). Panel C illustrates the convergence of mean yearly soil temperatures over 14 years at the interface of organic and mineral layers (upper 10cm) in OM2 and OM3 from 18 replicates per treatment.



3.2.3 Ecozone-wide, Global Responses to Harvesting

Modeling of bacterial and fungal OTU abundance patterns revealed three main trends among harvesting treatments: i) expansion in relative abundance in harvested plots, OM3 > OM2 ≈ OM1 > REF, ii) decline in relative abundance from unharvested levels, REF > OM2 ≈ OM1 > OM3, and iii) a predominance at either intermediate intensity, OM1 ≈ OM2 > OM3 ≈ REF (Figure 3.6). Overall, the pattern of 'expansion' (i), a term used to describe this phenomenon throughout this chapter, was observed in 14.1% and 12.5% of bacterial and fungal OTUs, respectively, while the pattern of 'decline' (ii) was observed in 12.1% and 9.4% of OTUs, and the predominance of OM1 or OM2 in a total of 10.6% and 13%, respectively. Not all ecozones showed the third trend (JP_{ON}, BS_{ON}, PP_{CA} and SBS_{BC}), and some showed a stronger expansion than others (LP_{TX} and IDF_{BC}). These patterns demonstrated that, over a decade after harvesting, the harvesting gradient produced a comparable gradient in the abundance of bacterial and fungal communities. Further, the retention of OM had selected for taxa not necessarily abundant in REF or OM3, corroborated by the fact OM1 and OM2 shared the greatest percentage of overlap of any two treatments and, conversely, REF and OM3 the least (Figure 3.2).

The alpha-diversity of bacterial and fungal communities significantly differed among harvesting treatments in a minority of ecozones and, depending on ecozone, exhibited increasing or decreasing trends in harvested plots (Figure 3.7). Five of the six statistically supported differences were indicative of increasing diversity in harvested plots. Fungal populations in mineral soils were consistently more diverse in harvested plots across all ecozones, though statistically significant in only SBS_{BC} and JP_{ON}. On average, fungal diversity was greatest in OM1 mineral soils everywhere except for LP_{TX}. Bacterial diversity was also greater in harvested plots in mineral soils in half of the six ecozones, but exhibited a reduction in diversity in the other half.

Harvesting produced broad differences in community structure at the phylum level in both fungal and bacterial communities (Figure 3.8). Populations of *Chloroflexi*, candidate phylum *AD3* and *Gemmatimonadetes* (mineral layer-associated) and *Cyanobacteria* (organic layer-associated) expanded with increasing OM removal across all ecozones. Conversely, populations of *Acidobacteria*, *Actinobacteria* and *Gammaproteobacteria* declined in harvested plots, though not always consistently across all ecozones. Samples from Texas (LP_{TX}), in particular, did not exhibit any broad changes at the phylum level. Fungal communities exhibited a consistent decline in the ratio of *Basidiomycota* to *Ascomycota*, everywhere except for LP_{TX} (Figure 3.9). Unclassifiable sequences increased with OM removal (Figure 3.10) with approximately double the amount of unclassified in OM3 compared to REF.

Figure 3.6. Snowflake plots showing the most common abundance patterns of OTUs among harvesting treatments. Each possible permutation in pattern is apparent by moving from the outermost coloured squares (greatest abundance) inwards step-by-step (ex. bottom-left of figure). Circles are scaled to the frequency of the pattern occurring relative to randomized data. Solid circles indicate that the pattern is more likely to occur and hollow circles less likely.

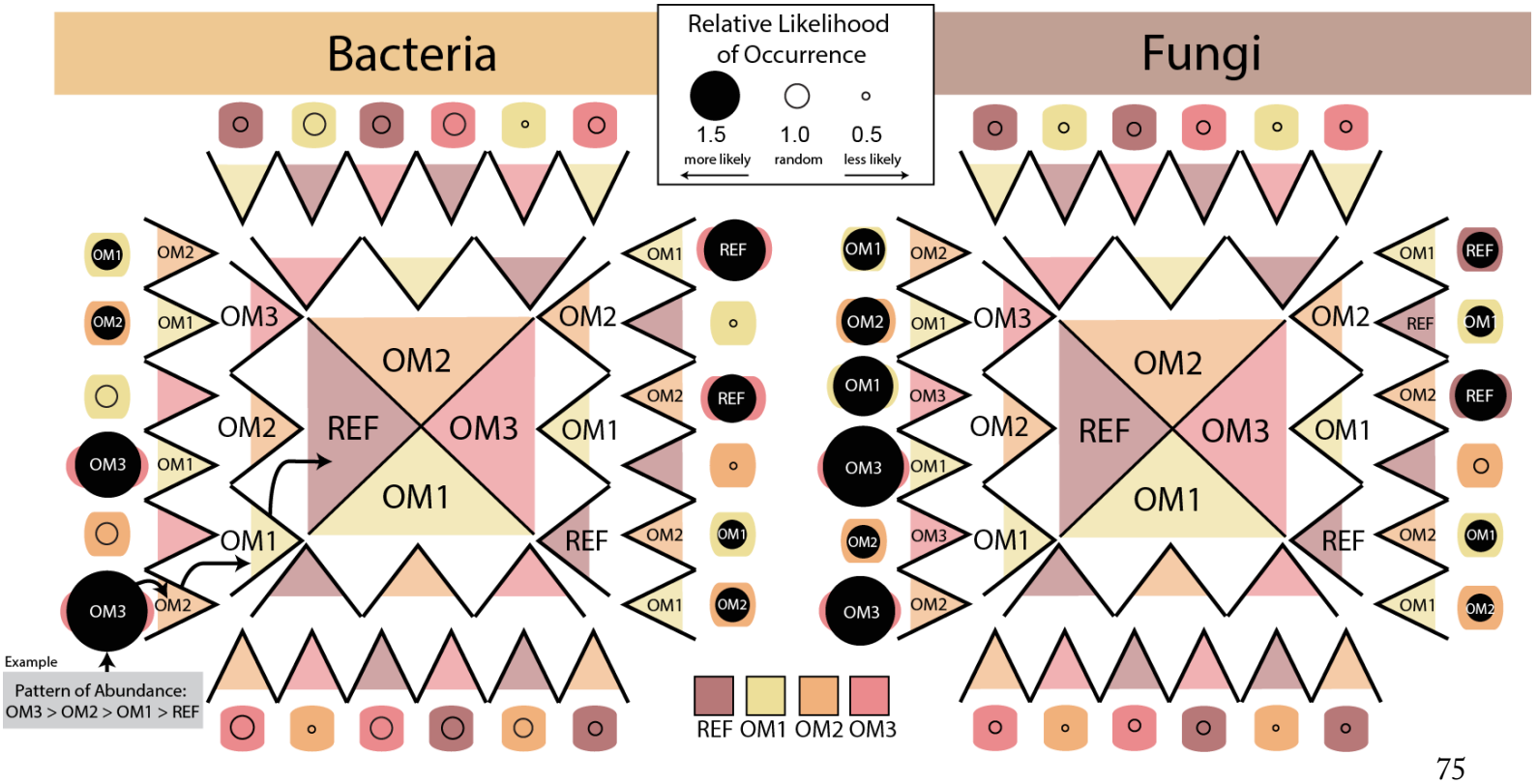


Figure 3.7. Box and whisker plots of Shannon diversity (H'), Chao1 richness and Simpsons's Evenness estimates of bacterial and fungal communities among harvesting treatments according to ecozone and soil layer. The estimation for a given sample was based on an average of 500 calculations on OTU tables rarefied to an equal sequencing depth. Tukey HSD supported, pairwise differences are grouped by lettering.

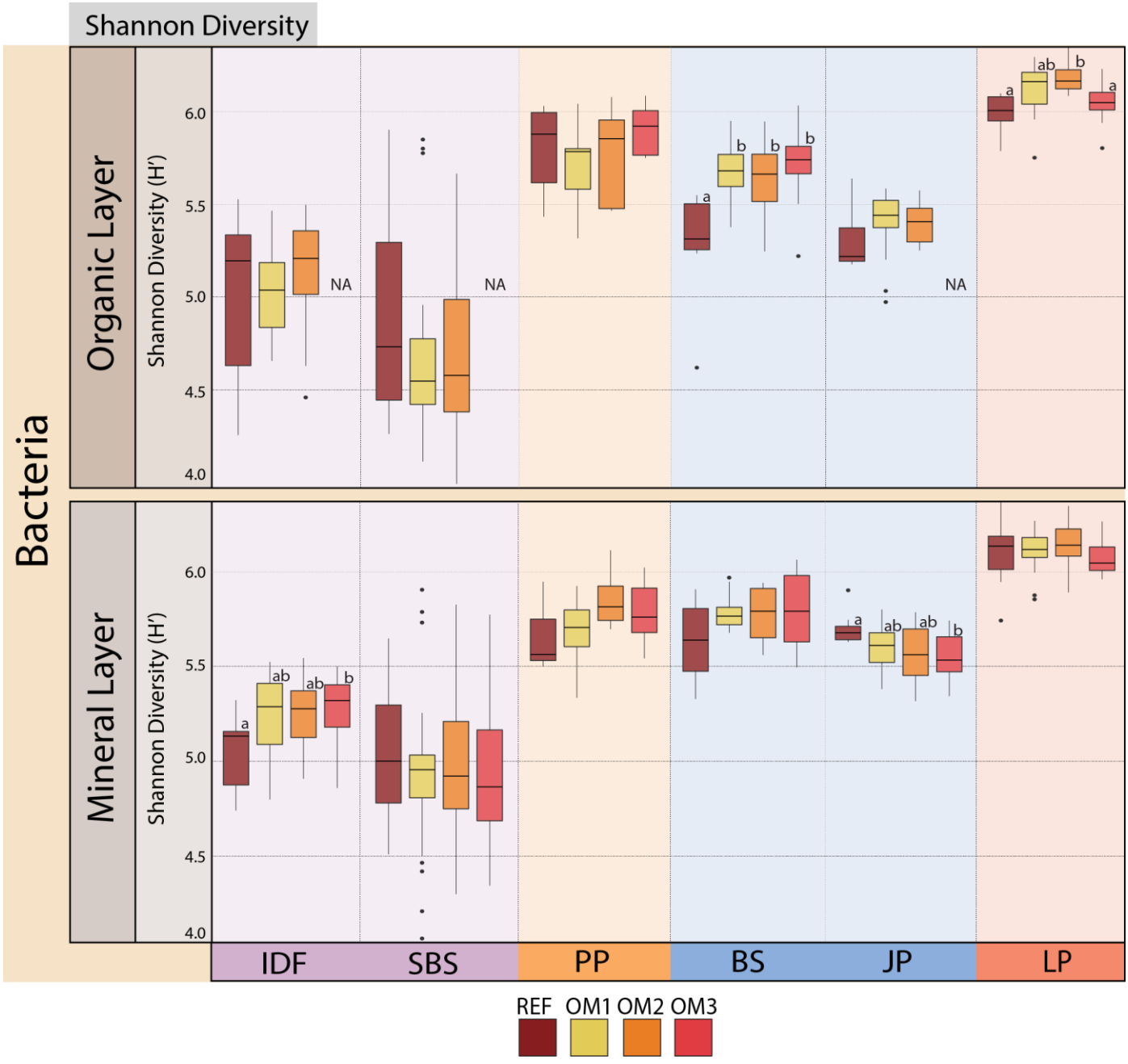


Figure 3.7 *... continued.*

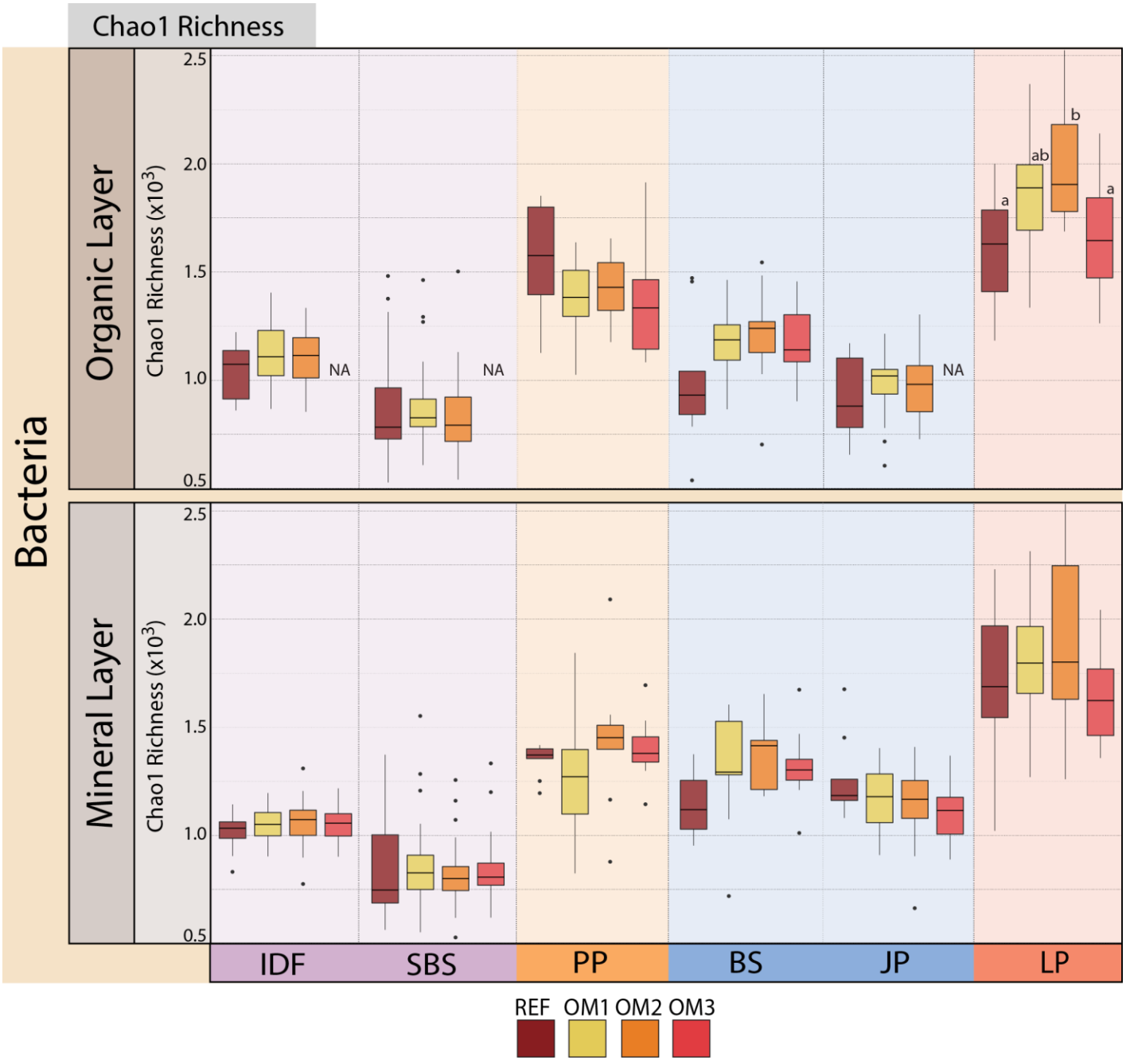


Figure 3.7 *... continued.*

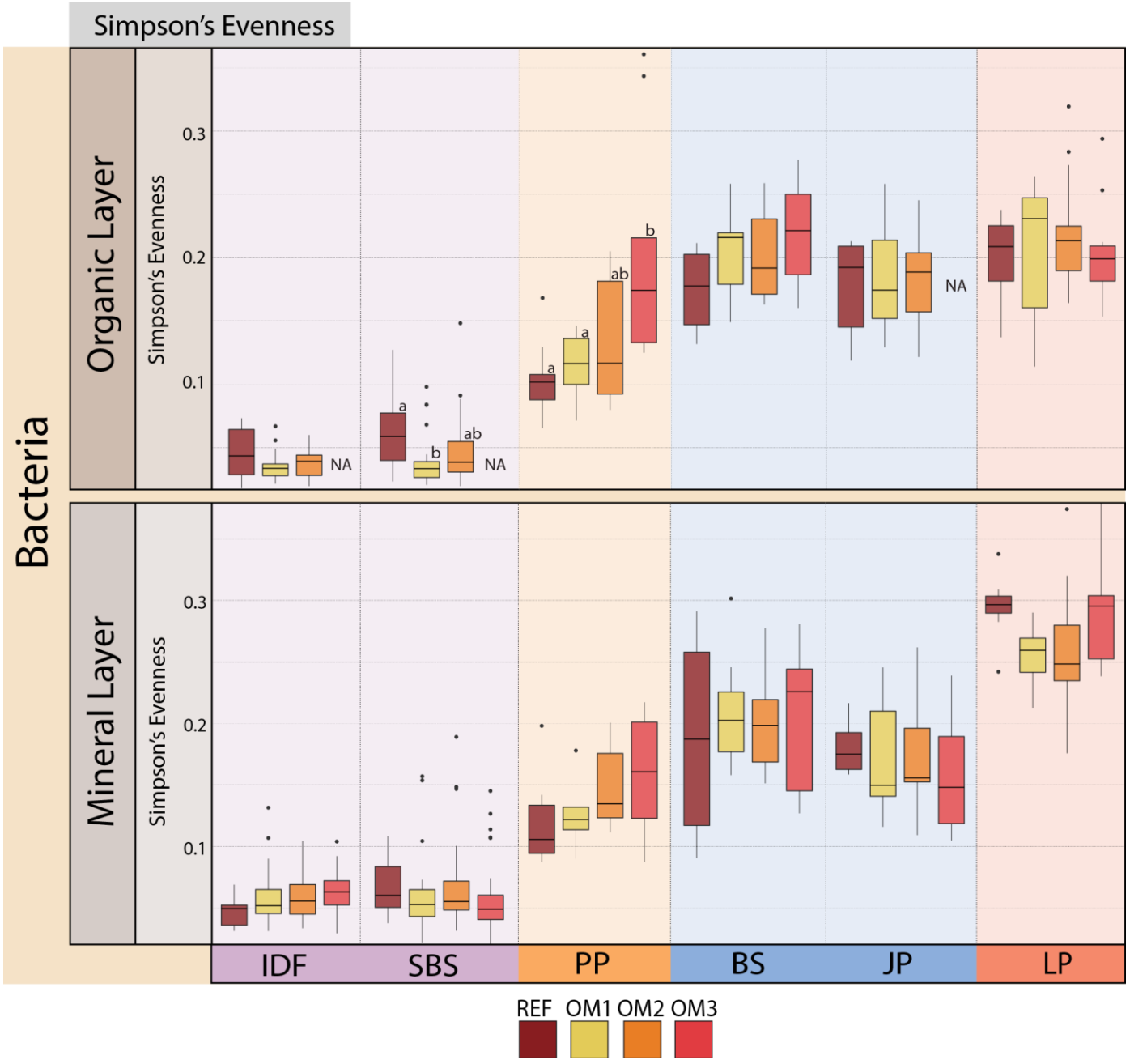


Figure 3.7 *... continued.*

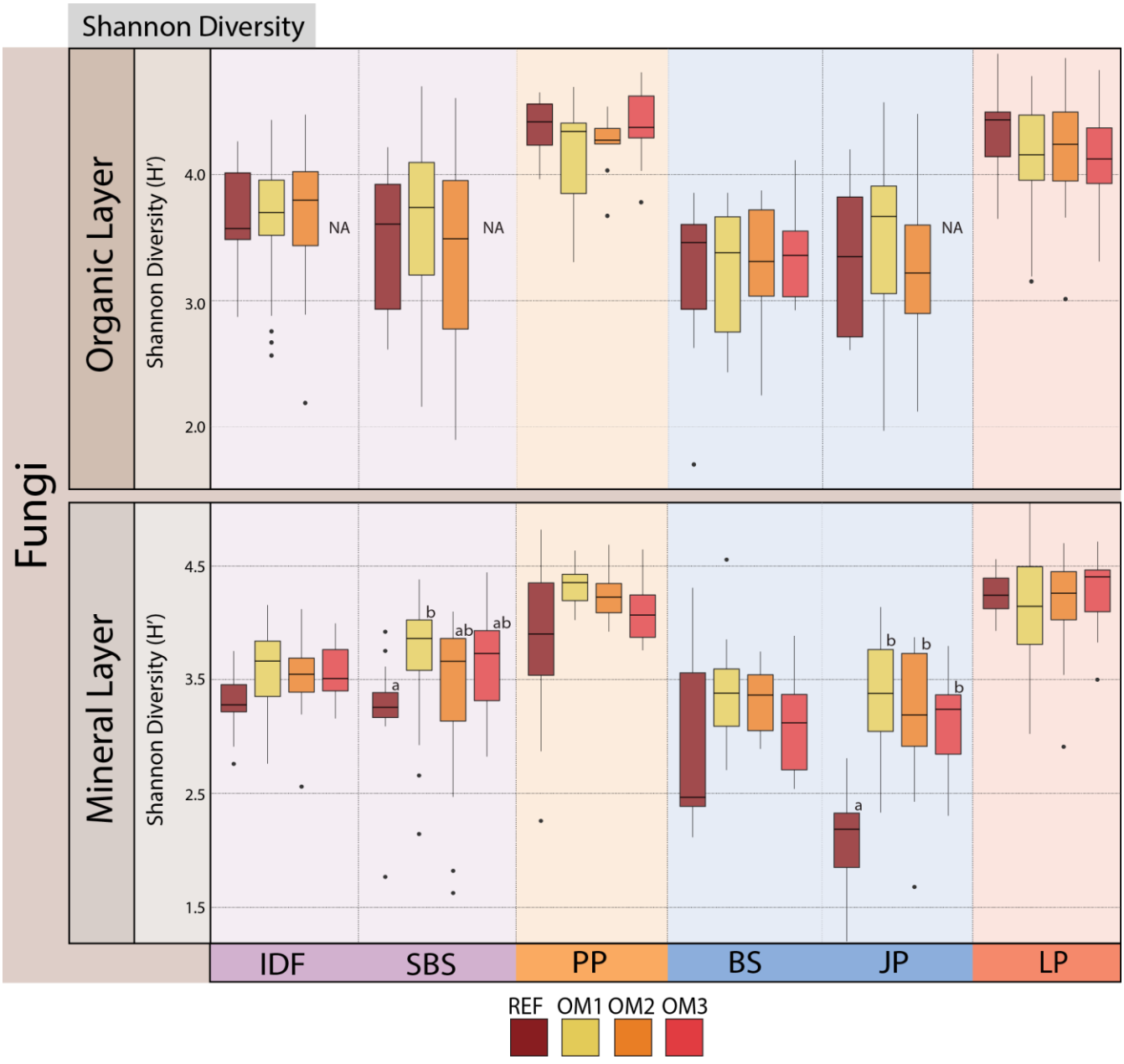


Figure 3.7 *... continued.*

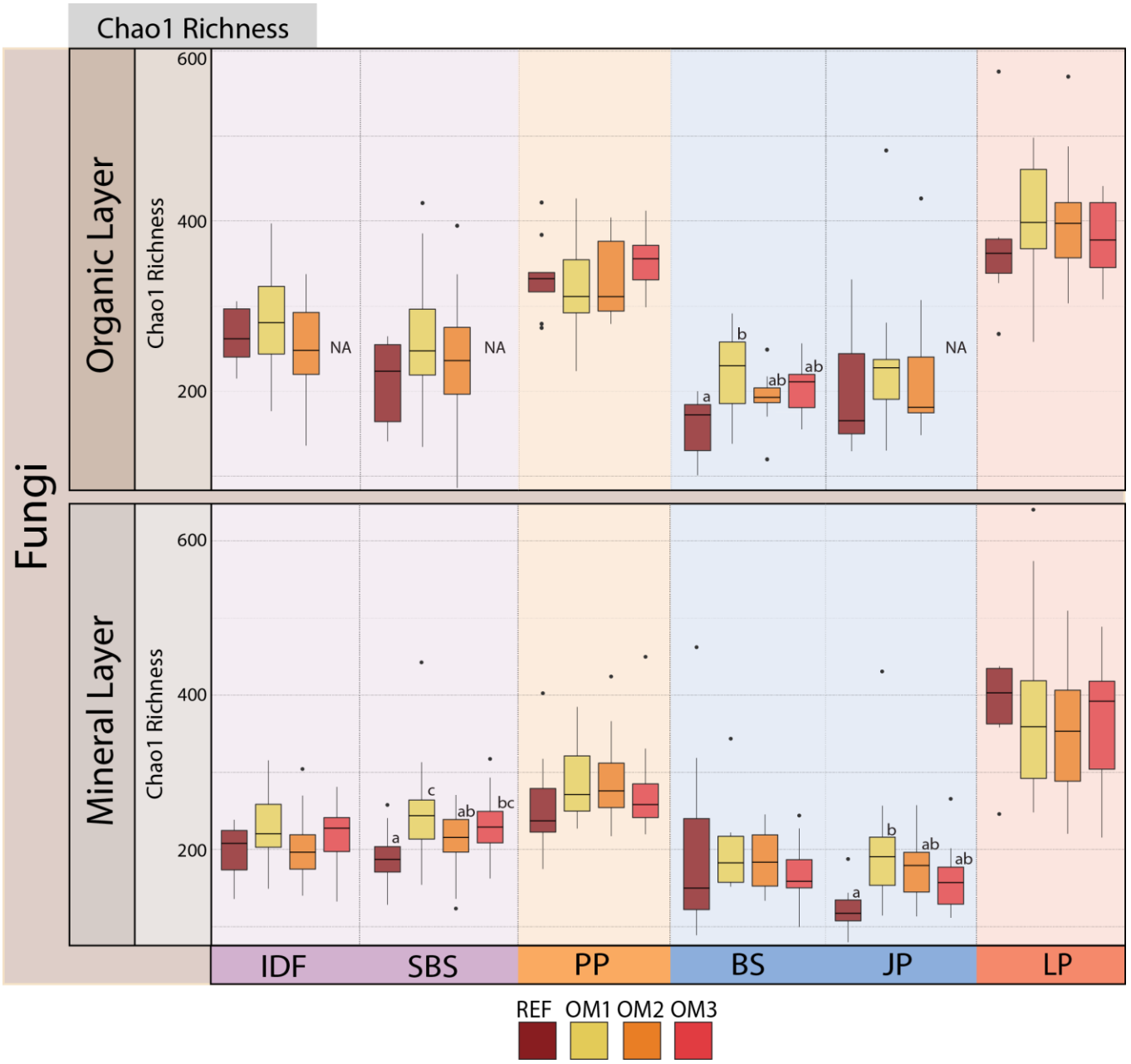


Figure 3.7 *... continued.*

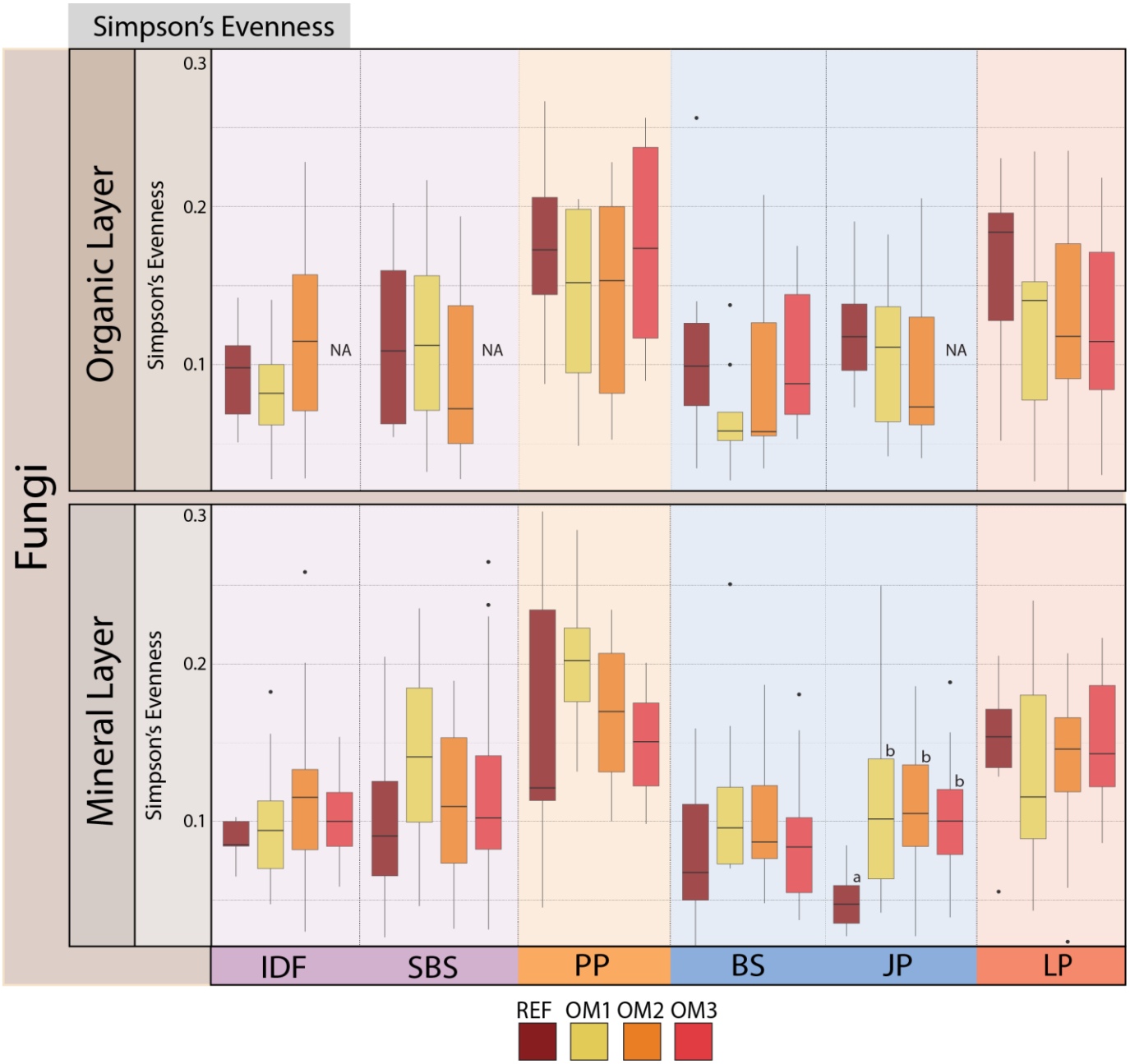


Figure 3.8. Barplots displaying the relative abundance (0 – 100%) of bacterial and fungal OTUs classified at the phylum level faceted by ecozone and harvesting treatments. The relative abundance of divisions within the phylum *Proteobacteria* are inset. Phyla with low abundances (< 0.075% of total reads) were filtered to reduce clutter.

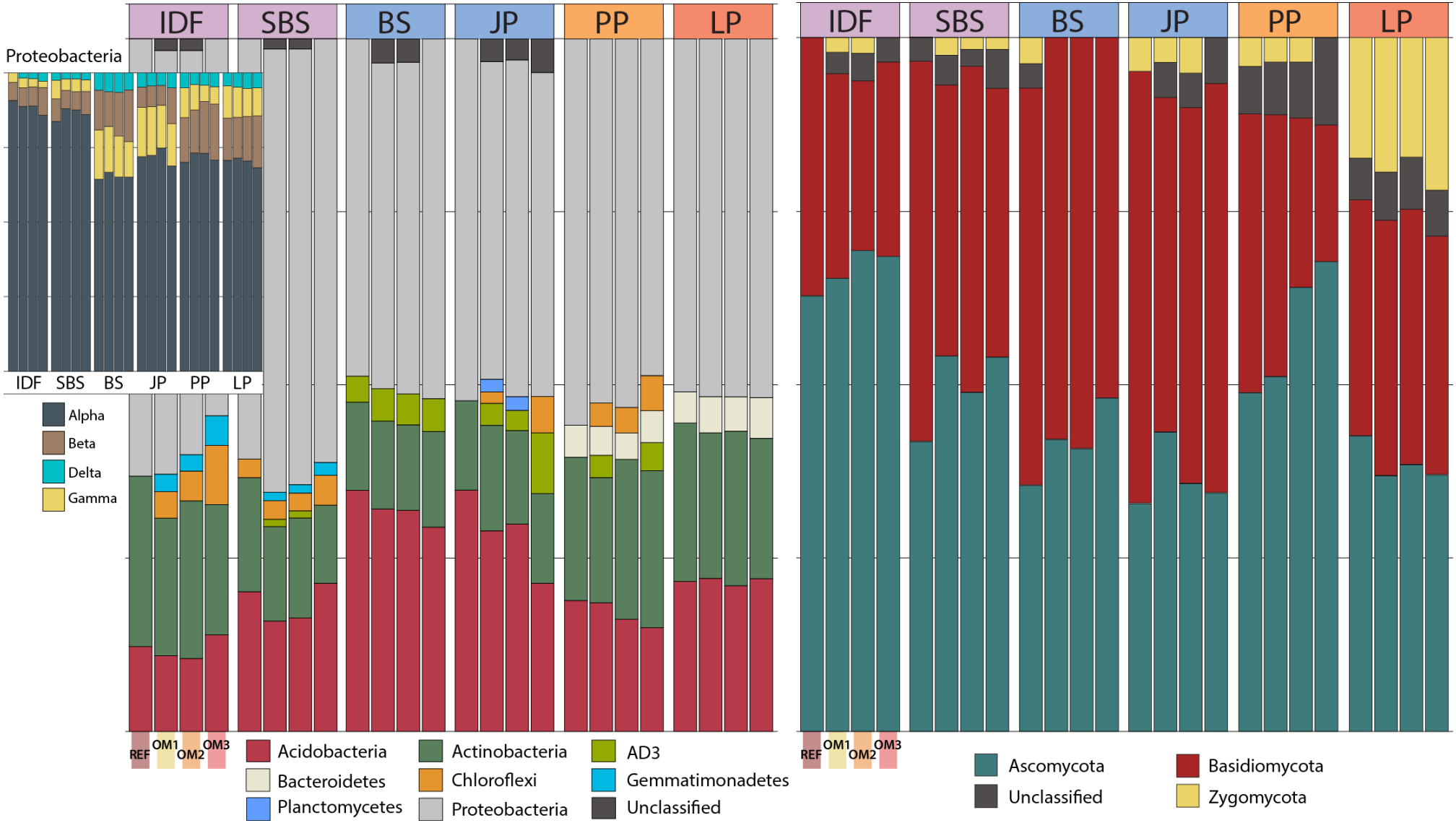


Figure 3.9. Relative abundances of *Basidiomycota* (top) to *Ascomycota* (bottom) according to ecozone and harvesting treatment. The log ratio of the ratio of their abundances is displayed by overlain patterned bars, illustrating the general decrease in *Basidiomycota* relative to *Ascomycota* according to OM-removal. Error bars correspond to one standard error of the mean.

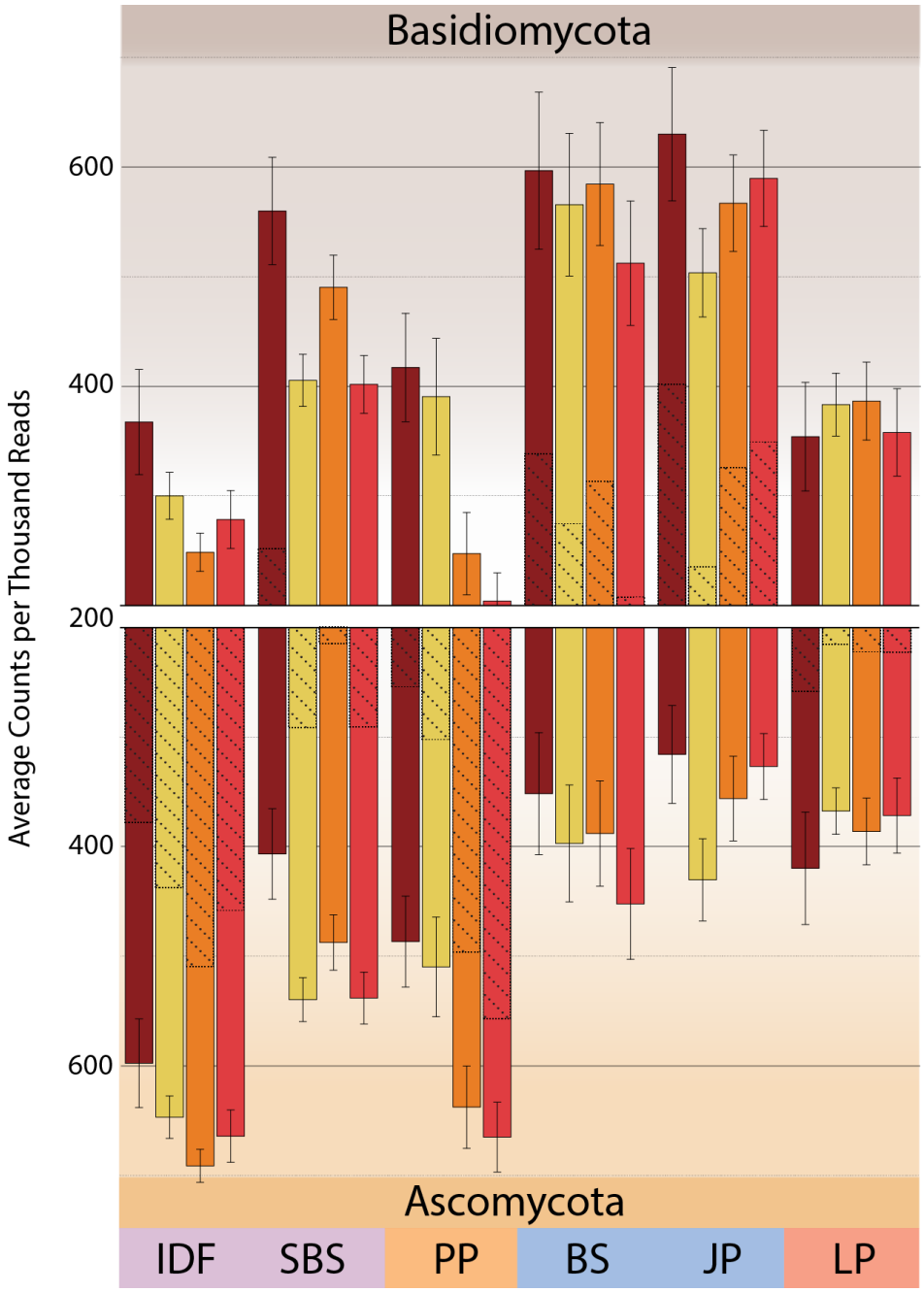
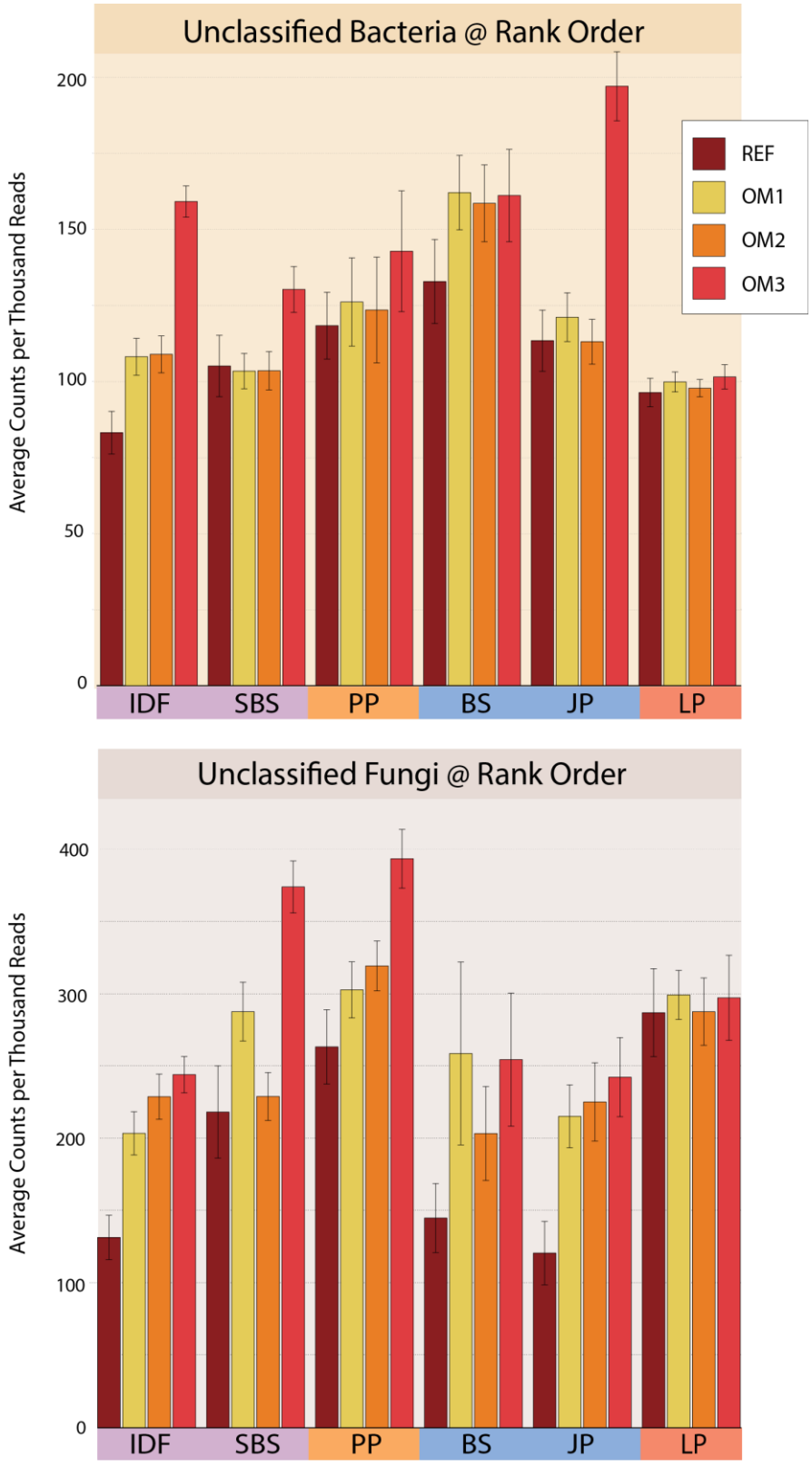


Figure 3.10. Abundance pattern of unclassified fungal and bacterial sequences at the rank order, exhibiting an increased proportion of sequences from poorly characterized or uncultured taxa in harvested plots. Error bars correspond to one standard error of the mean.



Fine-scale, OTU-based differences in community composition were identified through indicator analysis. Among the OTUs showing the greatest expansion following harvesting were radiation, desiccation and heat-tolerant taxa (shaded orange; Figure 3.11). Bacteria from the 'Terrabacteria group' (*Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria* and *Firmicutes*) were all more abundant in harvested plots, though certain families of *Actinobacteria* declined in abundance relative to unharvested plots (Figure 3.12A). Many of the stress-tolerant taxa were highly abundant (0.2-3.3% of total bacterial community), and included endolithic *Actinobacteria* (*Geodermatophilaceae*) and other *Actinobacteria* (*Gaiellaceae spp.* and *Arthrobacter*), *Firmicutes* (*Alicyclobacillus*, *Bacillus* and *Clostridium*), *Cyanobacteria* (*Nostoc*), a number of *Chloroflexi*, and members of other genera such as *Segetibacter*, *Flavisolibacter*, *Methylobacterium*, *Geothrix* and *Geobacter*. Similar growth of stress-tolerant fungal taxa was observed, including known pyrophilous fungi (Figure 3.12B), lichenized fungi (*Lecanorales*), lichenicolous fungi, and melanized, rock-inhabiting fungi, such as members of the genus *Phaeotheca* (Sterflinger, 2000), and desert-adapted taxa such as *Talaromyces* (Stolk *et al.*, 1965), *Hormonema* (Burford *et al.*, 2004) and *Preussia* (Rao *et al.*, 2016). *Glomeromycota*, a phylum containing arbuscular mycorrhiza, substantially expanded in harvested sites in BS_{ON} (undetected in REF and ~1% of total reads in OM3). A curated table of indicator taxa was prepared based on results from indicator analysis, representing taxa which responded most to harvesting (Table 3.1; full table in Table E.6). Indicator taxa with expanding populations were two-fold more common than taxa showing a decline in harvested plots. Fewer indicator taxa were observed for the mineral layer (n=38) than in the organic layer (n=51) and all but two showed patterns of expansion, whereas indicator taxa associated with organic layer showed relatively equal numbers of taxa indicative of expansion (n=24) or decline (n=27).

Figure 3.11. Dot plot displaying taxa containing OTUs indicative of harvesting treatments of bacteria (Panel A) and fungi (Panel B). The response ratio represents the average relative abundance of an OTU in all three OM treatments divided by the relative abundance in REF. OTUs are ordered from left to right by average abundance in harvested plots. All dots correspond to indicator OTUs with an indicator value > 0.5 and p-value <0.01. Dot area is scaled to average counts. A red dots indicates that the OTU met the criteria across all ecozones. Orange labels indicate members of the taxa have reported tolerance to radiation, desiccation and/or heat. Green labels indicate ecotomycorrhizal fungi (Panel B). OTUs are grouped by genus (x-axis).

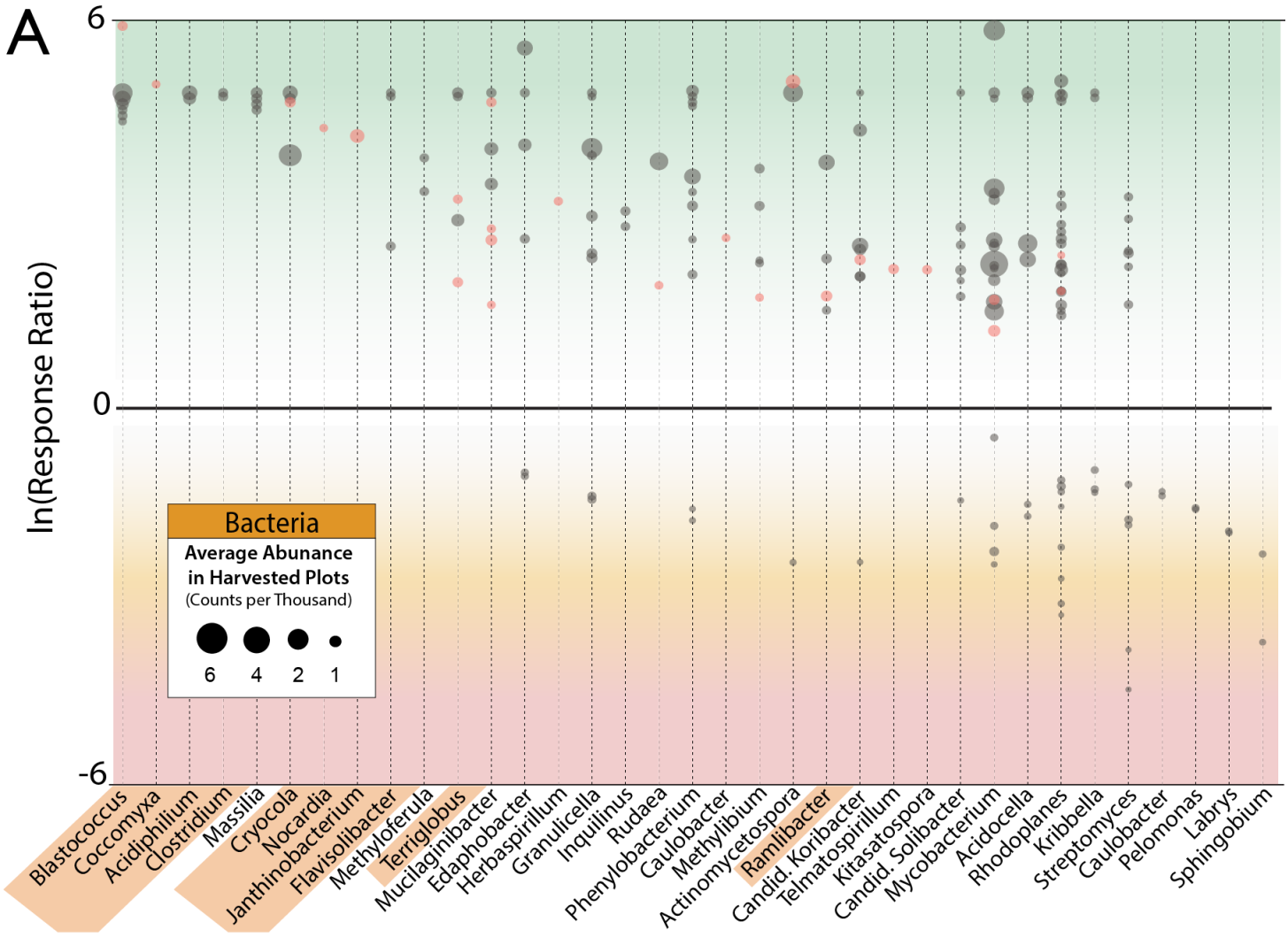


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Figure 3.11 *... continued.*

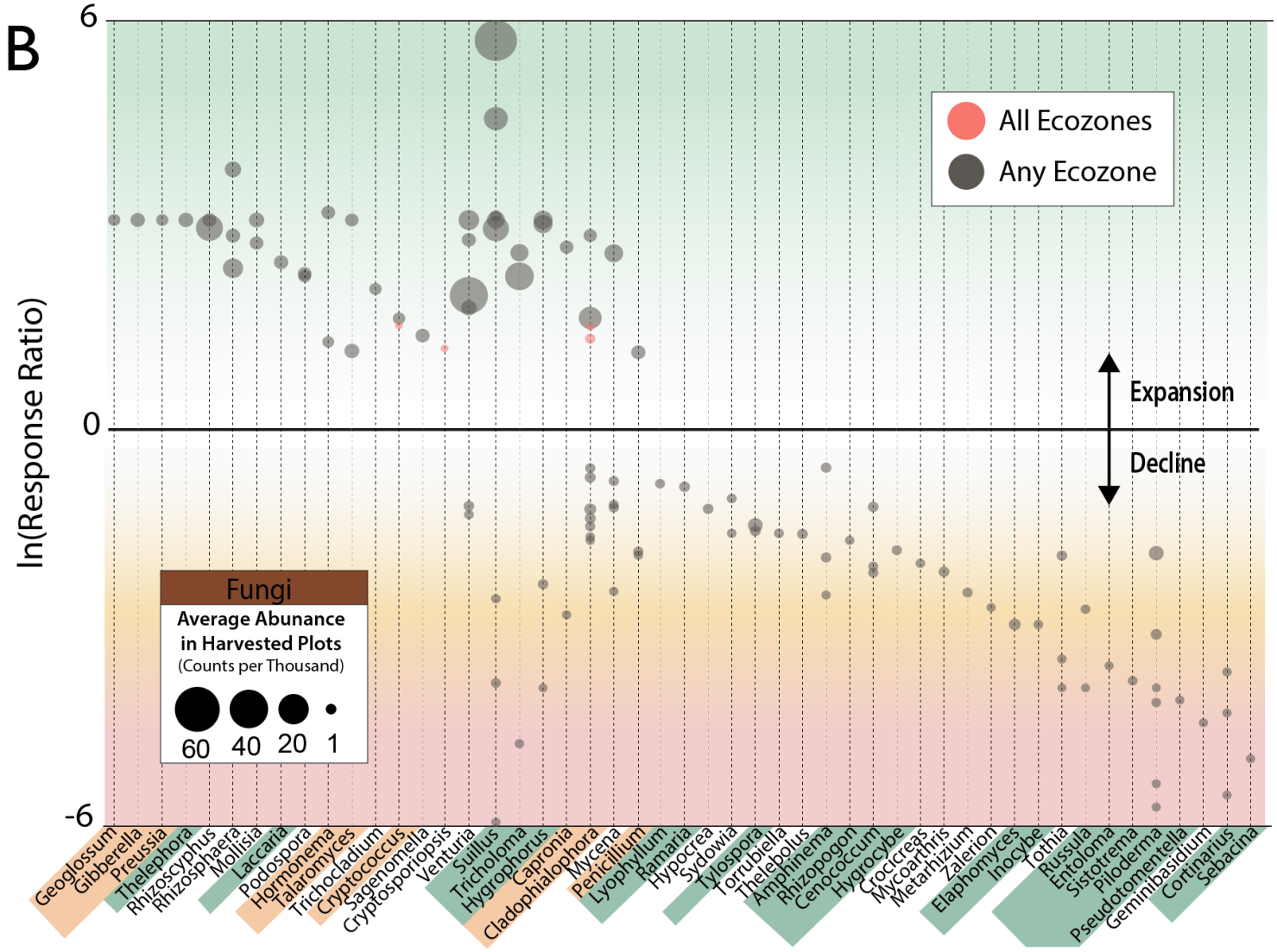

Figure 3.12. Expansion of stress-tolerant taxa evidenced by the increased abundances of phyla within the Terrabacteria group (*Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria* and *Firmicutes*) (A) and pyrophilous fungal genera (B). In (A), all phyla exhibited similar trends and were aggregated except for *Actinobacteria* which were plotted separately due to differences in response to harvesting at the family level. The following actinobacterial families exhibited declining populations (panel 1): *Actinospicaceae*, *Micromonosporaceae*, *Solirubrobacteraceae*, *Streptosporangiaceae*, *Thermomonosporaceae*, and *Streptomycetaceae*. The following actinobacterial families had expanded populations (panel 2): *Gaiellaceae*, *Geodermatophilaceae* and *Micrococcaceae*.

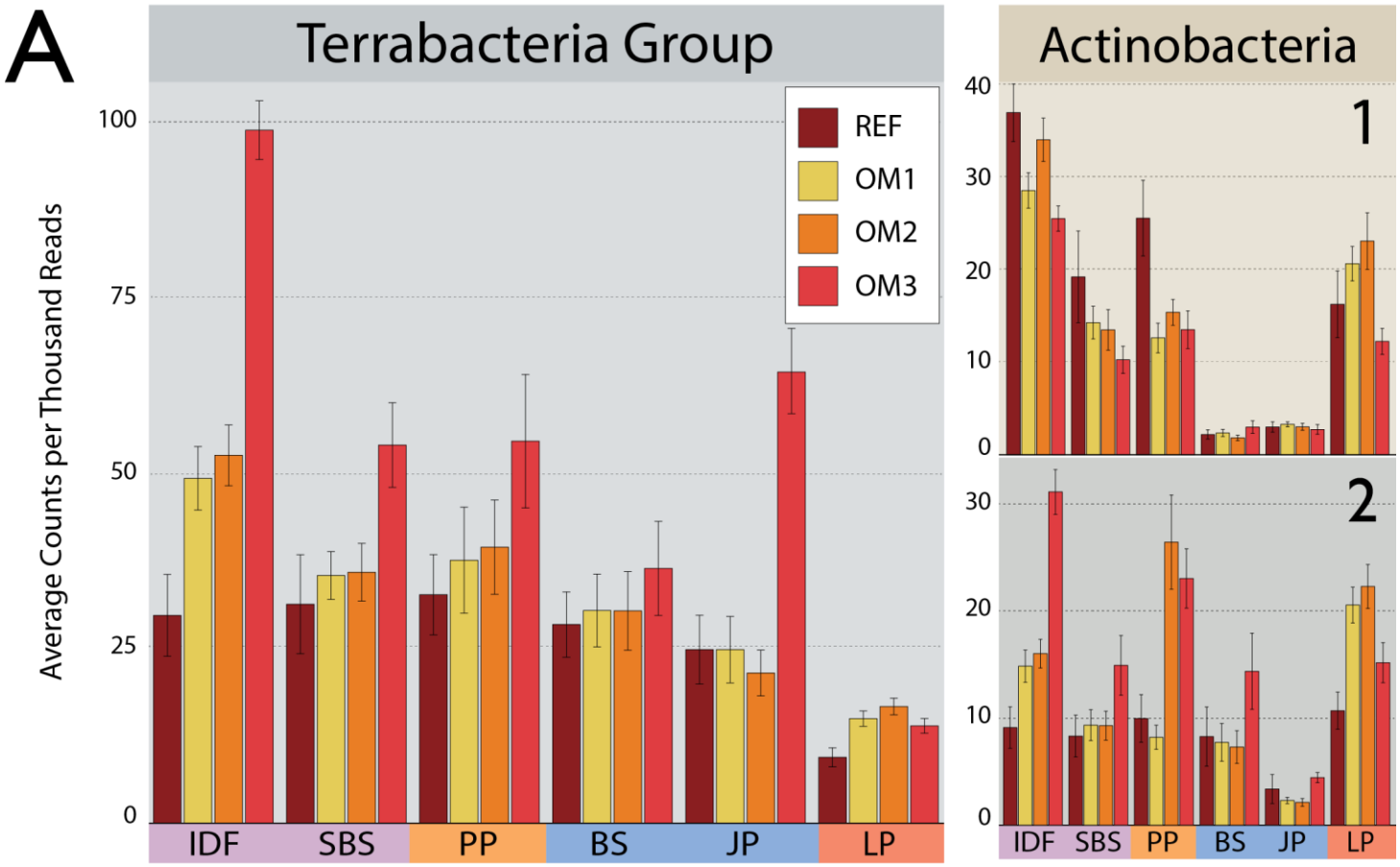

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Figure 3.12. *... continued*

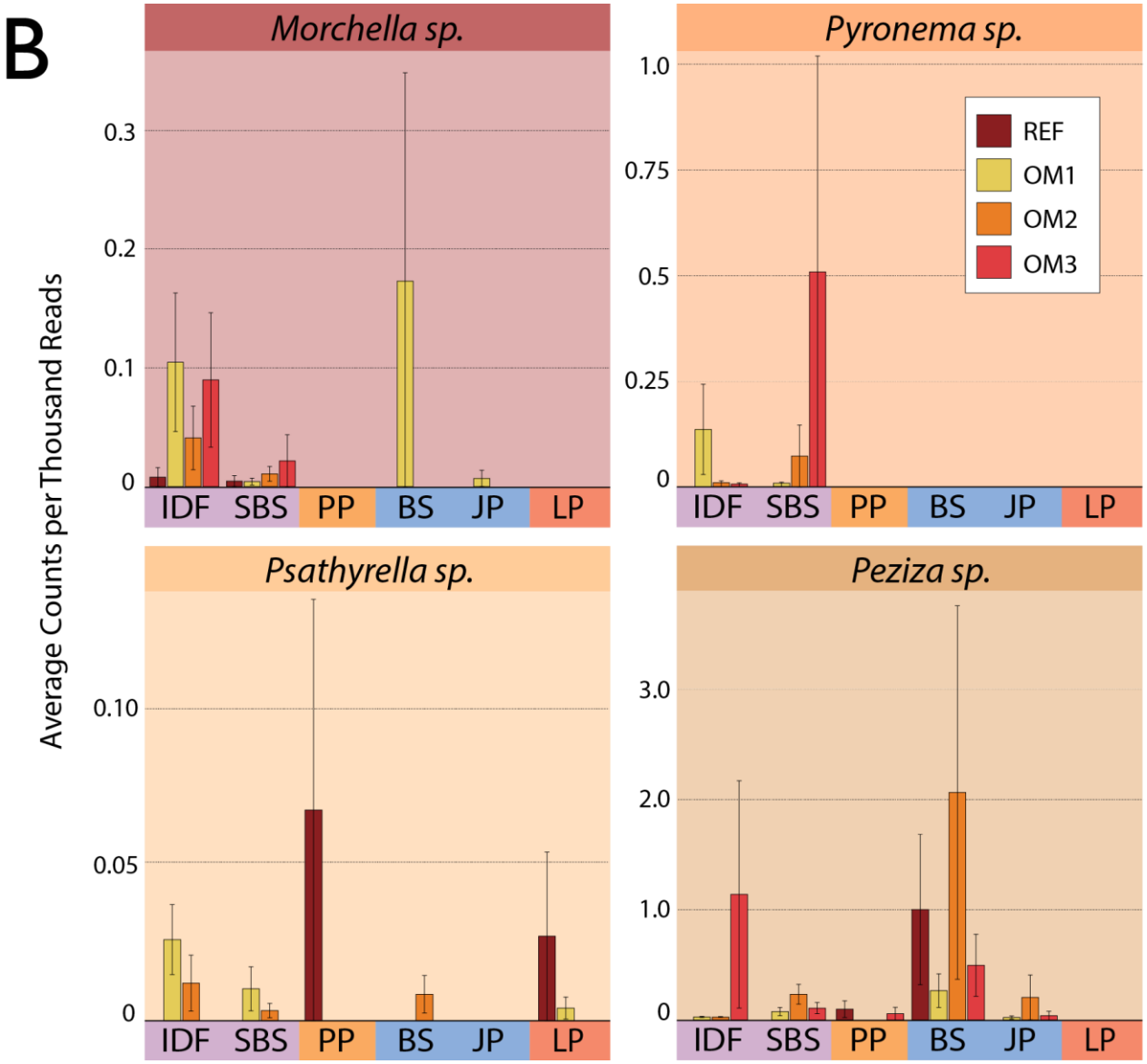


Table 3.1. A list of bacterial (A) and fungal (B) taxa that consistently expanded or declined in relative abundance in four or more ecozones in response to harvesting. Classification refers to the lowest possible classification with a bootstrap value > 80. Each classification is prefaced with its associated rank (i.e. "o__" corresponds to Order, etc.). Ecozones displaying trends are given. Mineral layer and organic layer-associations are noted by shaded squares. Horizontal barplots display the response ratio, which corresponds to the average abundance in OM3 divided by the average abundance in REF. Bars are coloured according to the maximum observed relative abundance of each taxon.

A

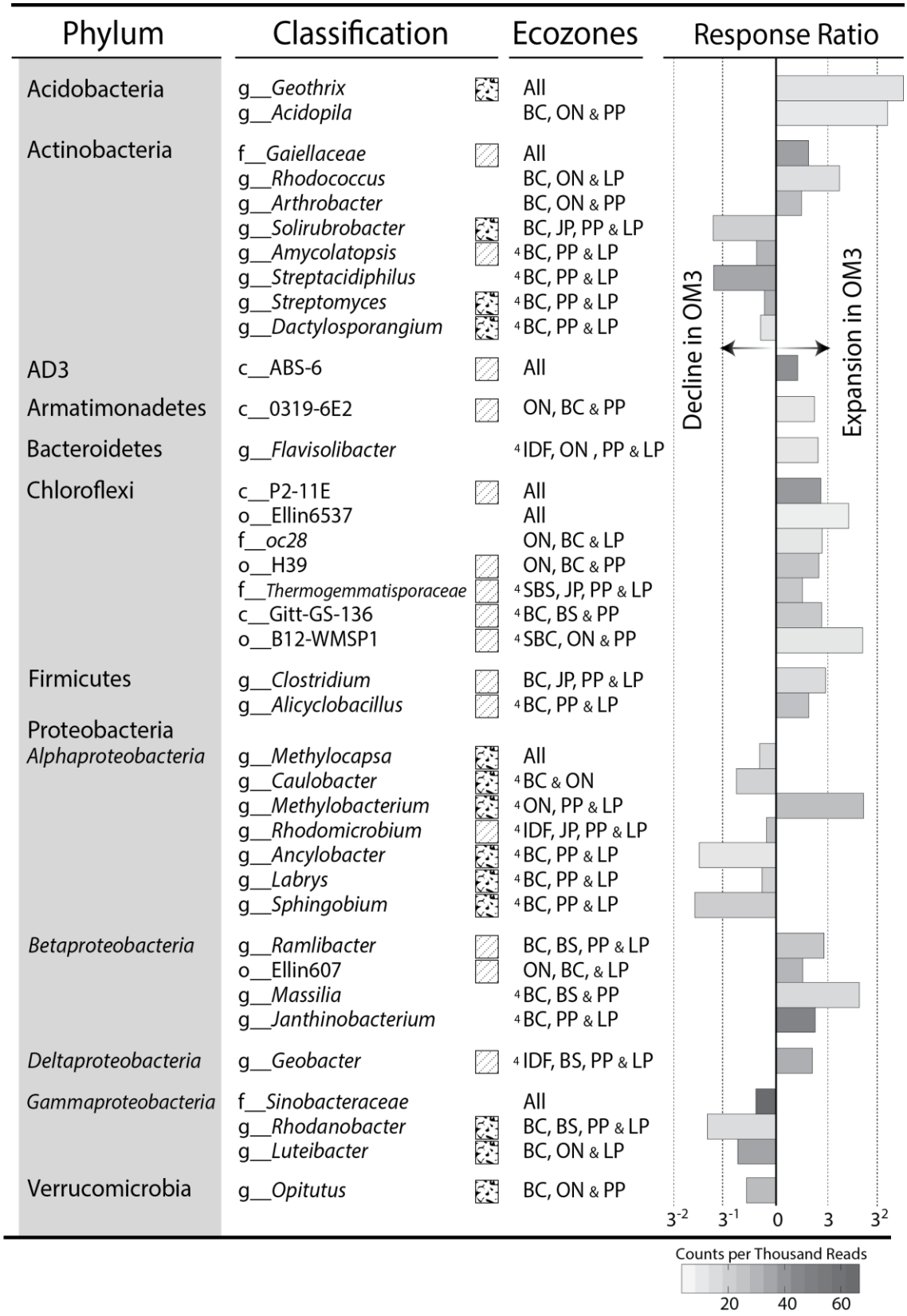
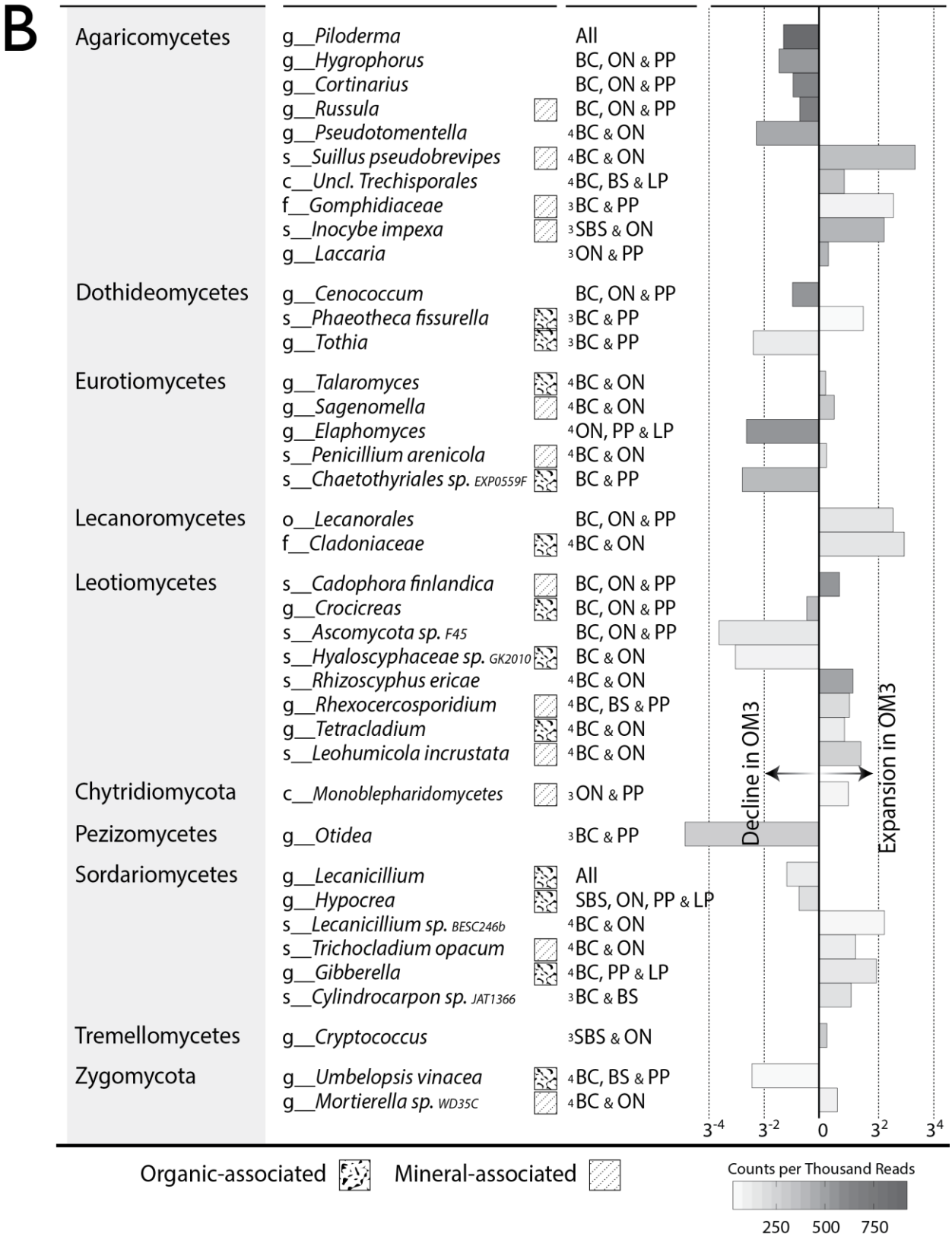


Table 3.1 *... continued.*



Declining relative abundances were observed in three or more ecozones for the following bacterial taxa: *Verrucomicrobia* (*Opitutus*), *Gammaproteobacteria* (unclassified *Sinobacteraceae*, *Rhodanobacter* and *Luteibacter*), *Alphaproteobacteria* (*Methylocapsa*, see Figure 3.13; *Rhodomicrobium* and *Ancylobacter*) and *Actinobacteria* (*Streptomycetaceae*, *Solirubrobacter* and *Dactylosporangium*). Fungi were more likely to be indicators of declining abundance (27/69) than bacteria (17/63). Ectomycorrhizal (EM) fungi were highly abundant (~10 – 50% of total ITS libraries) and far more likely to decline compared to known saprotrophic taxa (odds ratio 7.1, p=0.002). A majority of EM genera declined post-harvesting, such as *Russula*, *Cenoccocum*, *Cortinarius*, *Otidea*, *Piloderma*, *Hygrophorus*, and *Pseudotomentella*. However, some EM populations expanded in OM1 and/or OM2 (Figure 3.14) and a minority of EM taxa were consistently and heavily expanded in harvested plots, such as *Suillus* (3 to 6-fold increases), *Thelephora*, *Tomentella* and *Wilcoxina*.

The diversity of EM was generally reduced in harvested plots in both soil layers, though more so in organic layer soils. Diversity estimates based on OTU profiles or genus-level classifications both demonstrated a reduction in diversity of EM taxa in harvested plots, though the effect was more pronounced in classification-based estimations (Figure 3.15). All statistically supported differences exhibited decreases in the diversity of EM in harvested plots, except one instance in SBS_{BC} mineral soil where OM1.

Figure 3.13. Abundance patterns of the methanotrophic bacterial genus, *Methylocapsa*, exhibiting declining populations with increasing harvesting intensity. Error bars correspond to one standard error of the mean. *Methylocapsa* reads were originally classified as *Methylocella* using Green Genes, but further analysis of these sequences suggested they were more closely related to *Methylocapsa* based on best BLAST hits (97-98% match to NR_028923) as well as alignment and classification using both the Ribosomal Database Project and Silva 'All-Species Living Tree Project.'

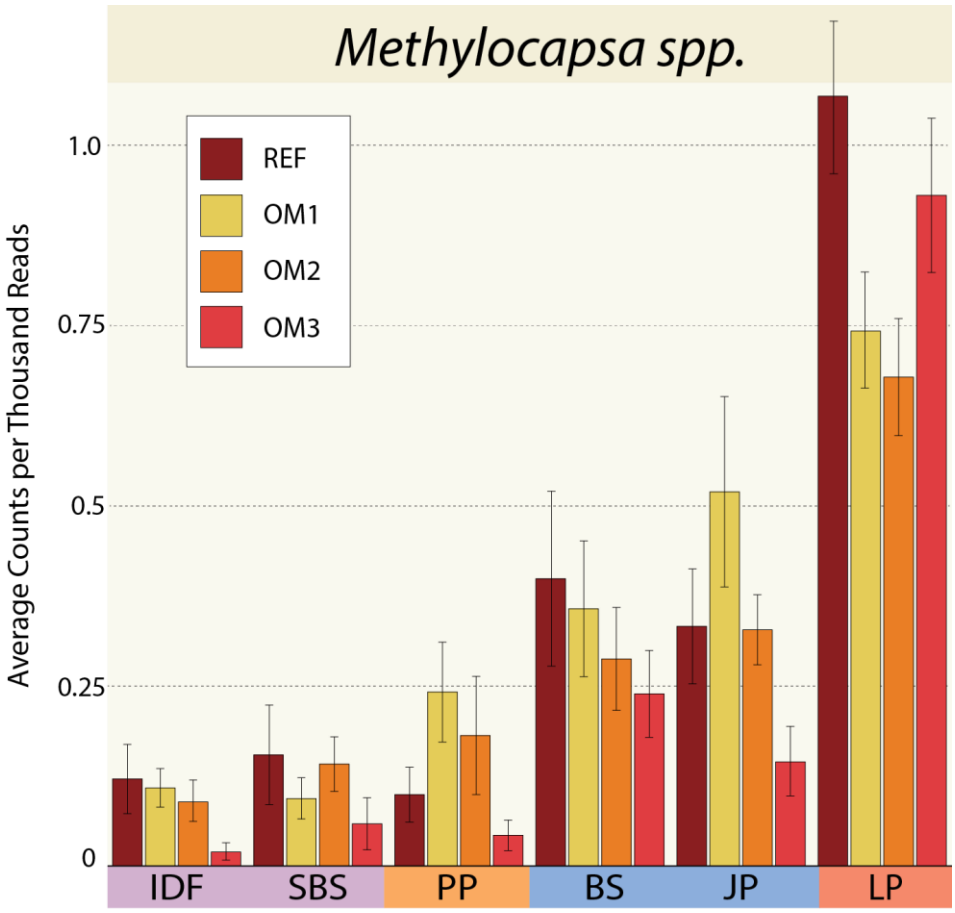


Figure 3.14. Relative abundances of ectomycorrhizal fungi according to soil layer, ecozone and harvesting treatment.

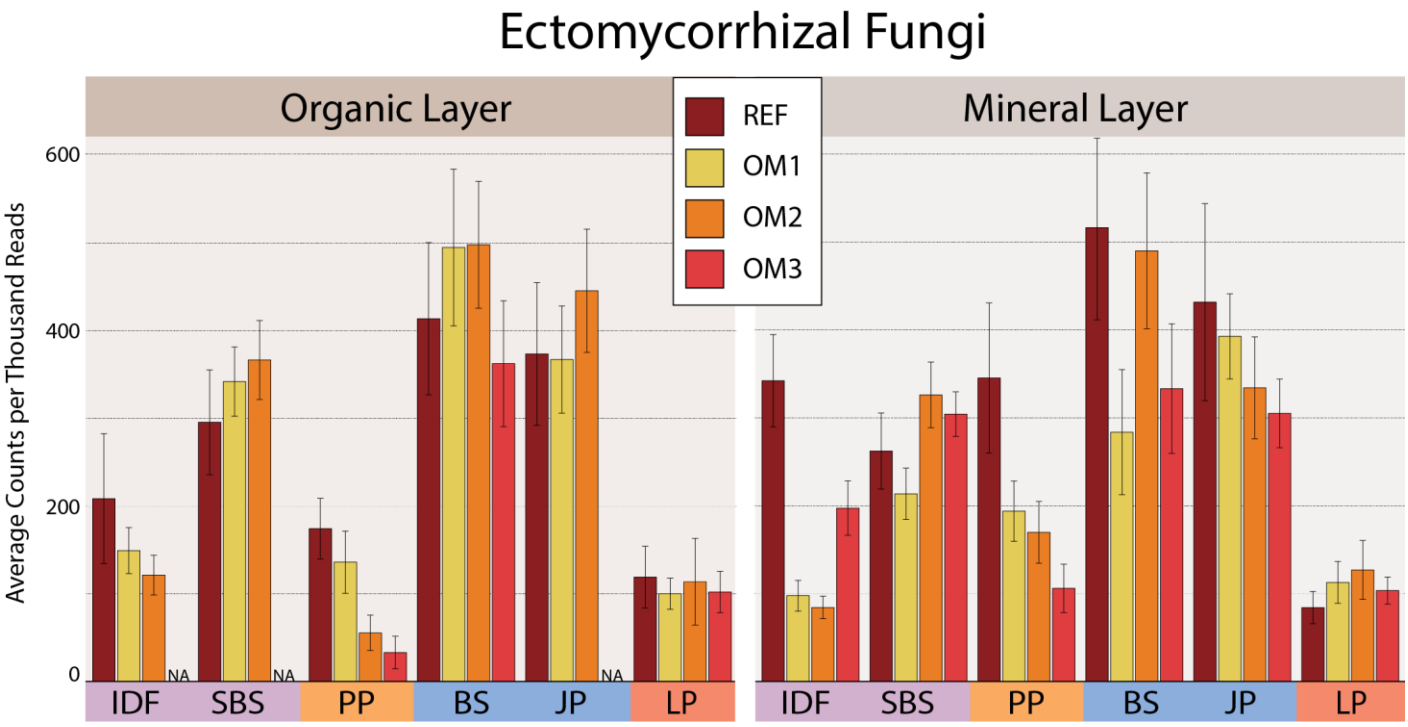


Figure 3.15. Box and whisker plots showing the average Shannon diversity estimate for all ectomycorrhizal fungi by soil layer, ecozone and harvesting treatments based on OTU profiles (Panel A) or genus levels classifications (Panel B). Estimations were based on an average of 100 calculations on data rarefied to equal sequencing depth. Statistically supported differences are grouped by lettering.

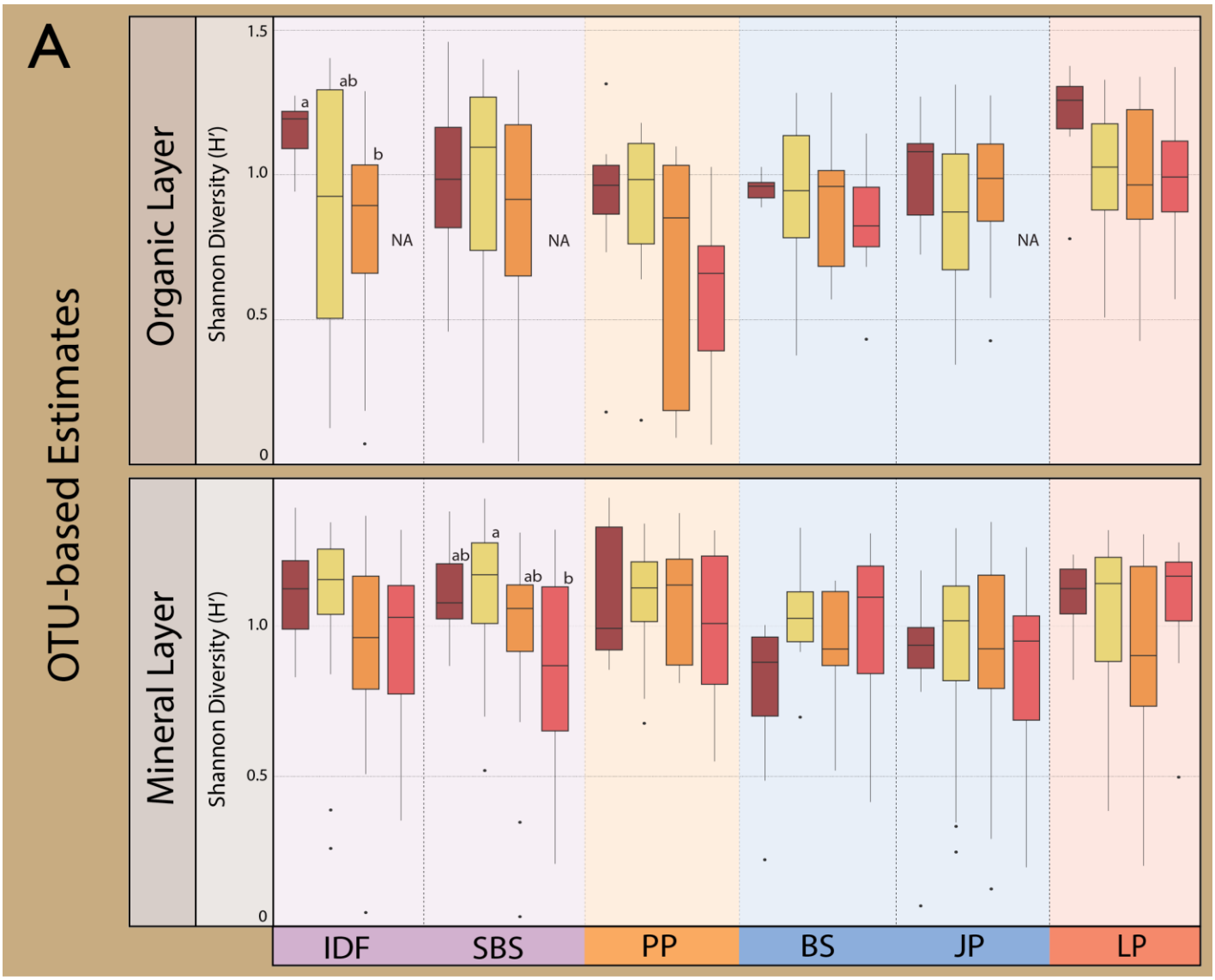
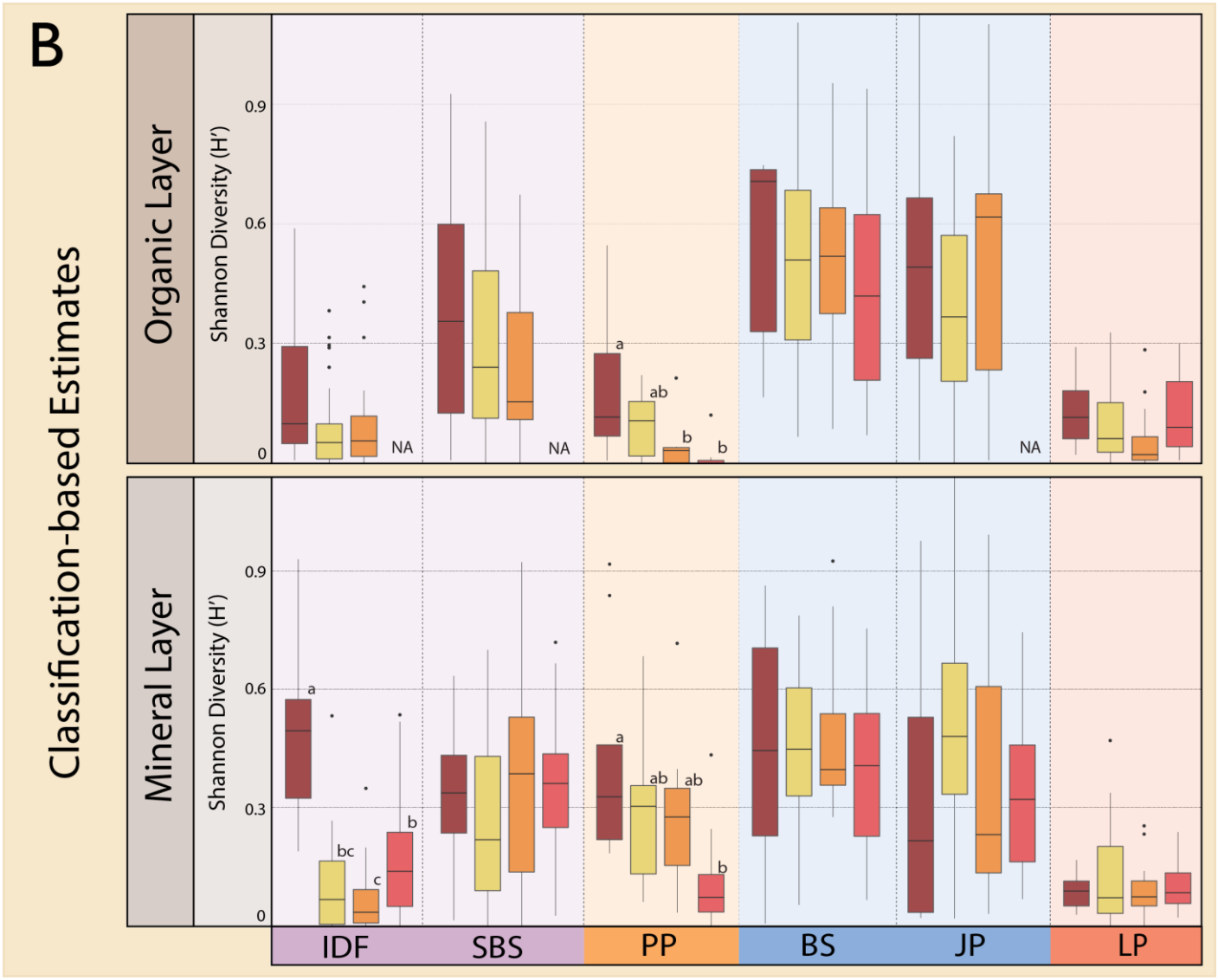


Figure 3.15 *... continued.*



Modeling of the abundance patterns (Figure 3.6) revealed that a sizeable proportion of OTUs exhibited higher relative abundance at intermediate levels of OM removal. Twenty-four fungal taxa and three bacterial genera were identified to be maximally abundant in OM1 or OM2 (Table 3.2) and all but one was more abundant in the organic layer. However, the trend in any given taxa was less consistent across ecozones in comparison to taxa indicative of expansion or decline. One EM species, *Thelephora sp. ECM1*, demonstrated a consistent increase at intermediate OM treatments in all ecozones except LP_{TX} (Figure 3.16A) and was positively correlated to C:N ratio ($r=0.25$, $p < 0.001$). Two other groups that were significantly correlated to C:N ratio showed predominance in OM1 and OM2 (Figure 3.16C and D): a group of unclassified *Agaricales* OTUs ($r=0.30$, $p < 0.0001$) and an OTU from *Dermataceae* ($r=0.36$, $p < 0.0001$). A species of unclassifiable *Agaricomycotina* demonstrated similar abundance patterns, but was not significantly correlated to C:N (Figure 3.16B). Each of these taxa exhibited the trend in both soil layers. The proportion of OTUs predominant at OM1 and OM2 was slightly higher among OTUs significantly correlated to C:N ratio, increasing by 4.4% and 3.5% in fungal and bacterial pyrotag libraries, respectively. A number of genera designated as 'wood saprotrophs' by FUNGuild had notably higher relative abundance in OM1 and/or OM2, including *Serpula*, *Coniophora*, *Gymnopilus*, *Perenniporia* and *Trechispora* (Figure 3.17).

3.2.4 Ecozone-specific Responses to Harvesting

The extent of ecozone-specific effects of harvesting was in line with the broad differences in community composition among ecozones. LP_{TX} shared the lowest overall overlap with other ecozones and, as one might expect, the fewest number of common taxa indicative of harvesting. In LP_{TX}, *Russula*, an EM fungus, expanded in OM3 in contrast to all other ecozones. Similarly, the EM genus *Amanita* was increased solely in harvested plots in LP_{TX} (Table E.6).

Table 3.2. A curated list of bacterial and fungal taxa showing population expansion with intermediate intensities of OM removal (i.e. OM1 and OM2) in contrast to REF and OM3 plots. Classification refers to the lowest possible classification with a bootstrap value > 80. Each classification is prefaced with its associated rank (i.e. "o__" corresponds to Order, etc.). Mineral layer and organic layer-associations are noted by shaded squares. Horizontal barplots display the response ratio, which corresponds to the average abundance in OM1 and OM2 divided by the average abundance in REF and OM3. Bars are coloured according to the maximum observed abundance of each taxon in any single library. For a complete list of indicator taxa see Table E.7.

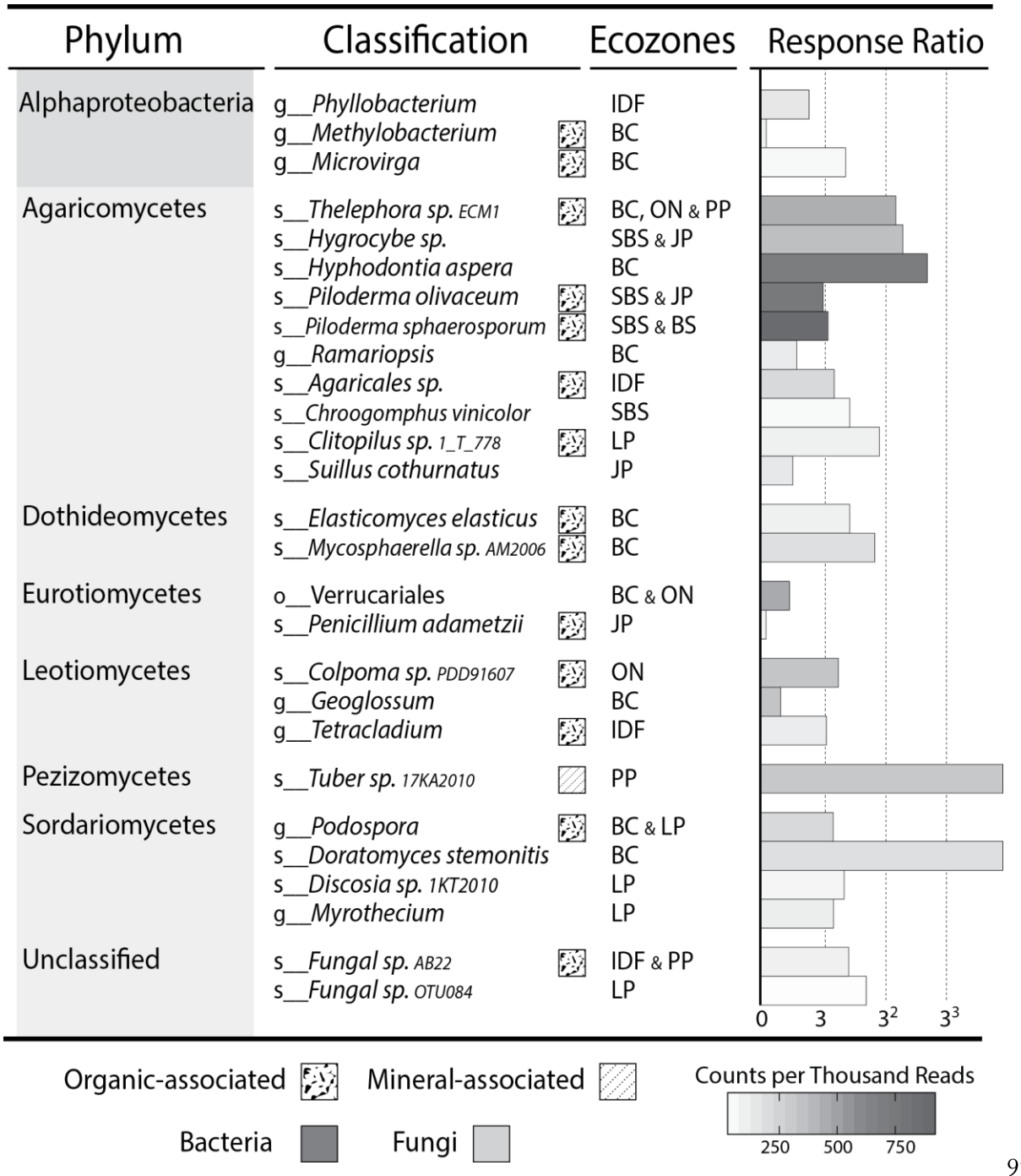


Figure 3.16. Abundance profiles of OTUs which displayed increased abundance at intermediate intensities of OM removal. Plots B and C correspond to a single OTU, while plots A and D correspond to 20 and 17 OTUs, respectively, all exhibiting the same pattern of abundance. Error bars correspond to one standard error of the mean. Plot E illustrates the phylogenetic relationship of OTUs classified as '*Thelephora sp. ECM1*' due to historic misclassification of putatively saprotrophic *Odontia* as *Thelephora* (sequences derived from Tedersoo *et al.*, 2014).

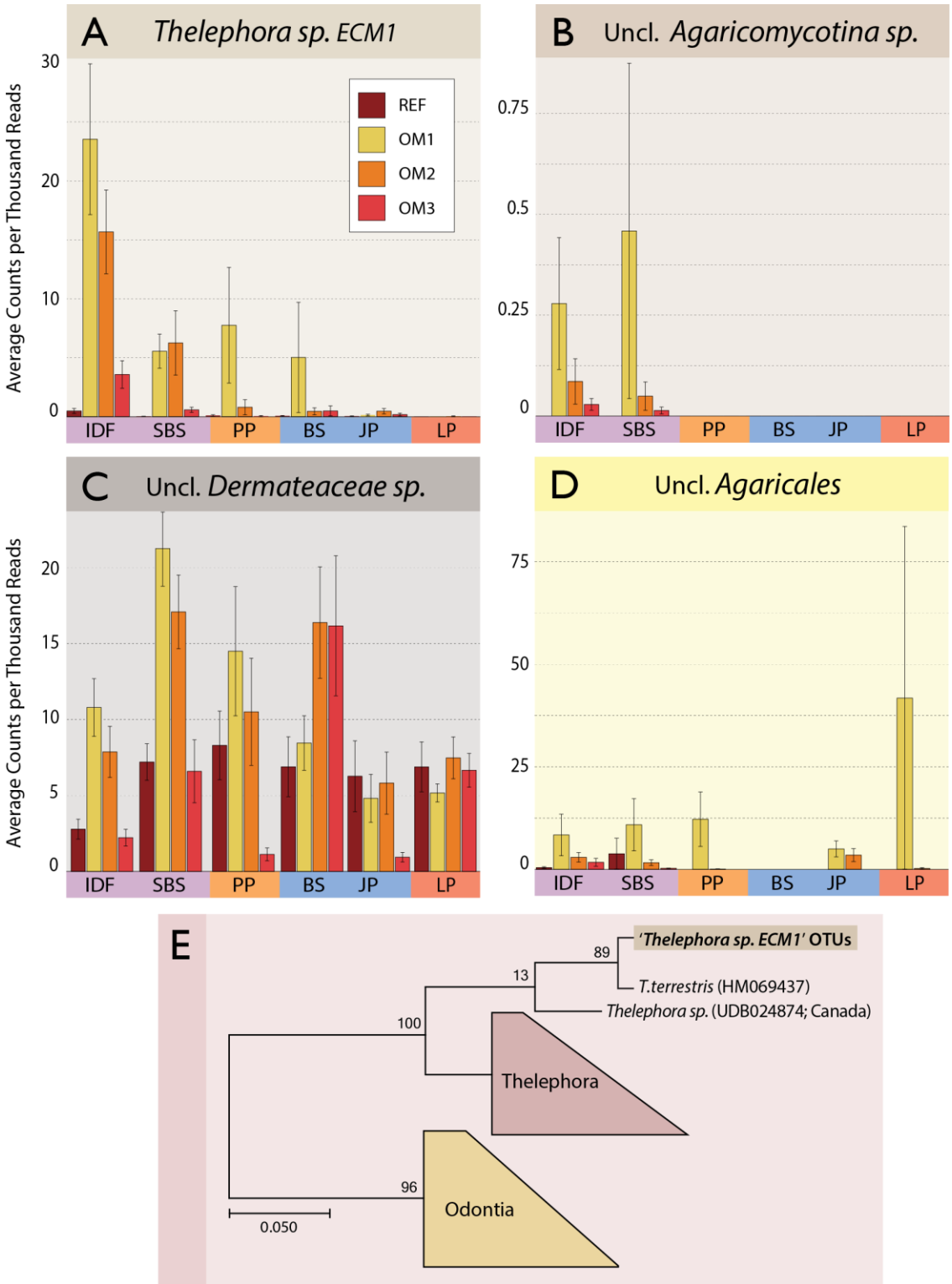
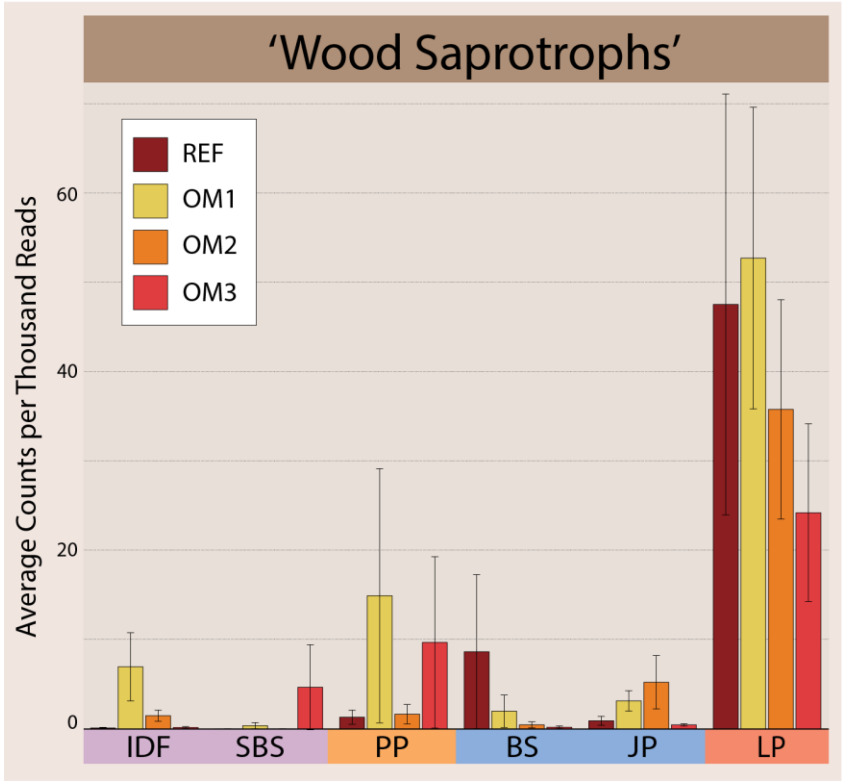
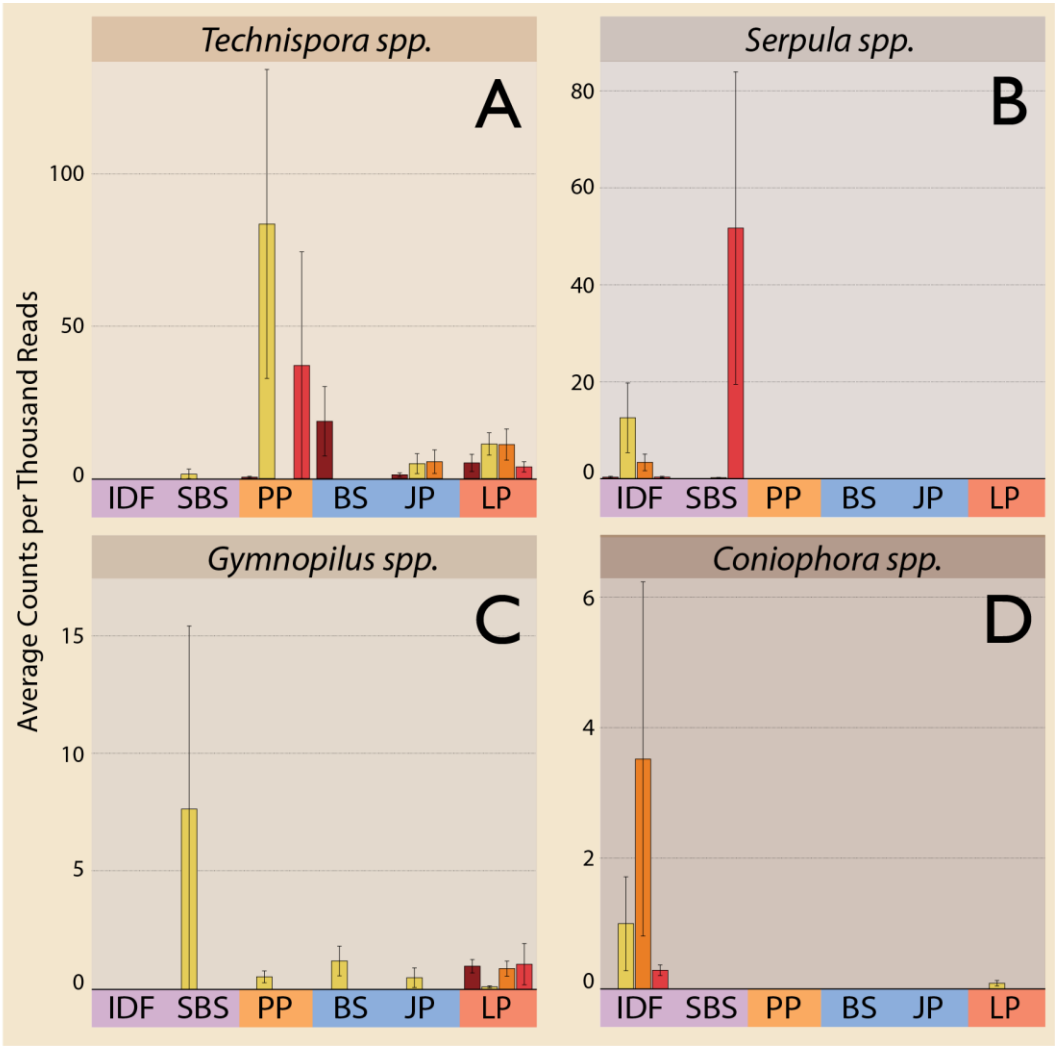


Figure 3.17. Aggregated abundances of all fungal genera designated 'wood saprotrophs' by FUNGuild, including plots for subsetted by genus for taxa associated with white rot (A), brown rot (B & D) or both (C). All exhibited characteristics of increased abundance in plots were a degree of organic matter has been retained.





Similarly, a number of bacterial indicator taxa were exclusive to PP_{CA}, such as *Spirosoma* and *Rubrobacter,* which have cultured representatives with extreme tolerances to radiation (Ferreira *et al.* 2009; Lee *et al.* 2014). The expansion of unclassifiable *Syntrophobacteraceae* (*Deltaproteobacteria*) was a strong feature in IDF_{BC}, and, to a lesser extent, in the SBS_{BC}, with two-fold greater relative abundance in harvested plots (TukeyHSD; $p < 0.001$).

Common responses were typical of proximal ecozones presumably reflecting the similarity of environmental conditions (Figure 3.18). However, a number of taxa exhibited contrasting responses along a North-South axis, such as *Terriglobus* (bacteria) and *Cladophialophora* (fungi) which declined in B.C., but expanded in PP_{CA} and LP_{TX} (Figure 3.19). Conversely, fungi from the order *Boletales*, driven by increases of *Suillus* and *Rhizopogon*, expanded in northern and declined in southern harvested plots. Northern and southern sites exhibited marked differences in soil temperature, precipitation and soil moisture (Table E.5). Harvested plots in northern ecozones had slightly more basic soil pH, a trend not observed in southern sites. Other populations exhibited an East-West divide with some taxa exclusive to eastern (*Cupriavidus* and candidate phylum *GAL15*) or western sites (*Limnohabitans, Nostoc* and *AKIW781*) and other taxa exhibiting contrasting responses according to geography, such as *Rudaea*, *Kitasatospora* and members of *Atheliales* (Figure 3.20).

A number of bacterial and fungal taxa exhibited patterns of both expansion and decline in response to harvesting (Figure 3.11). For a subset of these taxa, the variation could be attributed to different responses among closely related species. For example, fungal OTUs within the EM genus *Suillus* that increased in harvested plots formed a distinct clade (along with *S. variegatus*, *S. luteus* and *S. pseudobrevipes*) separate from OTUs that declined (grouping with *S. lakei* and *S. caerulescens*; Figure 3.21). *Rhizopogon* exhibited similar species-level differences in response to

harvesting, notably according to northern and southern location (Figure 3.22). Species of *Kitasatospora* also exhibited different responses, which also differed in their distribution in eastern and western ecozones (Figure 3.21). Two EM genera within the same family, *Tomentella* and *Pseudotomentella*, also exhibited different responses to the impacts of timber harvesting (Figure 3.23). However, the majority of OTUs within taxa that showed both declining and expanding populations did not display a clear phylogenetic basis for their abundance pattern.

Figure 3.18. Non-parametric multidimensional scaling of bacterial (left) and fungal (right) pyrotag libraries based on Bray-Curtis dissimilarities. Individual OTUs were mapped as black crosses (~44, 000 bacterial and 16,000 fungal OTUs). Samples were mapped as coloured circles. Samples from the organic layer in PP_{CA} and LP_{TX} were not mapped due to incomplete environmental data. Bacterial samples exhibit a clear split between organic (top) and mineral (bottom) layers. Experimental factors were fitted to ordination with the length of the arrow proportional to the correlation between variable and ordination.

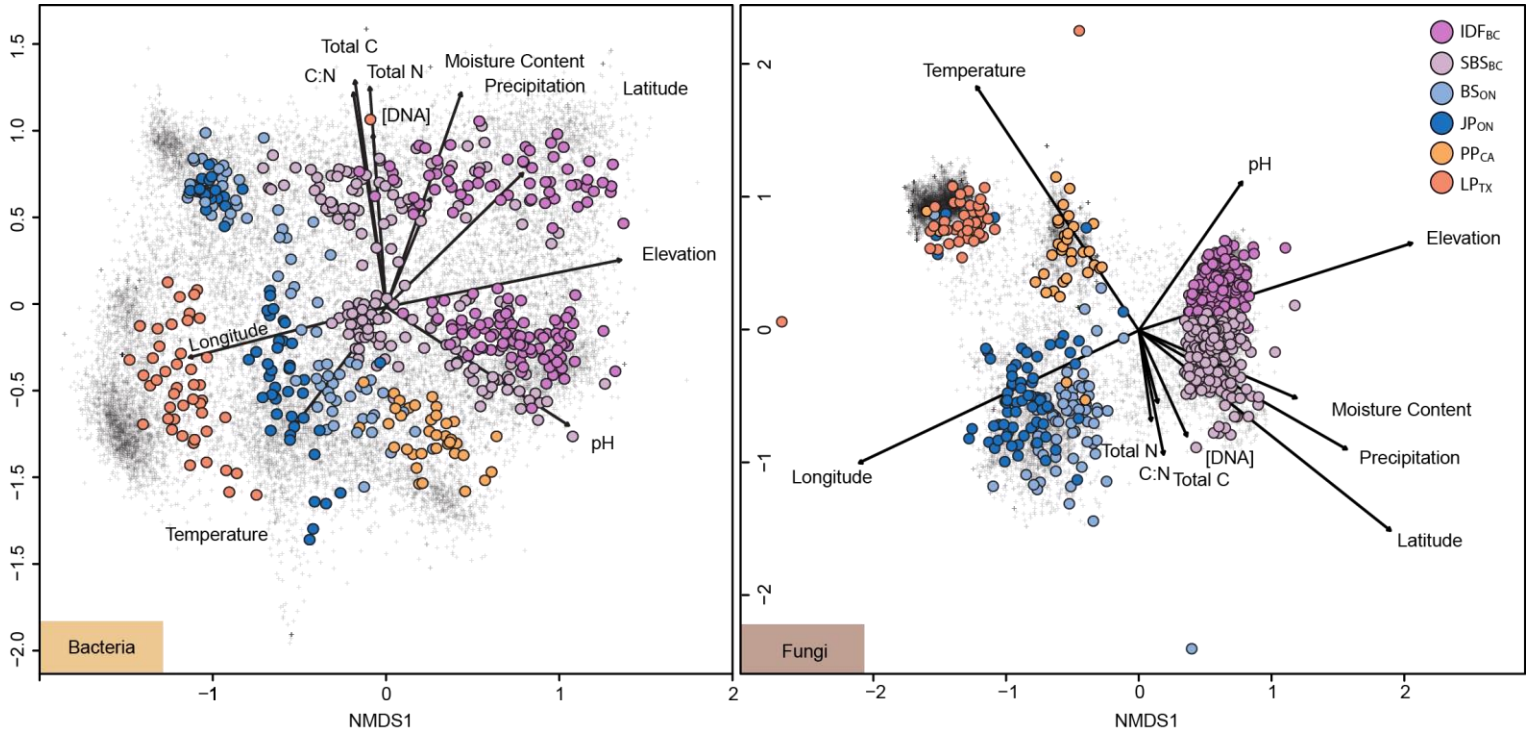


Figure 3.19. Abundances of four taxa with differing responses to harvesting (i.e. expansion or decline) between northern and southern sites. OM removal treatments are represented by conventional colours. Y-axis corresponds to average counts per thousand reads and error bars depict one standard error of the mean. For a more detailed phylogenetic breakdown of OTUs within genus *Rhizopogon*, consult Figure 3.22.

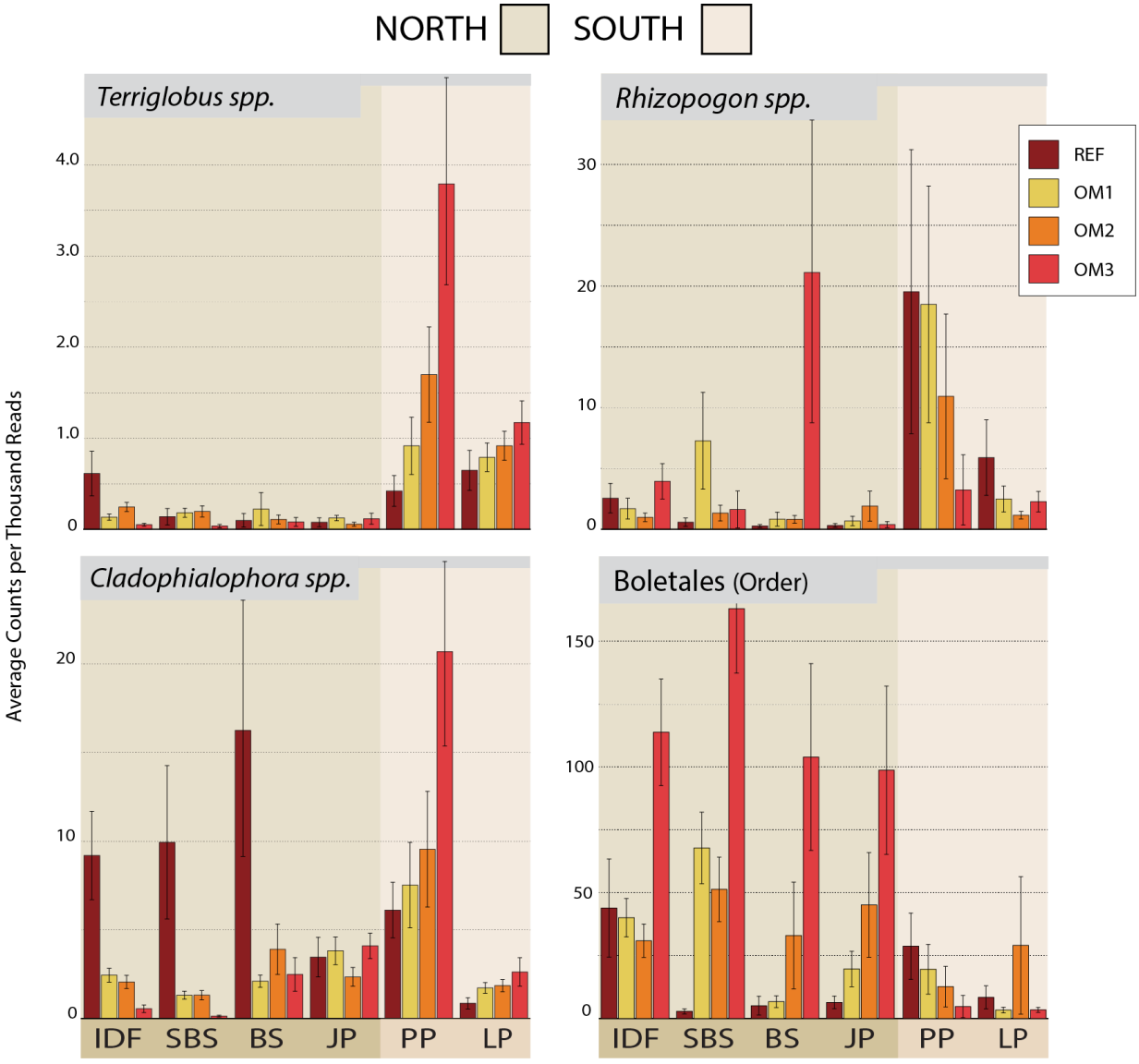


Figure 3.20. Abundance patterns for eight taxonomic groups exhibiting contrasting responses to harvesting (i.e. expansion or decline) between western and eastern sites. Error bars depict one standard error of the mean. The order AKIW781 is within the phylum *Chloroflexi*.

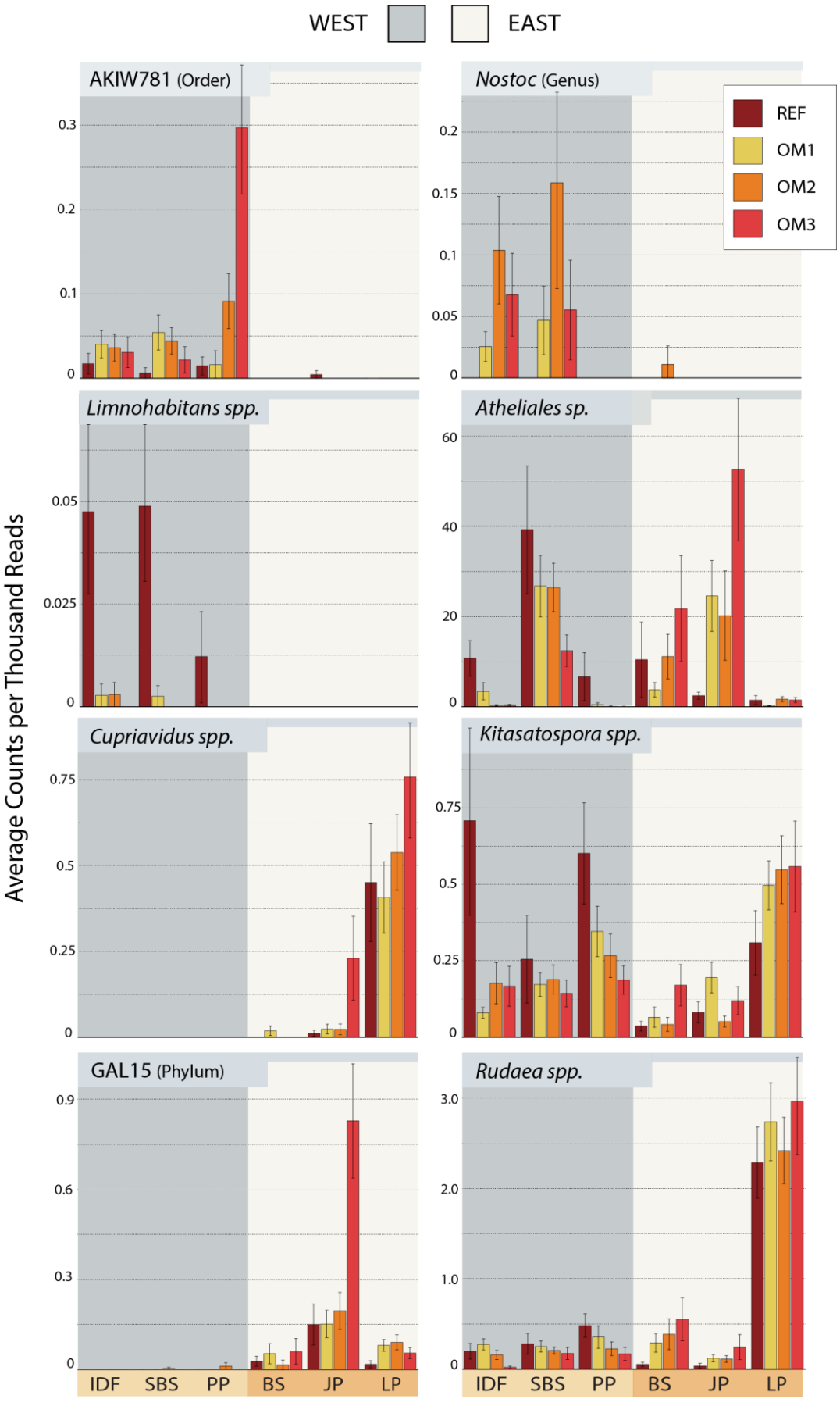


Figure 3.21. Phylogenetic tree of fungal genus, *Suillus*, and bacterial genus, *Kitasatospora*, accompanied by their abundances across OM removal treatments and ecozones. Inset barplots show the total counts for each genus, while barplots along the right-hand side show counts for each individual phylogenetic clades (marked with star). For simplification, the Y-axis of the right-hand plots, corresponds to percent relative abundance. On average, *Suillus spp*. accounted for 5% of ITS reads per library, while *Kitasatospora spp.* accounted for an average of 0.05% per library. Aligned sequences were trimmed to 355 bp (*Suillus*) and 250 bp (*Kitasatospora*) prior to tree building.

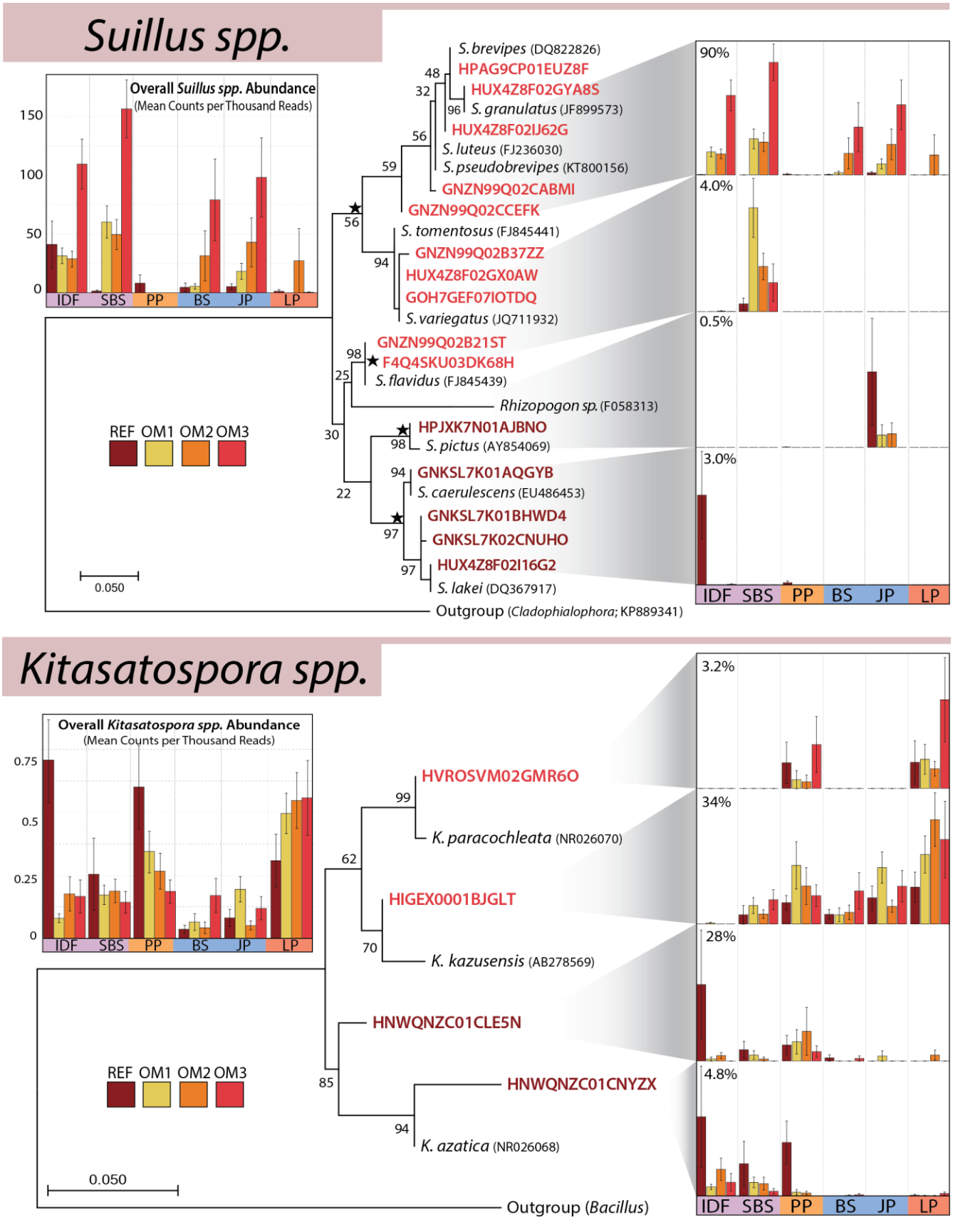


Figure 3.22. Phylogenetic tree of fungal genus, *Rhizopogon*, accompanied abundance by data across OM removal treatments and ecozones. The inset bar plot shows the relative abundance of all reads classified as *Rhizopogon*, while barplots along the right-hand side correspond to the percent abundance of individual phylogenetic clades. These 10 OTUs account for 82% of all reads of the 51 OTUs classified as *Rhizopogon*. On average, *Rhizopogon spp*. accounted for 0.7% of ITS reads per library. Aligned sequences were trimmed to 420 bp prior to tree building.

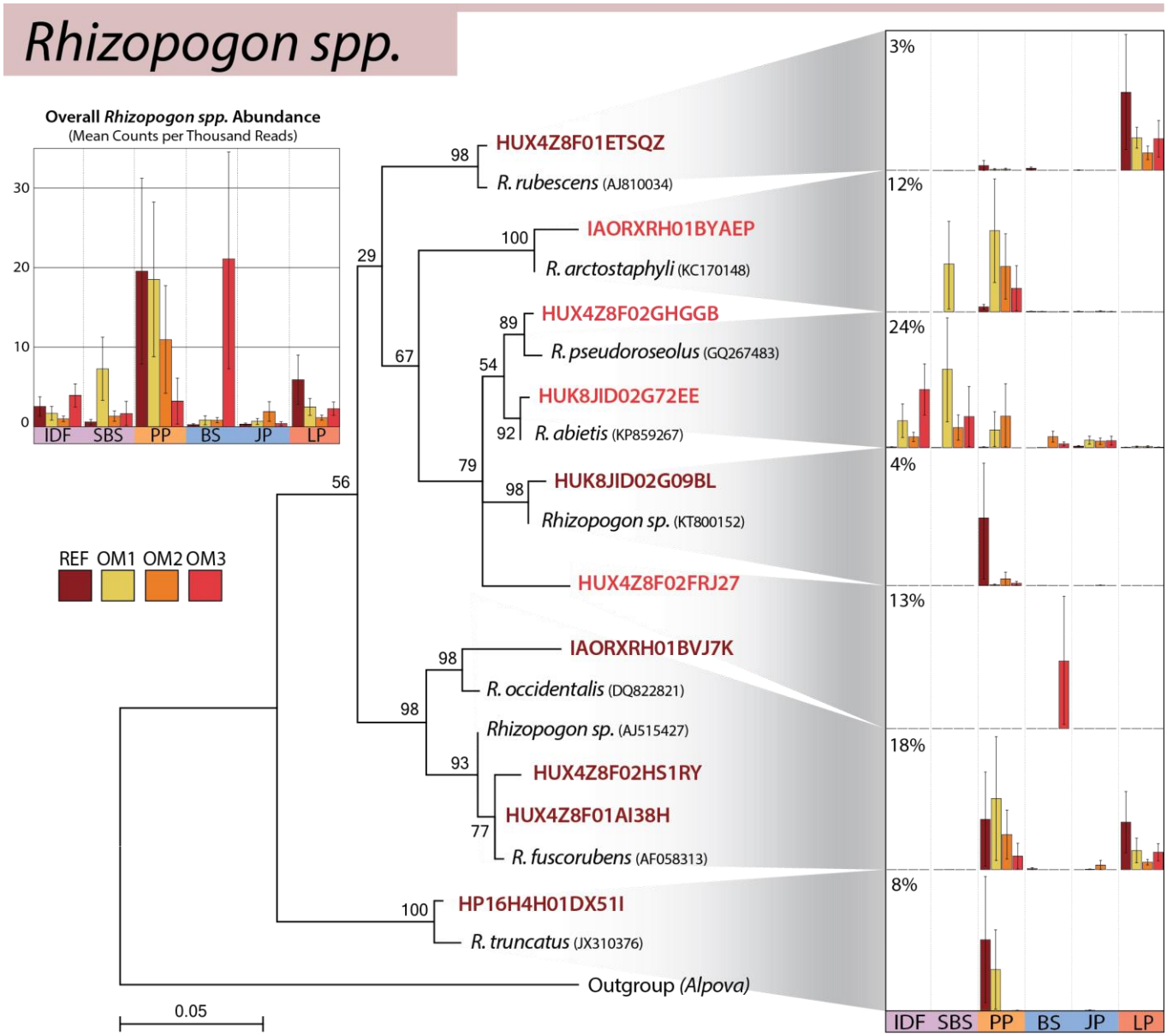
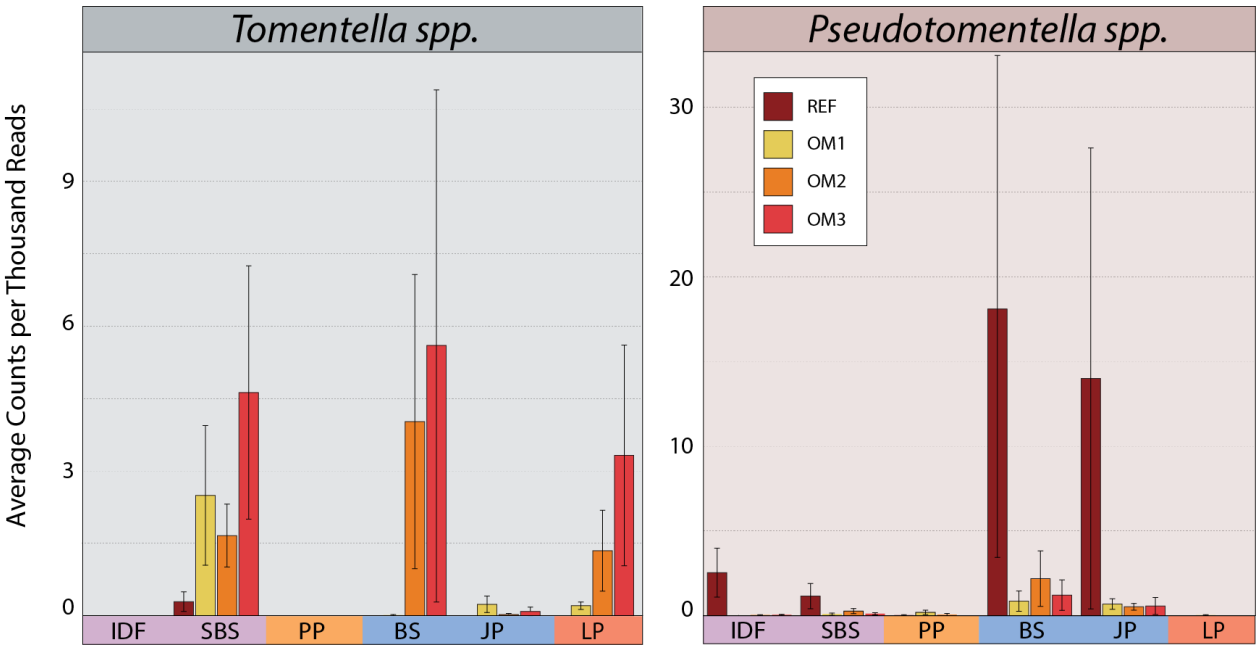


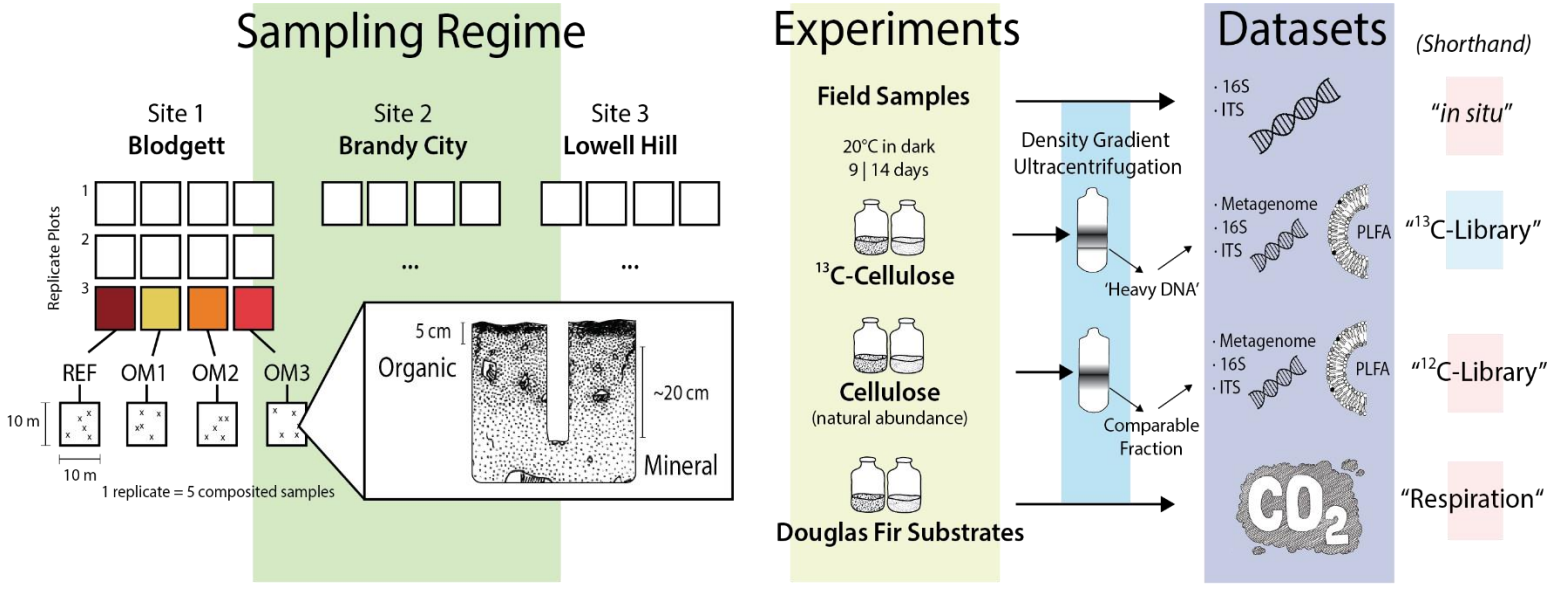
Figure 3.23. Differential responses to harvesting in two closely related ectomycorrhizal fungi from the family *Thelephoraceae*: *Tomentella* and *Pseudotomentella*. Error bars depict one standard error of the mean.



3.3 Impacts of Harvesting on Cellulolytic Populations

Figure 3.24 provides an overview of the sampling regime, experiments and data collected for analysis in Section 3.3. A total of 48 bacterial 16S rRNA gene and 46 fungal ITS region pyrotag libraries (half ^{13}C- and half ^{12}C-libraries) plus pyrotag libraries from PP_{CA} (section 3.2), 72 PLFA samples, 108 respiration assays, and 4 metagenomes were analyzed in this sub-chapter. All raw sequencing data described in Section 3.3 can be retrieved from the European Nucleotide Archive under the study accession (PRJEB9761) for 16S rRNA gene (ERS803692-ERS803739) and ITS pyrotag libraries (ERS803740-ERS803786), draft genomes (ERZ288956 - ERZ288966) and metagenomic libraries (ERS1099581- ERS1099584). All additional data, including sample metadata, other raw datasets, and representative FASTA sequences for putative cellulolytic taxa can be found in the Supplementary Data.

Figure 3.24. Overview of the samples, experiments and datasets utilized in studying the effects of timber harvesting on the cellulolytic community.



3.3.1 Effects of Harvesting on Respiration

The first test of whether timber harvesting affected microbial activity in PP_{CA} was to measure net respiration among treatments with or without the addition of Douglas-fir lignocellulosic substrate. Despite considerable variability, respiration in mineral soils was significantly reduced by harvesting (ANOVA, $p < 0.01$), but was not different between soils from OM1 and OM3 harvesting treatments (Figure 3.25). Respiration was greatest with the addition of raw lignocellulose, while amendments of 'cellulose + lignin' and cellulose-only supported similarly low level increases in respiration. Respiration in cellulose-amended soil from OM3 was particularly low, suggesting that cellulose-degrading populations were disproportionately affected by OM-removal ($t=-2.65$; $p=0.009$). Respiration was positively correlated with pH ($r=0.34$; $p < 0.001$) and weakly with the total carbon content of soil ($r=0.19$; $p=0.049$), but not with the total nitrogen content or C:N ratio. Organic layer soils respired 3-fold more CO_2 than mineral soils, but no significant harvesting treatment differences were observed in organic layer soils due to its highly variable background respiration, which confounded the detection of differences between microcosms with and without added substrate.

3.3.2 Characterization of SIP-Enrichment for Cellulose and Lignin

Prior to testing for the impacts of harvesting, time-course experiments were performed to characterize the rate and quality of ^{13}C-incorporation from labeled cellulose. Low-level enrichment was detected as early as four days into the incubation (Figure 3.26) and rose gradually with time (more details in Section 4.2.1). Despite the low amount of ^{13}C relative to ^{12}C (δ-^{13}C) in PLFAs from organic soils, the total amount of ^{13}C incorporated was comparable to mineral soils. Both DNA-based and PLFA-based quantitative methods revealed similar trends in enrichment (Figure 3.27).

Figure 3.25. Dot-plot showing soil respiration in mineral soils from reference and harvested treatments (coloured lines are averages; n=9). Dot area is scaled to carbon to nitrogen ratio of individual soil samples. In all cases, respiration was significantly different between reference and harvested plots (t=16.9; p < 0.01), but not between OM1 and OM3. An arrow depicts the interaction between OM3 and respiration on cellulose, which was statistically supported (t=-2.65; p=0.01).

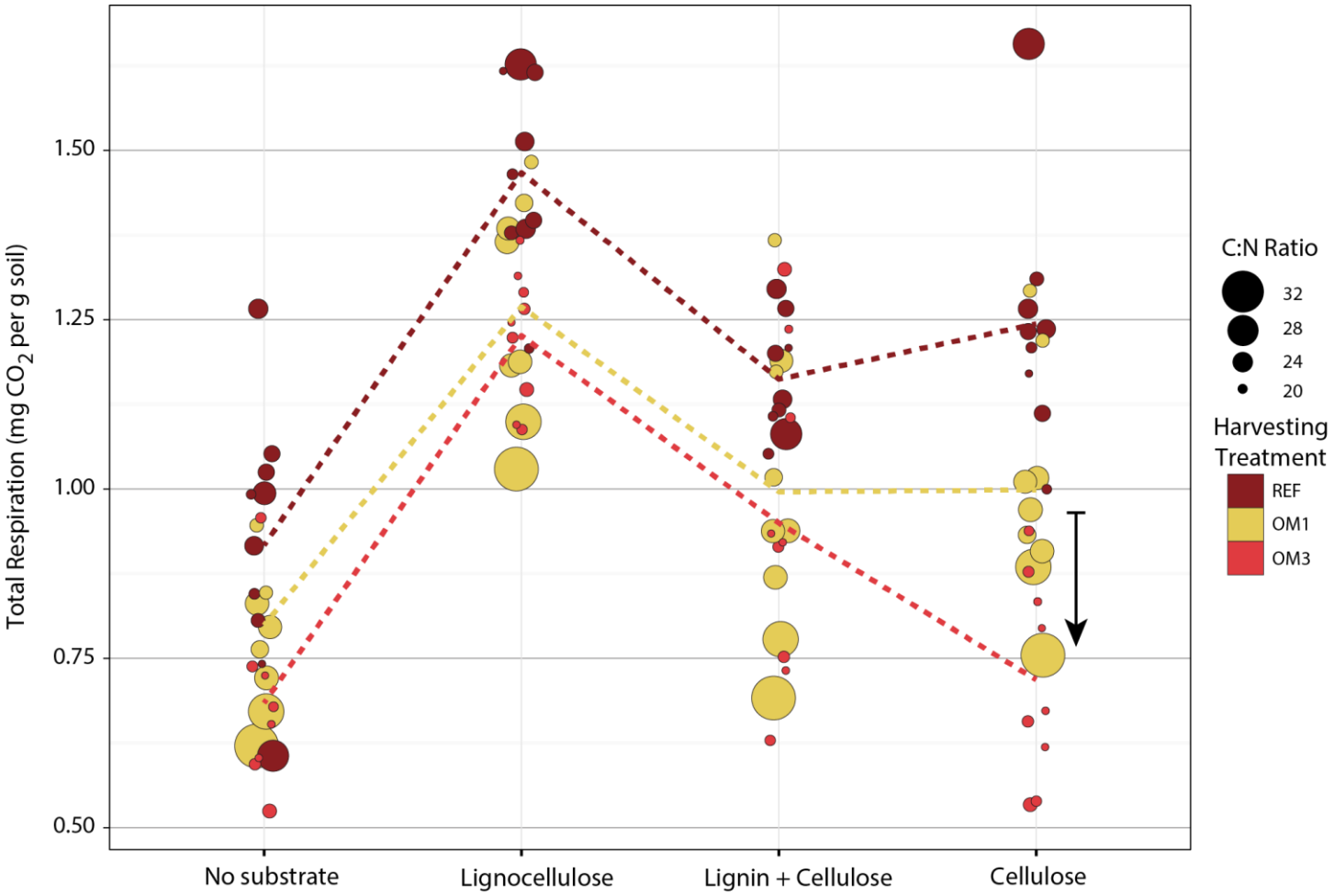
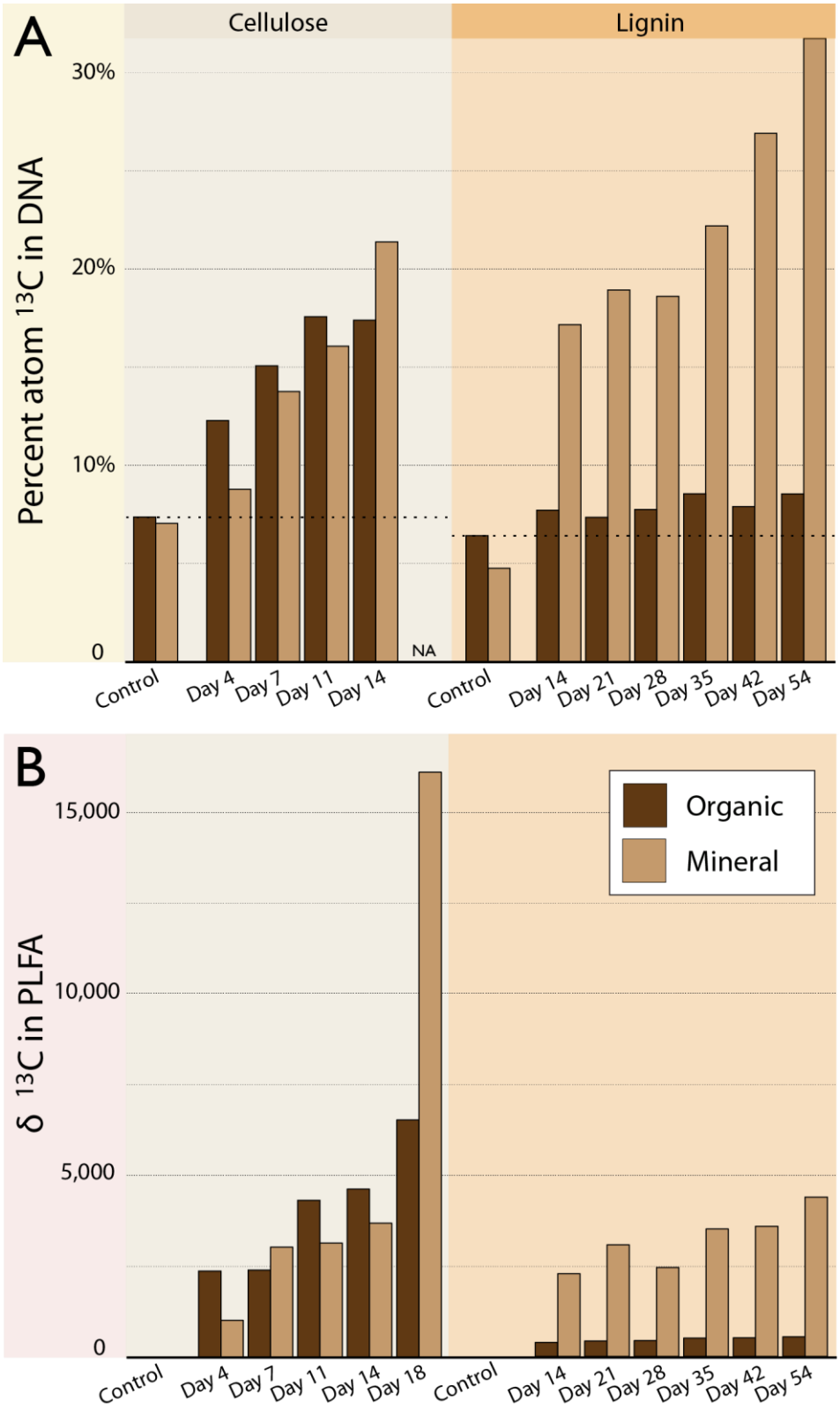


Figure 3.26. Time-course assay of enrichment of organic and mineral layer soils from LP_{TX} revealed by measurements of ^{13}C-incorporation into DNA (A) and PLFA biomass (B) by both cellulose and lignin substrates. Microcosms were sacrificed at each time-point rather than sub-sampled (n=1 for all time points). Natural abundances of ^{13}C in DNA are indicated by dotted lines.



3.3.3 Harvesting Effects on Cellulolytic Activity

The incorporation of ^{13}C into PLFAs was generally lower in soil from harvested plots (Figure 3.27; Table E.9). The trend was clearest in mineral soils, where REF had significantly higher δ-^{13}C enrichment in PLFAs and DNA than harvested plots; though, in organic soils, OM2 had the highest levels followed by REF. The total number of PLFAs showing ^{13}C-enrichment above natural abundance was reduced in harvested plots in both soil layers (Table E.9), though only statistically supported in the organic layer (Figure 3.28A; TukeyHSD, $p < 0.01$). Bacterial cellulolytic activity was higher in unharvested soils exemplified by the lower ratio of fungal to bacterial PLFAs relative to all harvested plots in both soil layers (Figure 3.28B; TukeyHSD, $p < 0.01$). This trend was evident in both ^{12}C and ^{13}C PLFA profiles, driven by an increase in fungal biomass in OM3 (1.8-fold higher than REF) as well as higher amounts of Gram-positive and Gram-negative bacterial biomass in the unharvested treatments, 1.4-fold and 1.3-fold higher than OM3, respectively. REF had the highest proportion of Gram-positive PLFAs (47%), relative to OM1 (35%) and OM2 (31%), followed by OM3 (28%). Overall, the organic layer was host to more microbial biomass, approximately 4-fold greater total abundance of PLFA biomass than mineral soils and exhibited greater total cellulolytic activity. Fungi showed double the amount of ^{13}C-enrichment relative to Gram-negative bacteria and quadruple that of Gram-positive bacteria in both soil layers.

Figure 3.27. Soil properties and microbial activity in microcosms incubated with ^{13}C-cellulose with harvesting treatments indicated by bar colours. Panels are scaled according to the highest value in each and treatments are ordered descending from highest to lowest value. Within groups of four treatments, paired values which have asterisks (in bold) are significantly different ($p < 0.05$), whereas values denoted by different letters are significantly different based on Tukey's Honest Significant Difference. Respiration data from organic layer soils was not included because of extreme variability. Table E.9 presents a more detailed version of these data, including standard error.

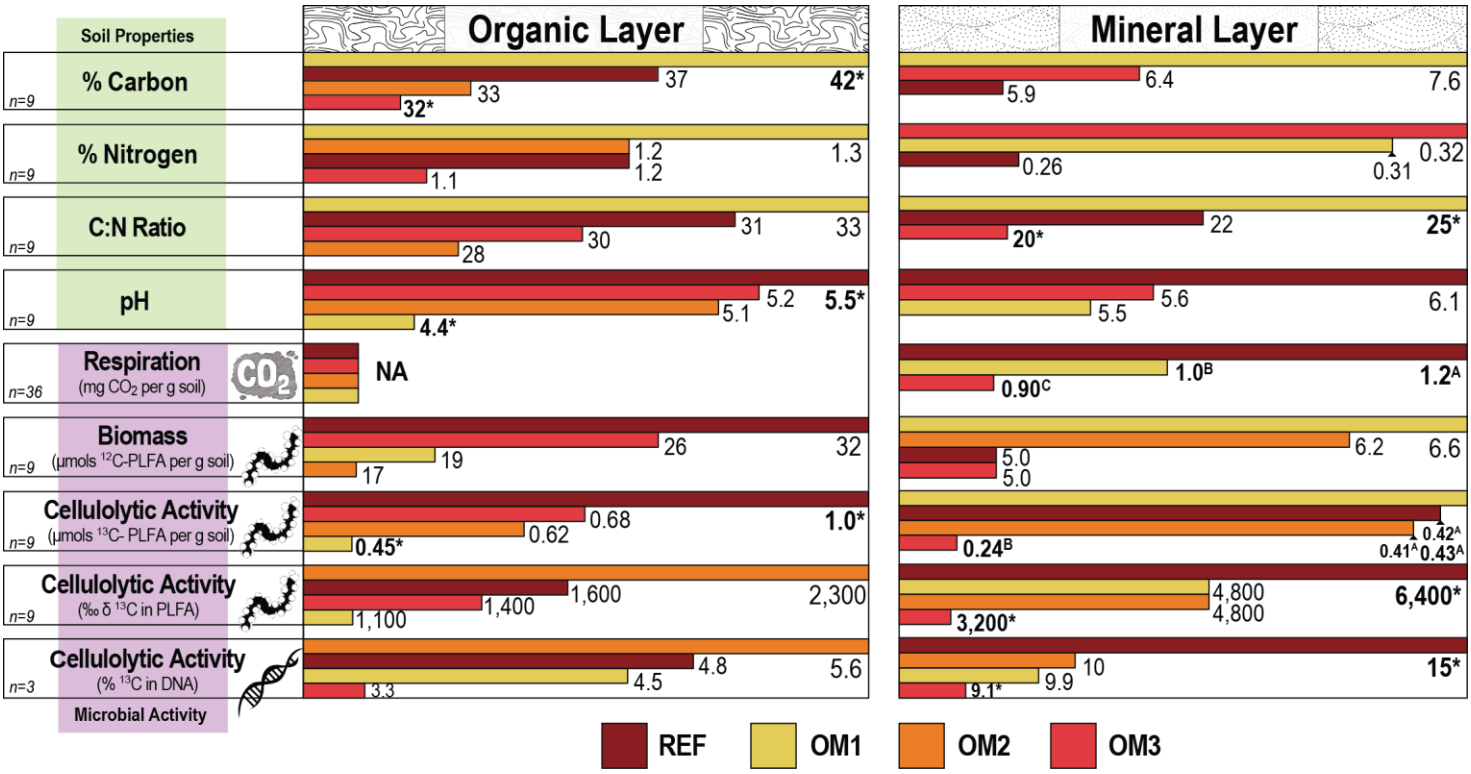
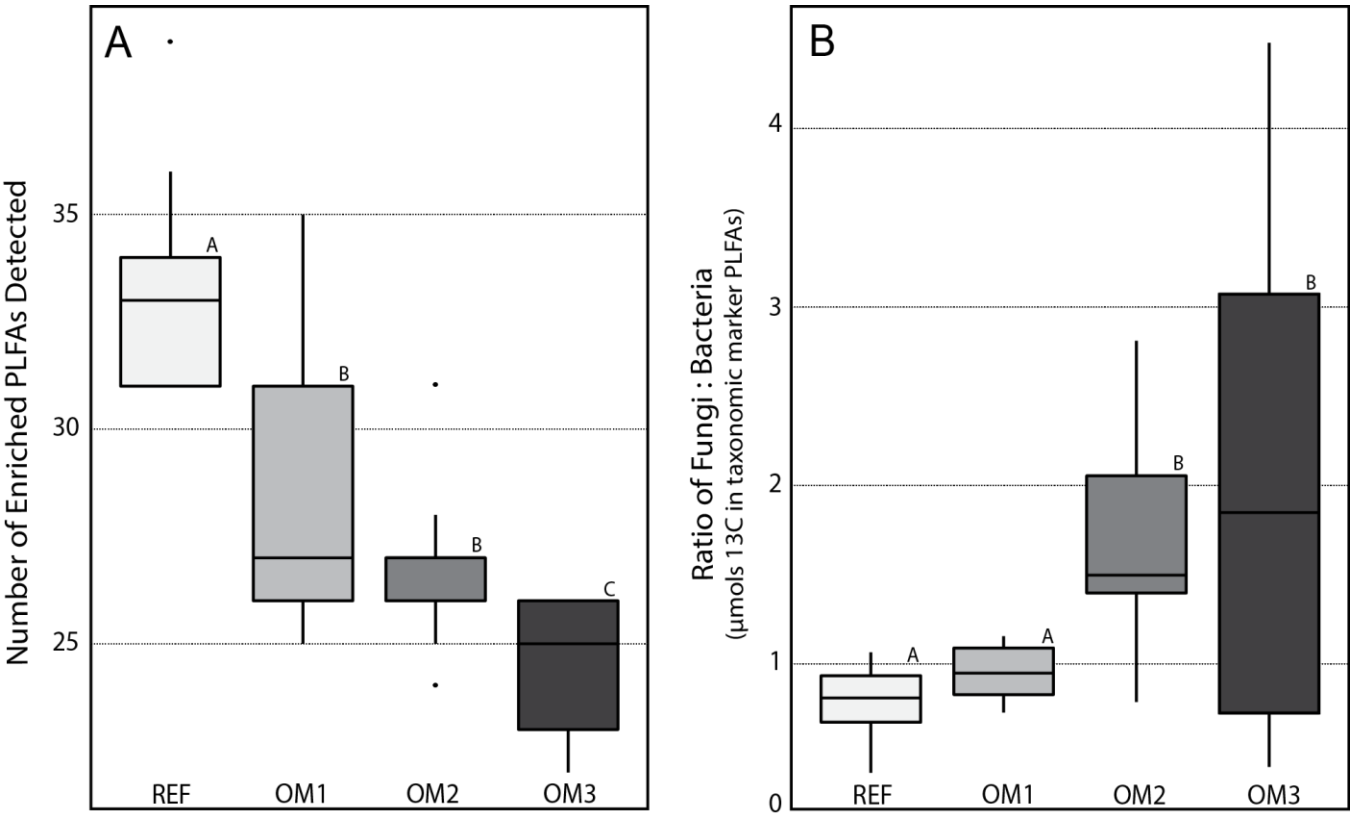


Figure 3.28. Trends in PLFAs in organic layer soil incubations with ^{13}C-cellulose: (A) average total number of ^{13}C-enriched PLFAs and (B) the ratio of fungal versus bacterial ^{13}C-PLFAs. Mineral soils showed similar, but less pronounced, trends (Table E.9). Statistically supported differences are grouped by lettering.



3.3.4 Harvesting Effects on Community Structure

SIP-cellulose proved effective in recovering DNA from actively cellulolytic populations. This was illustrated by the elevated levels of ^{13}C-carbon in total DNA extracted from soils incubated with ^{13}C-cellulose and the resulting 2.5-fold higher concentrations of DNA in heavy CsCl gradient fractions (Figure 3.29). Ordination of OTU profiles from the resulting pyrotag libraries exhibited distinct clustering of samples according to ^{13}C-enrichment and soil layer (Figure 3.30). Furthermore, shotgun metagenomes derived from ^{13}C-enriched DNA had substantial improvements in assembly over the ^{12}C-control library (Figure 4.1; next section).

Harvesting significantly changed which bacterial populations incorporated ^{13}C, accounting for ~9% of the total variation in ^{13}C-pyrotag profiles ($p < 0.05$), comparable to the variation explained by soil layers (Figure 3.31). Harvesting alone was not a significant factor in explaining differences among fungal ^{13}C-pyrotag profiles; rather, pH and harvesting had an interacting influence ($p=0.053$, $R^2=0.11$). Harvesting treatments did not affect the alpha diversity (Shannon-Weaver diversity) or beta diversity (UniFrac) of ^{13}C-pyrotag libraries. Harvesting did alter the predominant taxa incorporating ^{13}C from cellulose but did not cause the complete loss or gain of cellulolytic taxa. Soil from harvested plots yielded ^{13}C-libraries with significantly diminished abundances of cellulolytic *Verrucomicrobia* (*Chthoniobacter* and *Opitutaceae*), *Streptomycetaceae*, *Burkholderia*, unclassified *Rhizobiaceae* and *Caulobacter* (Table 3.3). Overall, populations of *Verrucomicrobia*, *Streptomycetaceae* and *Caulobacter* were sufficiently abundant and active (i.e. enriched by SIP) to yield partial or complete draft genomes (Table E.11).

Figure 3.29. Comparisons of (A) ^{13}C-enrichment of soil DNA extract and (B) total DNA recovery from fraction F_1-F_7 between microcosms fed ^{12}C- and ^{13}C-cellulose. Consult Wilhelm *et al.* 2014 for additional comparisons and characterizations of successful separation and recovery of enriched nucleic acids.

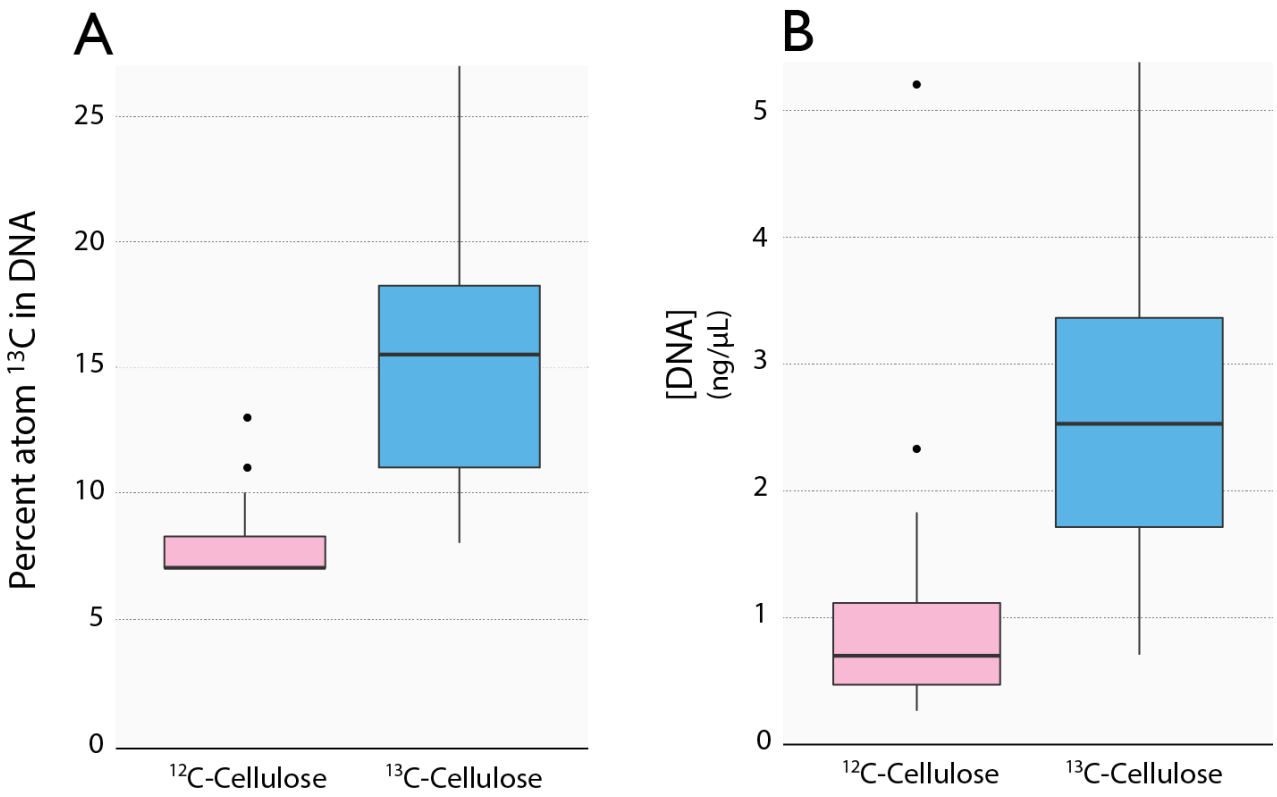


Figure 3.30. Non-parametric multidimensional scaling of 16S rRNA gene pyrotag libraries based on Bray-Curtis dissimilarities. Ovals indicate the distribution of samples (grey crosses) that clustered according to ^{13}C-enrichment (separated along x-axis) and soil layer (separated along y-axis). Blue and pink coloured circles represent the ordination of bacterial classes and are scaled to normalized abundances in ^{12}C- (pink) and ^{13}C-libraries (blue). Only classes with relative abundances greater than 0.15% are shown. Candidate taxa without designated classes are identified as *FBP* (division of *Armatimonadetes*) and *WPS-2* (phylum).

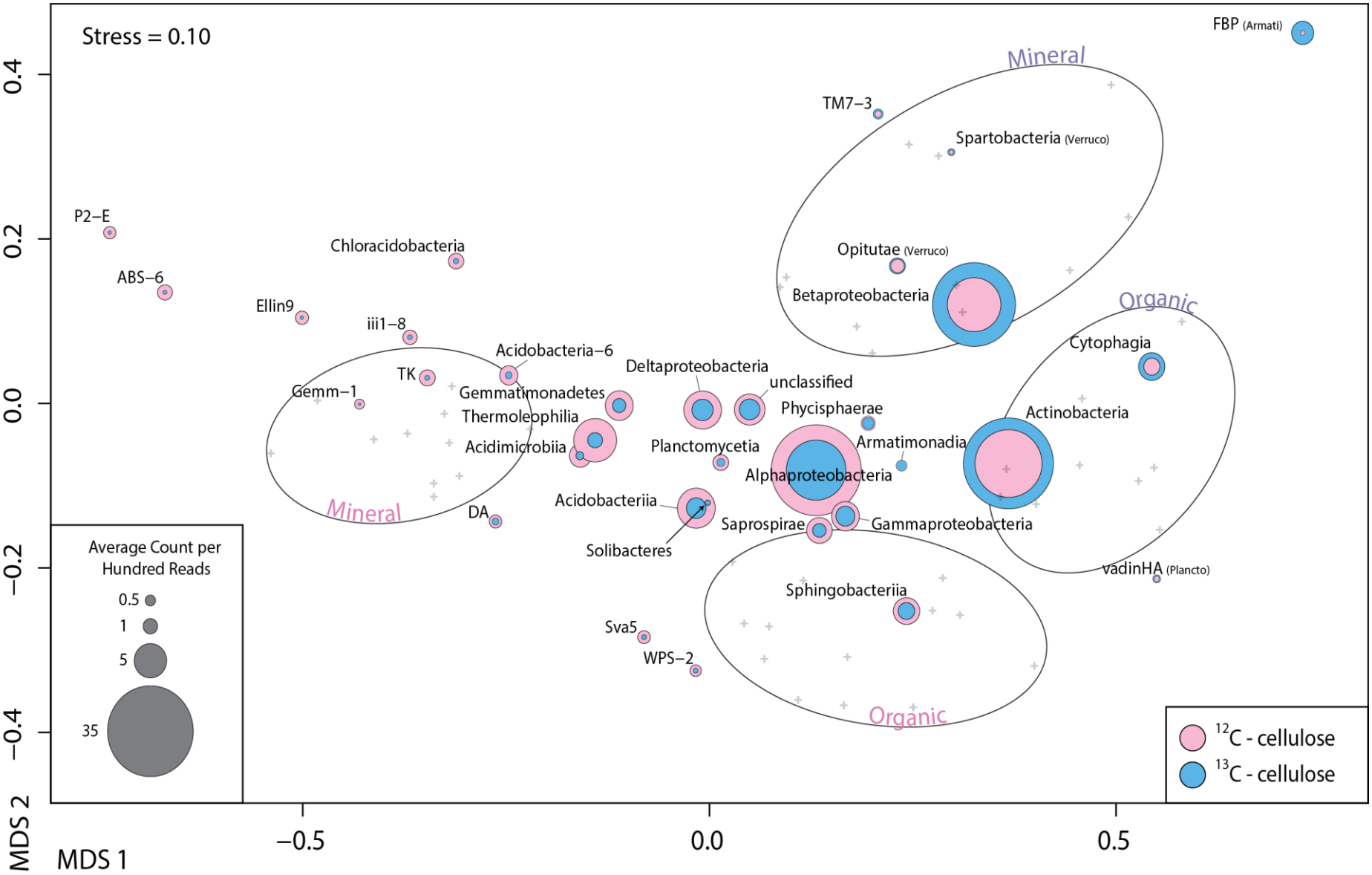
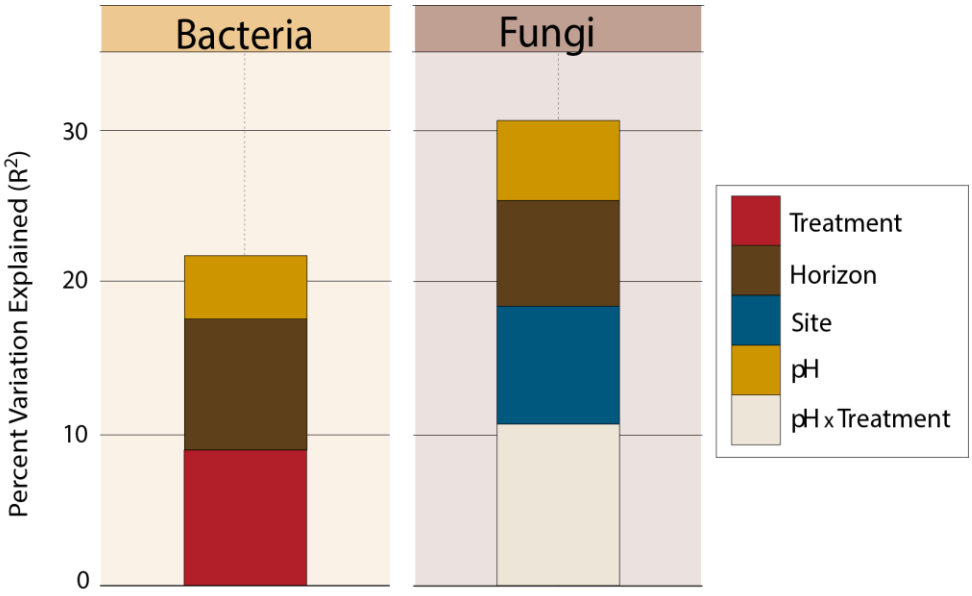


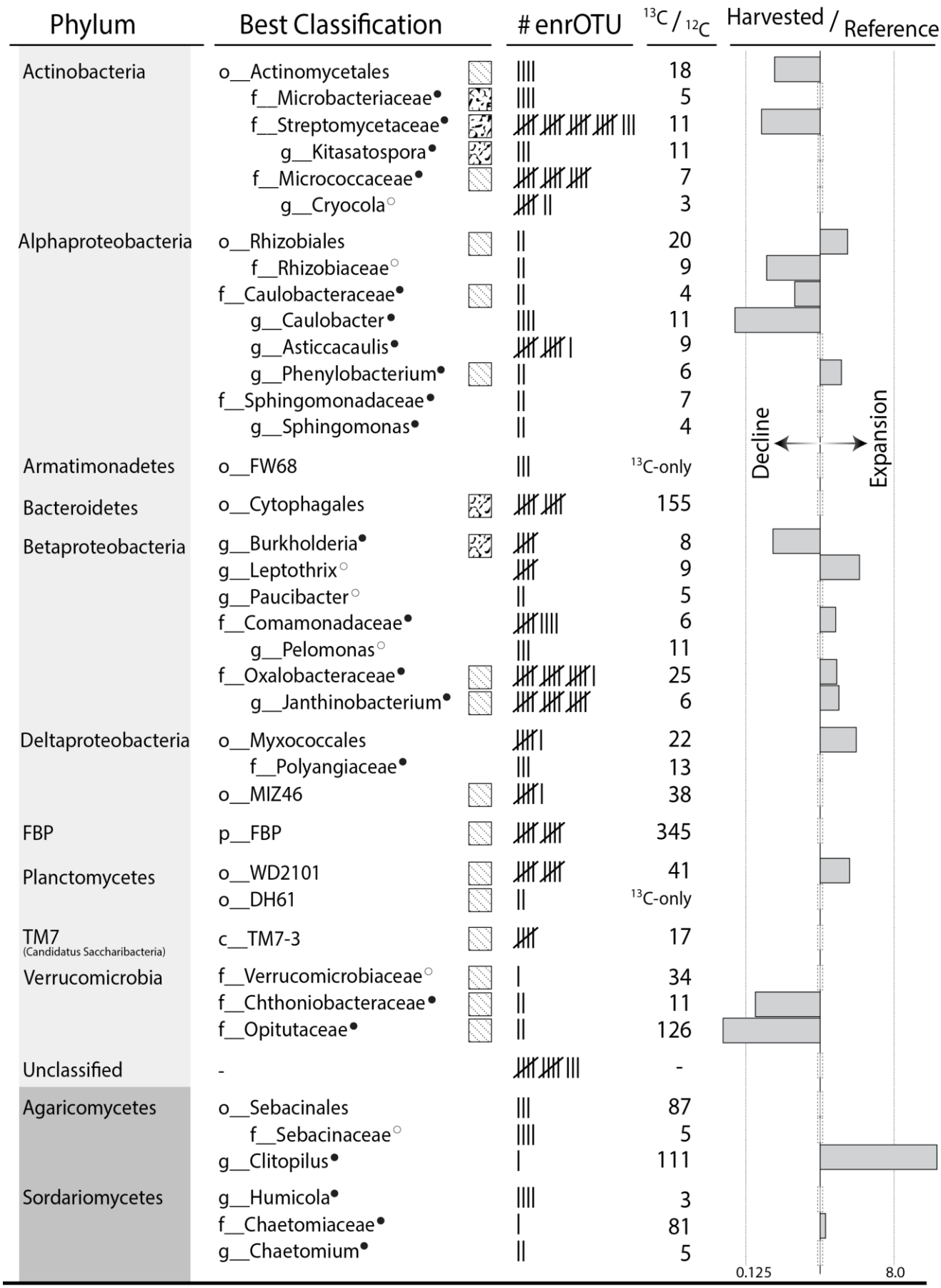
Figure 3.31. Bar plot of the percent variance (R^2) explained by each factor based on perMANOVA (n_{perm}=1000) using Bray-Curtis dissimilarities for either 16S rRNA gene or ITS pyrotag libraries. Only factors with statistical support ($p < 0.05$) factors were included in the plot. The full tabular perMANOVA results are included.



Fungi	Df	Sum of Sqs	F.Model	R^2	p-value
pH	1	0.56	2.64	0.062	0.005
Treatment	3	0.92	1.43	0.102	0.091
Horizon	1	0.70	3.28	0.078	0.006
C:N	1	0.36	1.66	0.040	0.095
Site	2	0.77	1.80	0.086	0.041
pH x Treatment	3	0.99	1.54	0.110	0.053
pH x Horizon	1	0.19	0.90	0.021	0.500
Treatment x Horizon	2	0.61	1.42	0.068	0.130
pH x C:N	1	0.12	0.57	0.014	0.850
Treatment x C:N	3	0.35	0.81	0.039	0.68
Residuals	16	9.00	-	0.381	-

Bacteria	Df	Sum of Sqs	F.Model	R^2	p-value
pH	1	0.63	1.90	0.046	0.002
Treatment	3	1.30	1.30	0.094	0.036
Horizon	1	1.24	3.74	0.090	0.001
C:N	1	0.36	1.09	0.026	0.301
Site	2	0.76	1.14	0.055	0.182
pH x Treatment	3	1.08	1.08	0.078	0.278
pH x Horizon	1	0.38	1.15	0.028	0.224
Treatment x Horizon	2	0.57	0.85	0.041	0.831
pH x C:N	1	0.29	0.88	0.021	0.666
Treatment x C:N	3	0.83	0.83	0.060	0.887
Residuals	19	6.32	-	0.459	-

Table 3.3. List of putatively cellulolytic bacterial and fungal taxa determined by differential abundance between ^{13}C- and ^{12}C-libraries 16S rRNA gene and ITS pyrotags (^{13}C:^{12}C). Harvested/reference indicates taxa significantly ($p < 0.05$) more abundant in microcosms with soil from harvested plots (right bar) or reference plots (left bar) based on log response ratio (the natural log of the mean abundance in soil from harvested plots divided by the mean abundance in soil from reference plots). Mineral layer and organic layer-associated taxa are noted by shaded squares. Taxa with isolates possessing previously described cellulolytic activity are denoted by solid circles. Classification refers to the lowest possible taxonomic rank for the group of OTUs with a bootstrap value of > 80. Each classification is prefaced with its associated rank (i.e. "o__" corresponds to Order, etc.). The "# enrOTU" represents the total number of 13C-enriched OTUs assigned to a corresponding taxon. A full list of enrOTUs with corresponding accession numbers can be found in Supplementary Data.


Phylum
Best Classification
enrOTU
13C / 12C
Harvested / Reference
Actinobacteria
o__Actinomycetales 18
f__Microbacteriaceae● 5
f__Streptomycetaceae● 11
g__Kitasatospora● 11
f__Micrococcaceae● 7
g__Cryocola○ 3
Alphaproteobacteria
o__Rhizobiales 20
f__Rhizobiaceae○ 9
f__Caulobacteraceae● 4
g__Caulobacter● 11
g__Asticcacaulis● 9
g__Phenylobacterium● 6
f__Sphingomonadaceae● 7
g__Sphingomonas● 4
Armatimonadetes
o__FW68
13C-only
Bacteroidetes
o__Cytophagales 155
Betaproteobacteria
g__Burkholderia● 8
g__Leptothrix○ 9
g__Paucibacter○ 5
f__Comamonadaceae● 6
g__Pelomonas○ 11
f__Oxalobacteraceae● 25
g__Janthinobacterium● 6
Deltaproteobacteria
o__Myxococcales 22
f__Polyangiaceae● 13
o__MIZ46 38
FBP
p__FBP 345
Planctomycetes
o__WD2101 41
o__DH61
13C-only
TM7
(Candidatus Saccharibacteria)
c__TM7-3 17
Verrucomicrobia
f__Verrucomicrobiaceae○ 34
f__Chthoniobacteraceae● 11
f__Opitutaceae● 126
Unclassified
- -
Agaricomycetes
o__Sebacinales 87
f__Sebacinaceae○ 5
g__Clitopilus● 111
Sordariomycetes
g__Humicola● 3
f__Chaetomiaceae● 81
g__Chaetomium● 5
Decline
Expansion
0.125
8.0
Bacteria
Fungi
Organic-associated
Mineral-associated
● Previously reported cellulolytic activity by members of group

Their reduced abundances in soil from harvested plots was supported by the proportion of metagenomic reads which mapped back to these genomes (Figure 3.32) as well as abundance patterns in ^{13}C- and ^{12}C- pyrotag libraries (Figure 3.33). Their reduced *in situ* abundances in harvested plots were also apparent (Figure 3.33). Conversely, a number of different cellulolytic taxa were enriched in soils from harvested plots, including a number of *Betaproteobacteria* and members of *Myxococcales, Planctomycetes* and the Basidiomycota *Clitopilus* (Table 3.3).

The decline of Actinobacteria relative to fungi in soil from harvested treatments was corroborated in PLFA, ^{13}C- and *in situ* pyrotag and shotgun metagenomic data. Abundances of the order, *Actinomycetales*, as well as the family *Streptomycetaceae* were reduced in harvested treatments (Figure 3.33 & Figure 3.34). Read mapping to the *Kitasatospora* draft genome (family *Streptomycetaceae*) further supported this trend (Figure 3.32). The predominant cellulolytic fungi, from the family *Chaetomiaceae* (*Sordariomycetes*), had expanded populations in soils from harvested plots in both ^{13}C- and *in situ* pyrotag libraries (Figure 3.33). *Chaetomiaceae* were highly abundant in all ITS libraries with a total percent of 2%, 3%, and 9% in *in situ*, ^{12}-C and ^{13}C-libraries, respectively. Read mapping to the recovered fungal draft genome, most closely related to the genome of *Myceliophthora thermophila* (family *Chaetomiaceae*), supported the increased abundances of *Chaetomiaceae* in microcosms from harvested plots (Figure 3.33 & Figure 3.34). Both *Sordariomycetes* and *Actinobacteria* populations were consistently negatively correlated to C:N ratio, though not always supported by a strong p-value (Figure 3.35; Figure 3.36). C:N ratio did not significantly differ among harvested plots, suggesting that C:N ratio, as a proxy for litter quality, did not explain differences in these two populations (Figure 3.37). Abundances of Actinobacteria were positively correlated with pH (Figure 3.38), which could not be said for *Sordariomycetes*.

Figure 3.32. Draft genomes recovered from metagenome assemblies from ^{13}C-enriched DNA. Bars indicate the percentage of reads contributed by metagenomes from each treatment group. Genome size corresponds to size of bubble (also written) and completeness to the bubble fill. For additional details on completeness, taxonomic uniformity and accession numbers, consult Table E.11.

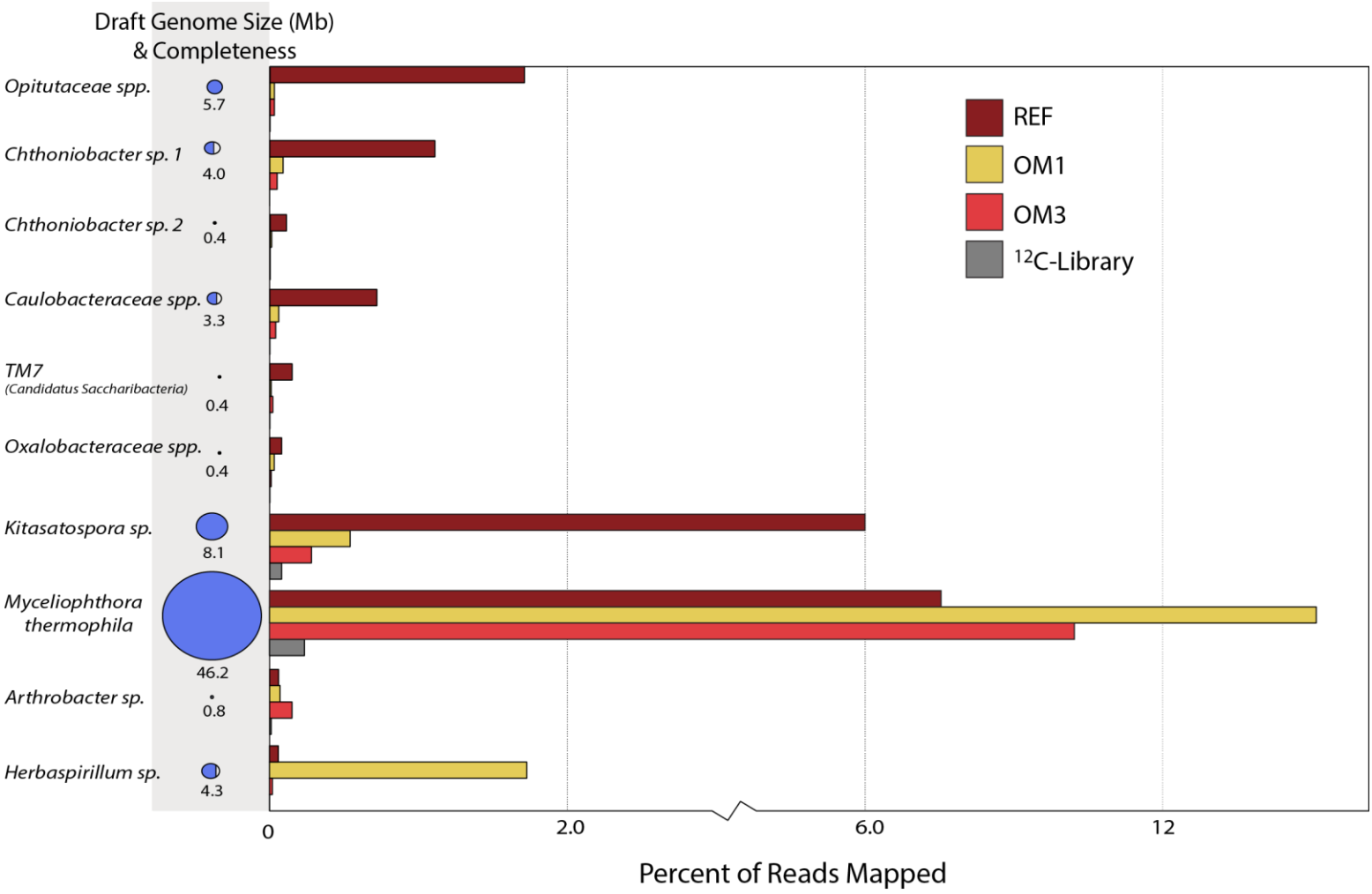


Figure 3.33. The abundance in the three types of 16S rRNA gene or ITS pyrotag libraries (z-axis) that are indicators of either reference (beige) or harvested (red) treatments. Taxa were designated as either identified in this study as cellulolytic (blue) or previously reported to be desiccation and/or heat tolerant (pink). Abundances of taxa with asterix (*) represent per mil, rather than per cent abundance.

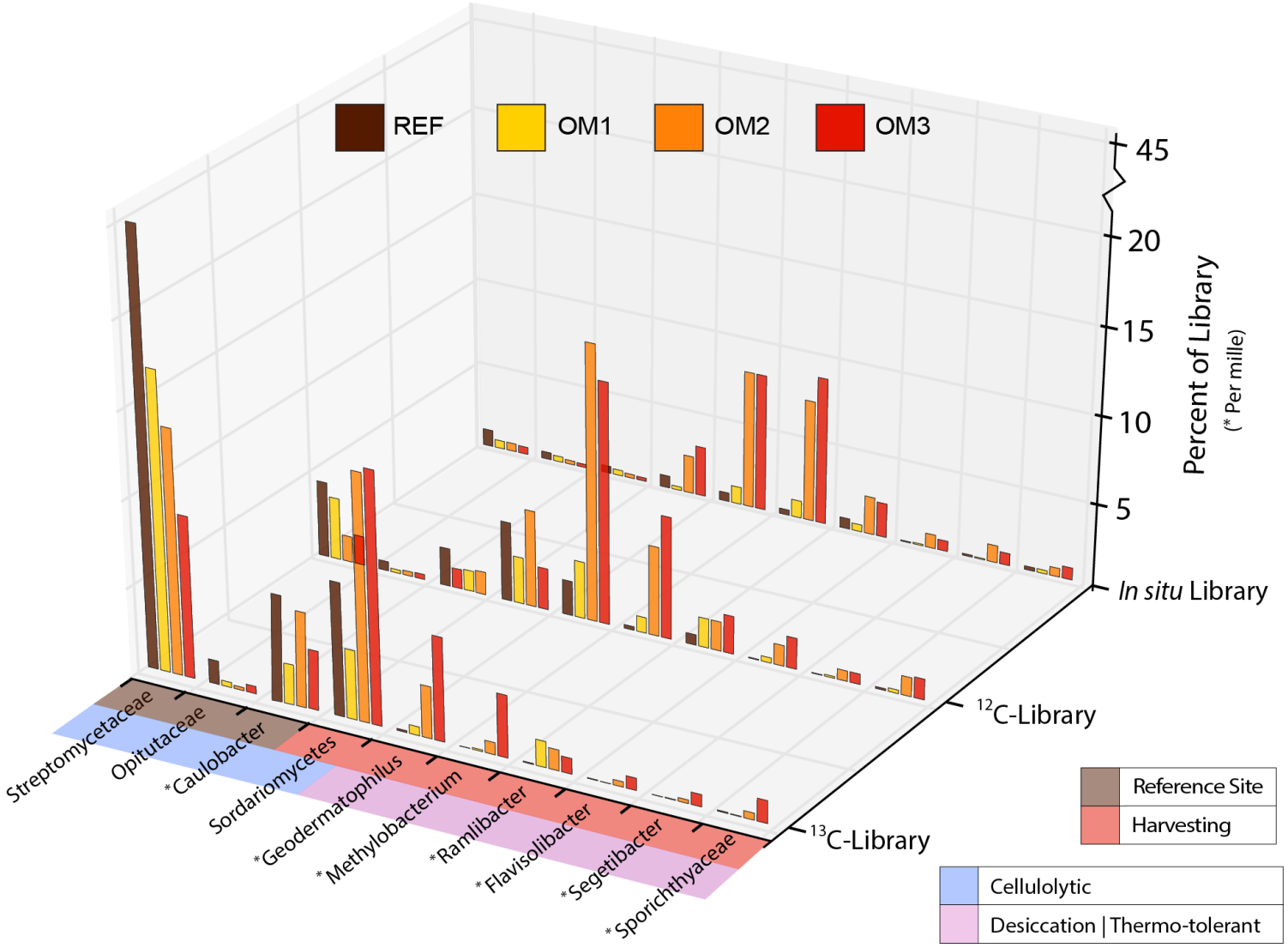
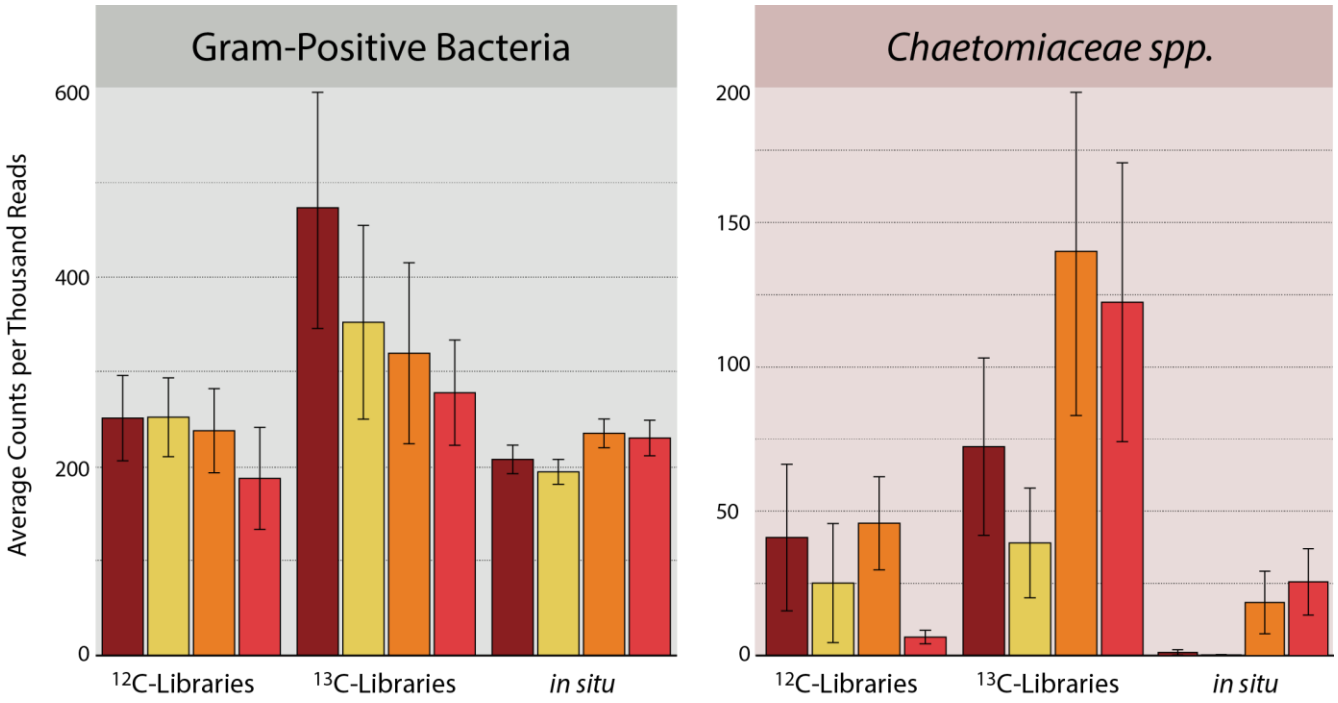


Figure 3.34. Abundance patterns of Gram-positive bacteria (*Firmicutes* and *Actinobacteria*) and the main group of cellulolytic fungi from family *Chaetomiaceae*. The identical pattern is observed when data is aggregated at class *Sordariomycetes* (see Figure 3.33). Error bars correspond to one standard error of the mean.



Indicator species analysis revealed bacterial taxa with consistently expanded populations in soils from harvested plots across all pyrotag libraries (Figure 3.33). These taxa were previously identified in Section 3.2.3, demonstrating consistency between broadly different datasets. As previously noted, the majority of these indicator taxa have been isolated from desert environments and are reported to be tolerant of heat, radiation and desiccation: *Geodermatophilus* (Montero-Calasanz *et al.,* 2014; Sghaier *et al.,* 2016), *Sporichthya* (Eppard *et al.,* 1996), *Ramlibacter* (de Luca *et al.,* 2011), *Flavisolibacter* (Joo *et al.,* 2015), *Methylobacterium* (Nogueira *et al.,* 1998; Romanovskaya *et al.,* 2002) and *Segetibacter* (Liu *et al.,* 2014).

Figure 3.35. Linear regressions of Sordariomycetes abundance and carbon to nitrogen ratio (C:N). This figure contains six of the twelve possible plots (6 ecozone x 2 horizons) which showed notable statistical support. Plots that have not been shown had p-values ranging from 0.12 – 0.6 with no discernable positive or negative trends.

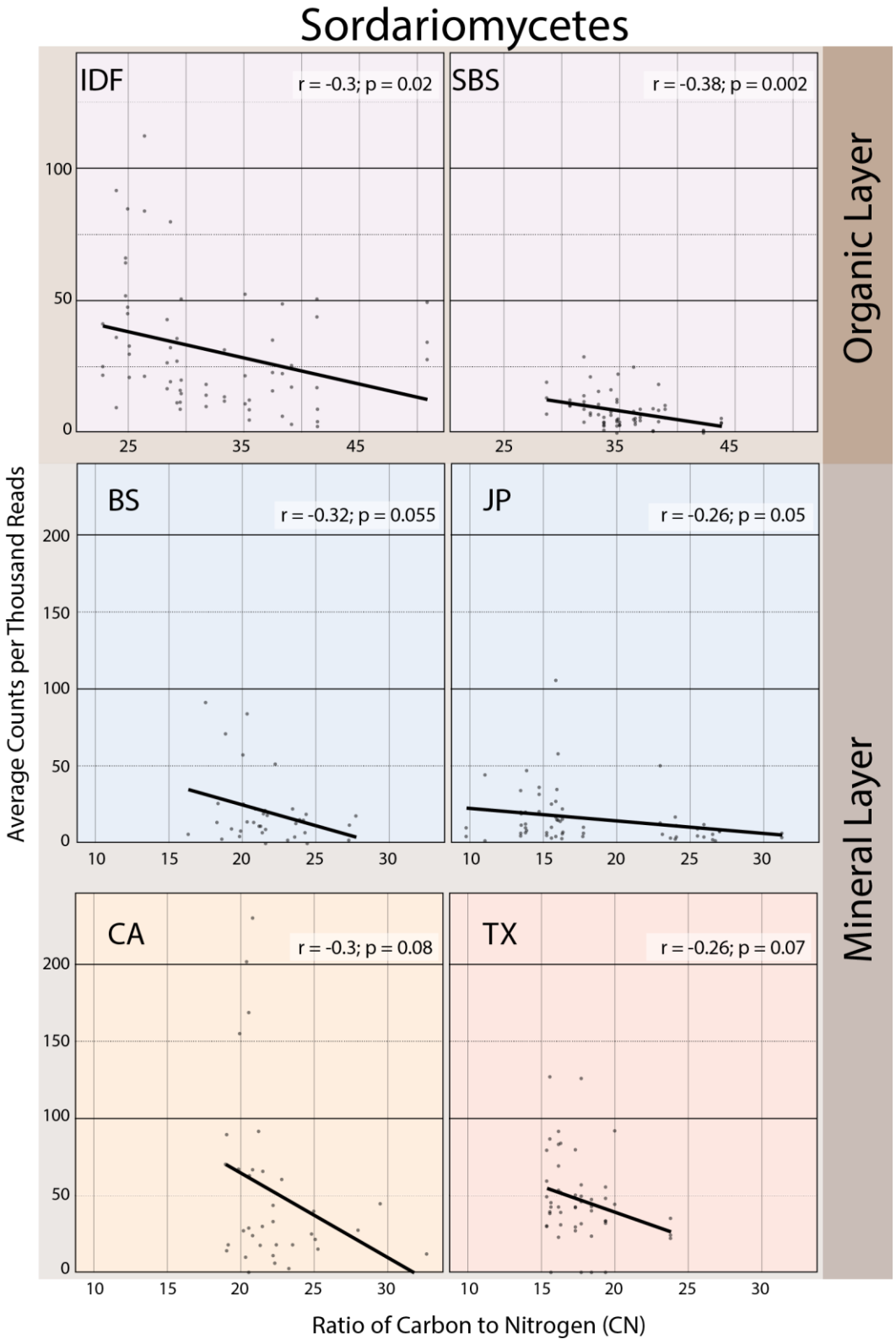


Figure 3.36. Linear regressions of Actinobacteria abundance and carbon to nitrogen ratio (C:N). This figure contains five of the twelve possible plots which showed notable statistical support. Plots that have not been shown had p-values ranging from 0.35 – 0.93 with no discernable positive or negative trends.

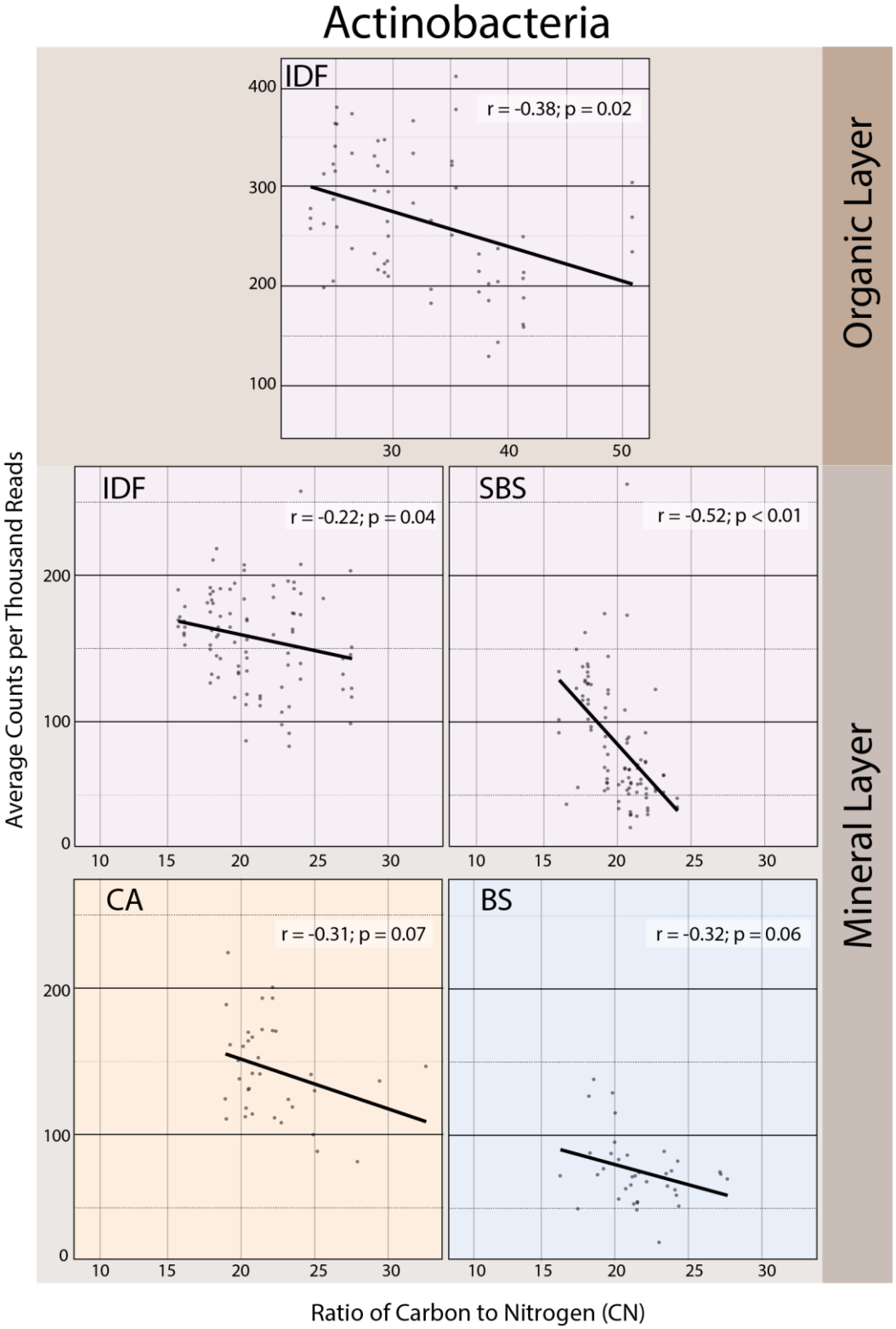


Figure 3.37. Dot plot showing the differences in C:N ratio among harvesting treatments at all ecozones. Lines correspond to the mean of each group. Few contrasts were statistically supported, so they were not plotted. The following contrasts within an ecozone were 'significant': $OM2_{TX;Min}$-$OM1_{TX;Min}$ (p=0.04); $OM2_{CA;Min}$-$OM1_{CA;Min}$ (p=0.01); $OM3_{CA;Min}$-$OM1_{CA;Min}$ (p < 0.001) and $OM2_{SBS;Org}$-$REF_{SBS;Org}$ (p=0.04).

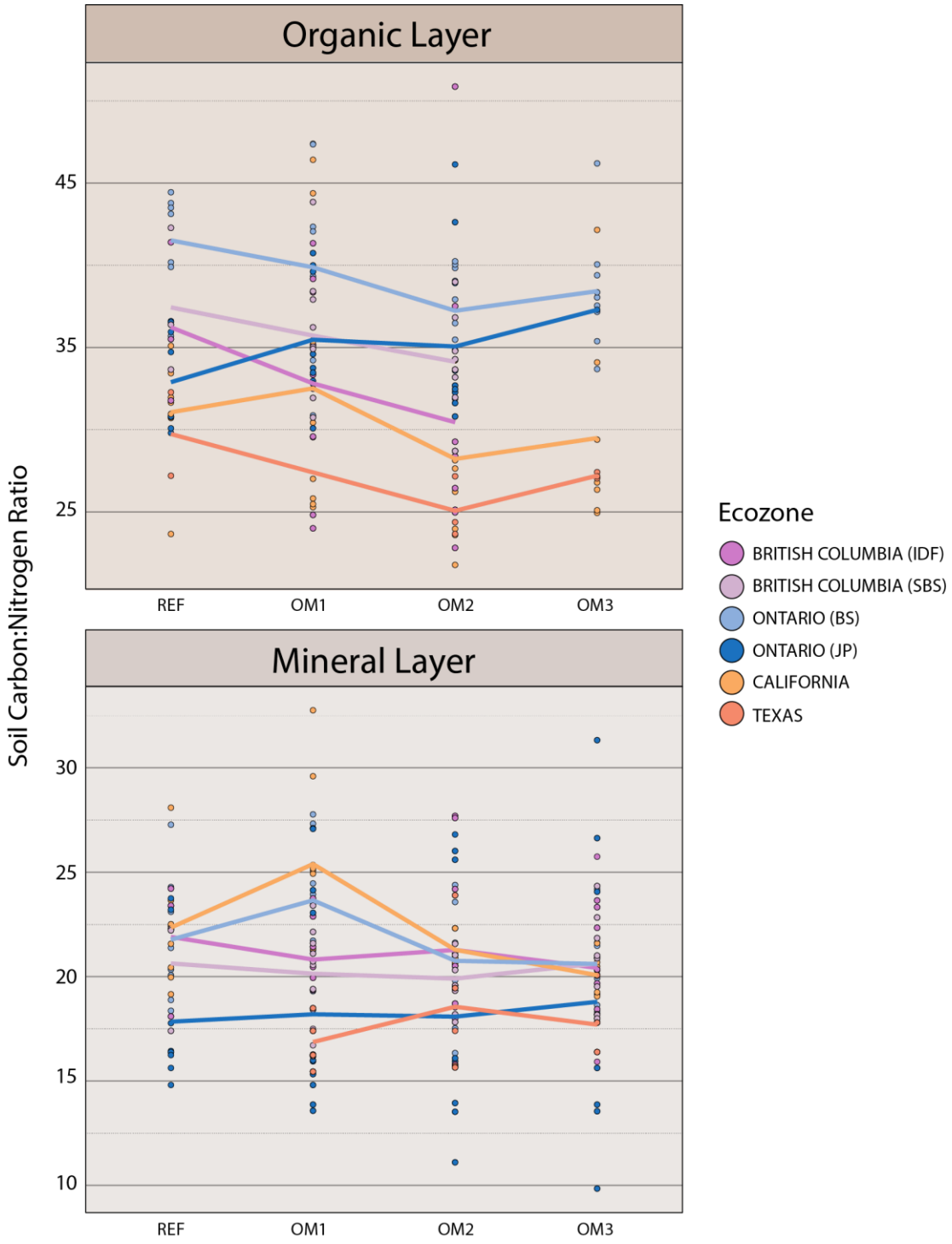
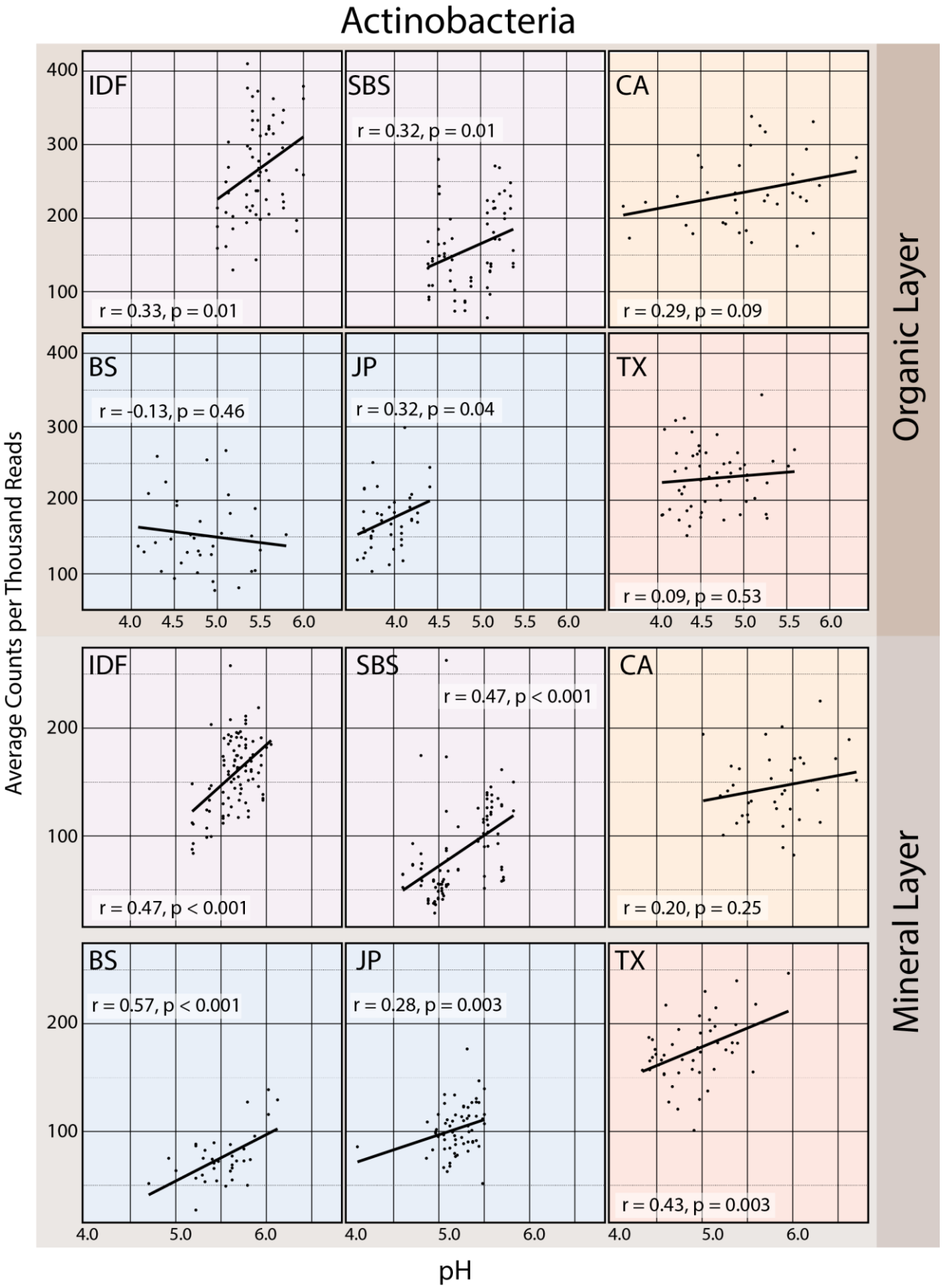


Figure 3.38. All possible linear regressions of actinobacterial abundance with pH among ecozones and soil layers. No statistically supported correlation occurred between pH and abundance of *Sordariomycetes*.



3.3.5 Description of Cellulolytic Taxa

Chapter 4 provides a detailed description of lignocellulolytic taxa, yet, an initial characterization of cellulolytic populations in PP_{CA} was needed to assess harvesting impacts. Therefore, a preliminary description based exclusively on Chapter 3 datasets is presented here.

We identified a total of 234 bacterial ^{13}C-enriched OTUs (enrOTUs) representing nine phyla. EnrOTUs were classified to *Actinomycetales, Armatimonadetes, Cytophagales, Myxococcales, Planctomycetes, Rhizobiales, Opitutaceae* and *Oxalobacteraceae* (Table 3.3). Non-metric dimensional scaling confirmed broad differences between ^{13}C- and ^{12}C-libraries as well as distinct cellulolytic communities in each soil layer (Figure 3.30). The organic-rich soil layer was comprised of previously known cellulose-degrading phyla, *Cytophaga* and *Actinobacteria*, while the mineral layer consisted of *Betaproteobacteria* and less characterized phyla such as *Armatimonadetes* (candidate division *FBP* and order *FW68*), *Verrucomicrobia* (classes *Opitutae*, *Spartobacteria*) and *Candidatus Saccharibacteria* (formerly *TM7*).

The delineation of fungal enrOTUs was less successful due to sparse overlap amongst OTUs in ITS libraries, which were typically dominated by a few highly abundant OTUs. This is illustrated by the poor separation of samples by NMS (Figure 3.39) and the relatively small number of fungal enrOTU identified (n=16). These included unclassified *Ascomycota* and members of *Agaricomycetes* and *Sordariomycetes* (Table 3.3), though clustering in NMS suggest the involvement of *Dothideomycetes* and a large proportion of unclassified ITS sequences. The massive difference in proportion of reads classified as *Ascomycota* between ^{13}C- versus ^{12}C-metagenomes (0.8% and 10.6%, respectively) and enrichment of fungal PLFAs both suggested that *Ascomycota* were the predominant cellulose-degraders under our experimental conditions (Figure 3.40). *Clitopilus* was the only cellulolytic *Basidiomycota* to be identified.

Basidiomycotal sequences were between ~10^3 and 10^4 times less abundant than *Ascomycota* in ^{13}C-pyrotag libraries and the ratio between the two did not significantly vary according to harvesting treatment in either ^{12}C- or ^{13}C-libraries, but the relative abundance of *Ascomycota* did significantly expand in harvested plots *in situ* (Figure 3.41), as previously noted among other ecozones (Figure 3.9).

One third of enrOTUs were detected *in situ*, demonstrating that low abundance cellulolytic populations were enriched for by SIP. enrOTUs occupied at most 1.4% of total reads and at least 0.08% with a median abundance of 0.42% among all samples. The most abundant single enrOTU (uncl. *Streptomycetaceae*) occupied, at most, 0.6% of a given sample with a mean abundance of 0.1%, followed by enrOTUs from *Janthinobacterium* (max: 0.5%), *Burkholderia* (0.4%), and uncl. *Microbacteriaceae* (0.3%). Of all enrOTU detected *in situ*, 90% were detected in the organic layer. For fungi, six out of the sixteen enrOTU were detected *in situ* with a surprisingly low maximum abundance of 0.3%, but based on taxonomic binning of OTUs, members of *Chaetomiaceae* averaged ~2% of *in situ* libraries.

Metagenome assembly was greatly improved by SIP with the percentage of reads assembled in the ^{13}C-metagenomes amounting to 17% (OM3), 23% (OM1), and 29% (REF) compared to less than 1% in the ^{12}C-metagenome. Improved assemblies enabled the recovery of ten taxonomically uniform partial genomes of putatively cellulolytic bacteria (Figure 3.32; details in Table E.10). The most complete genomes were related to *Myceliophthora thermophila* (*Ascomycota*), *Kitasatospora sp.* (*Actinobacteria*), *Opitutaceae spp.* (*Verrucomicrobia*), *Herbaspirillum sp.* (*Betaproteobacteria*), *Chthoniobacter sp.* (*Verrucomicrobia*) and *Caulobacteraceae spp.* (*Alphaproteobacteria*).

Figure 3.39. Non-parametric multidimensional scaling of ITS pyrotag libraries based on Bray-Curtis dissimilarities. Ovals indicate the distribution of samples (grey crosses) that clustered according to ^{13}C-enrichment and soil layer. Coloured circles represent the ordination of fungal classes of greater than 0.15% overall relative abundance and are scaled to their normalized abundances in ^{12}C- (pink) and ^{13}C-libraries (blue).

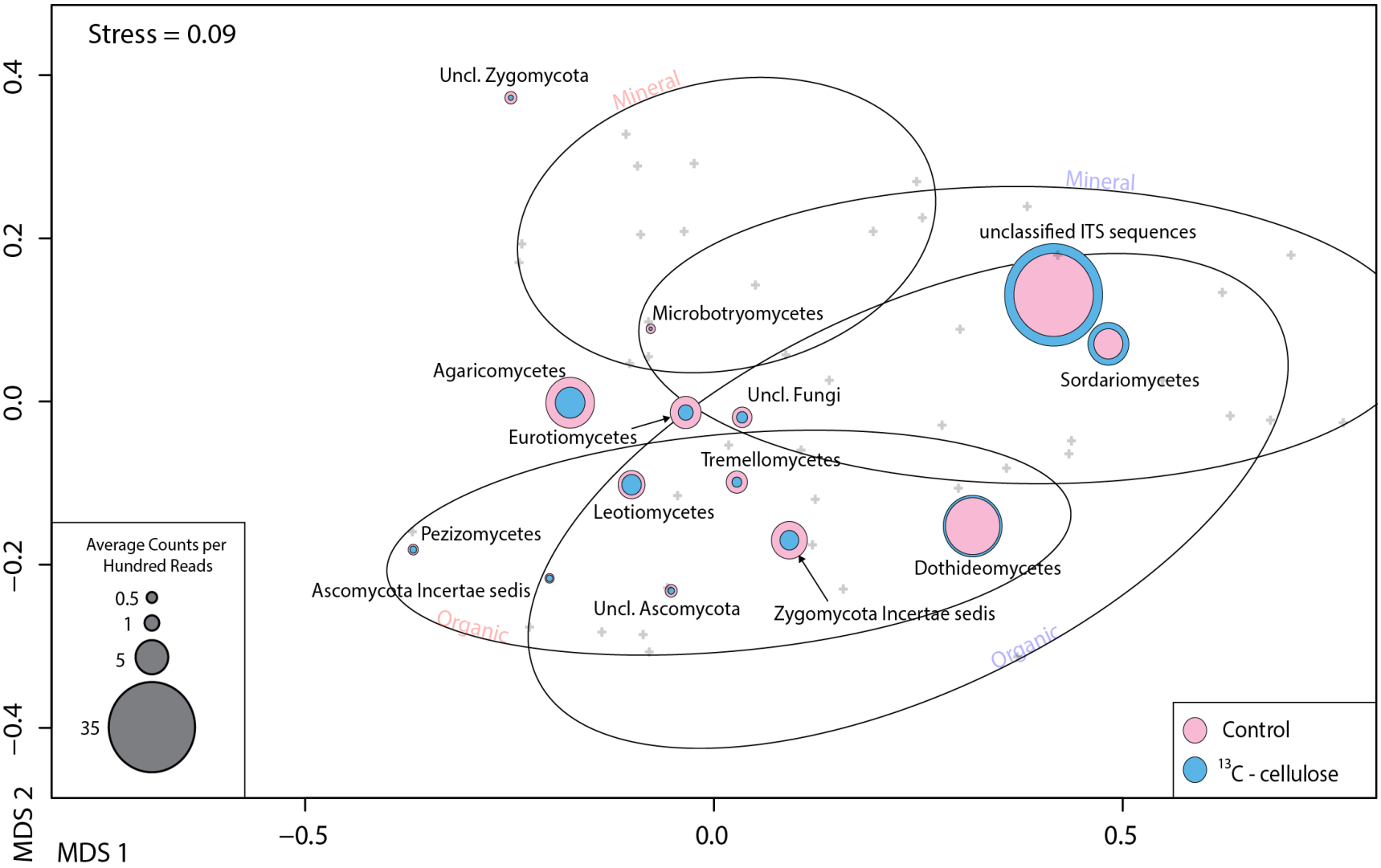


Figure 3.40. Lowest-common ancestor classification of all unassembled metagenomic reads at the phylum level. Phyla with an abundance of reads < 0.5% of the total were excluded.

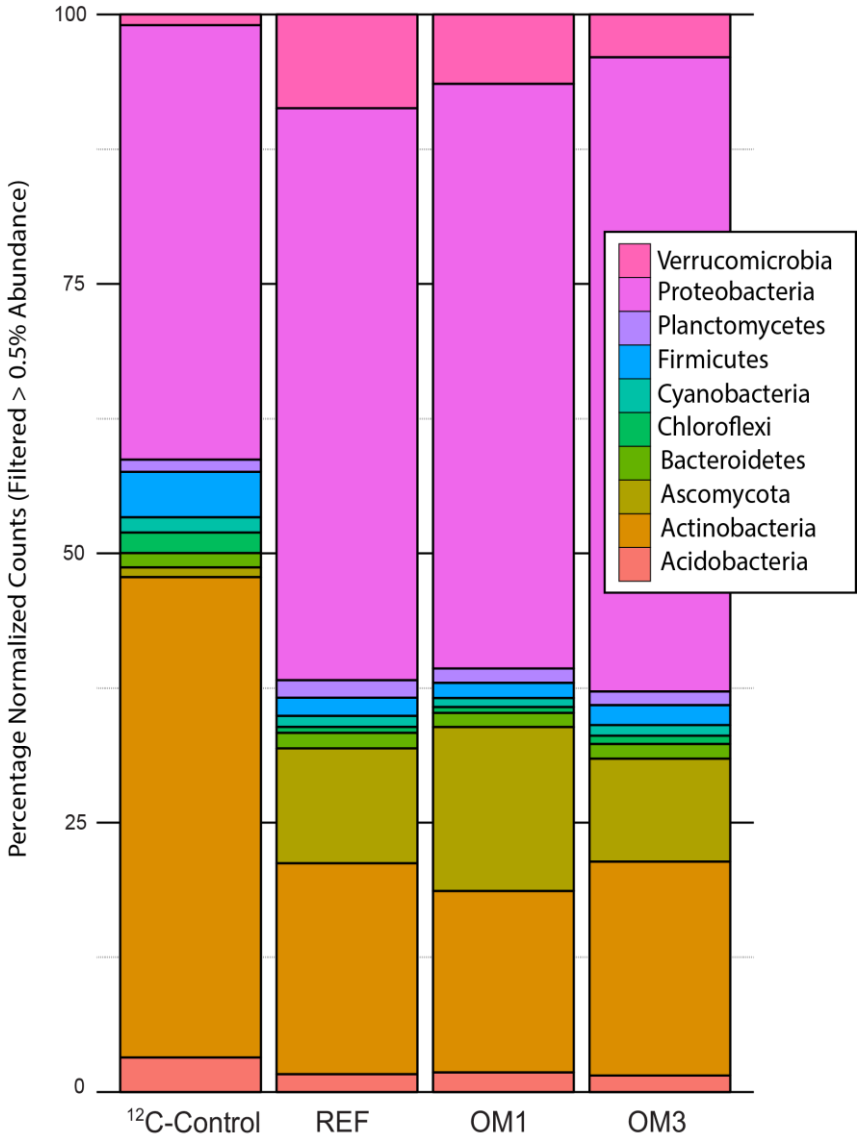
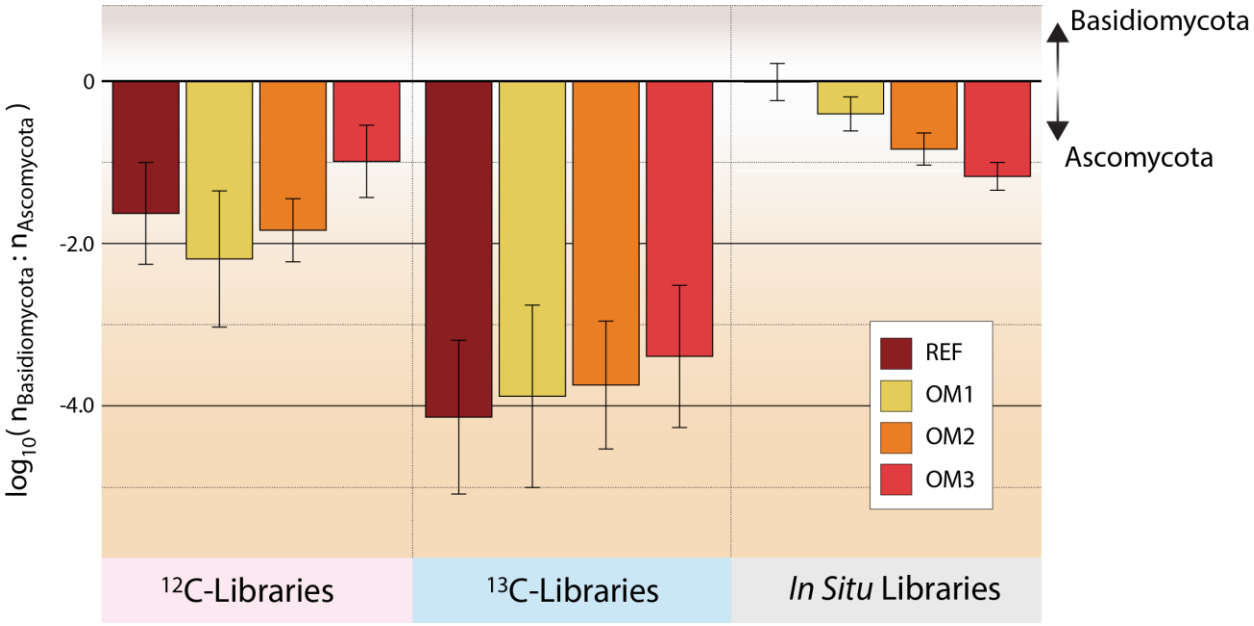
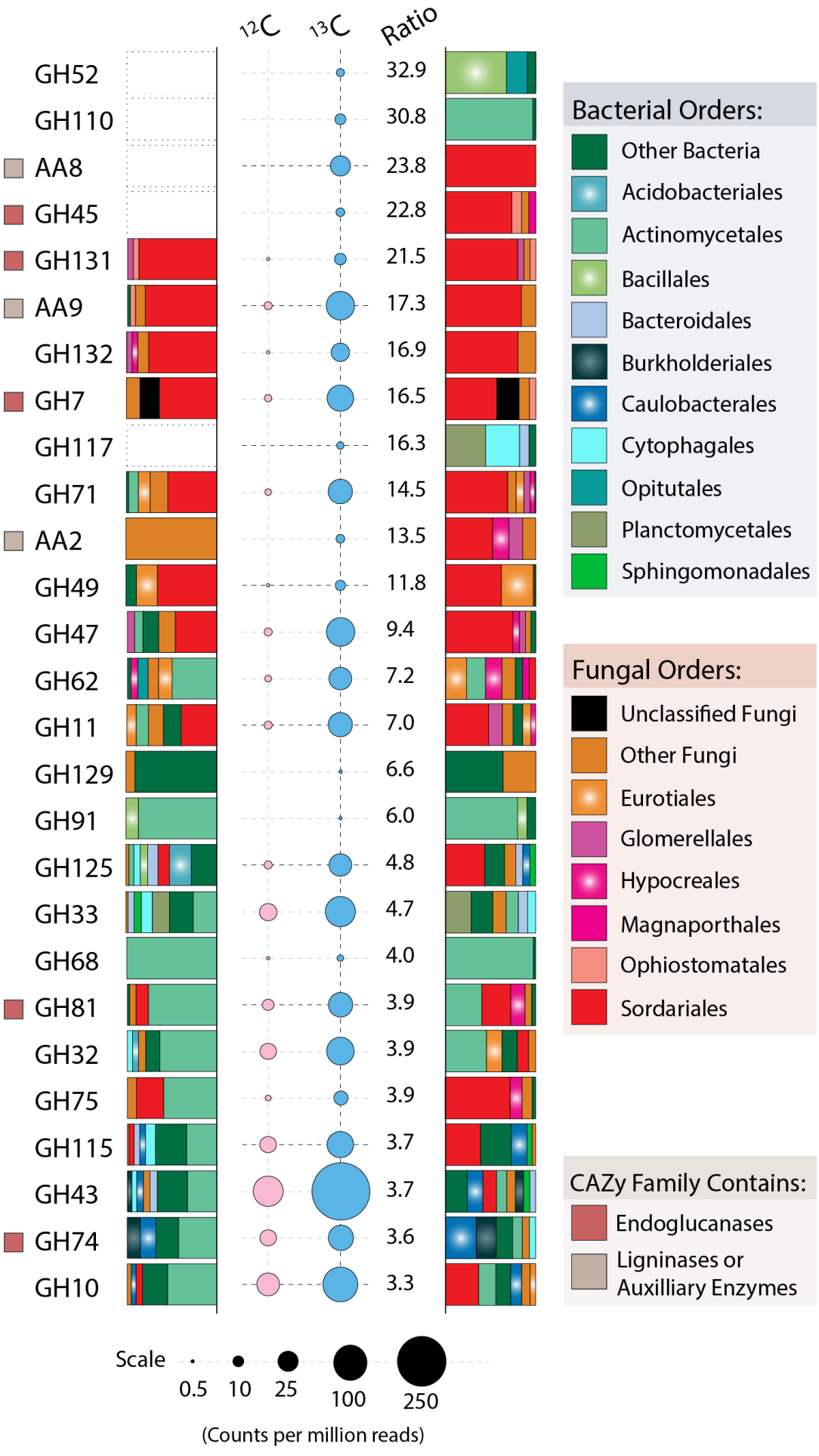


Figure 3.41. Relative abundances of *Basidiomycota* versus *Ascomycota* in pyrotag libraries. The y-axis corresponds to the log of the ratio of Basidiomycota to Ascomycota. Only *in situ* libraries showed a statistically significant trend.



Unassembled ^{13}C-metagenomes contained double the relative abundance of glycosyl hydrolase genes (GH) and three-fold more GH families with reported endoglucanase activity than the ^{12}C-metagenome. Six endoglucanase-containing families and lytic polysaccharide monooxygenases (AA9) were among the most enriched CAZy gene families in ^{13}C-metagenomes (Figure 3.42). Lignin-modifying enzymes, peroxidases (AA2) and iron reductase domains (AA8), were also highly enriched in ^{13}C-metagenomes and were classified to the order *Sordariales* with the exception of one catalase-peroxidase identified in the *Herbaspirillum sp.* draft genome. The majority of differentially abundant GH enzymes were actinobacterial and fungal (*Sordariales*), while a lesser number were from *Bacillales*, *Bacteroidales*, *Burkholderiales*, *Cytophagales*, *Opitutales*, and *Planctomycetes*.

Figure 3.42. Taxonomic affiliations of CAZy genes enriched in ^{13}C- (blue) versus ^{12}C-control (pink) metagenomes. Bubble area is scaled to counts per million among quality-filtered, unassembled reads, and the ratio corresponds to the relative counts between ^{13}C and ^{12}C metagenomes. CAZy gene families without a bubble had fewer than 0.5 counts per million reads. A beige square denotes lignin-modifying activity, while a red square denotes endoglucanase activity, based on www.cazy.org. Taxa comprising fewer than 5% of reads for any given family were binned as either 'Other Bacteria' or 'Other Fungi.'


12C
13C
Ratio
GH52 32.9
GH110 30.8
AA8 23.8
GH45 22.8
GH131 21.5
AA9 17.3
GH132 16.9
GH7 16.5
GH117 16.3
GH71 14.5
AA2 13.5
GH49 11.8
GH47 9.4
GH62 7.2
GH11 7.0
GH129 6.6
GH91 6.0
GH125 4.8
GH33 4.7
GH68 4.0
GH81 3.9
GH32 3.9
GH75 3.9
GH115 3.7
GH43 3.7
GH74 3.6
GH10 3.3
Bacterial Orders:
Other Bacteria
Acidobacteriales
Actinomycetales
Bacillales
Bacteroidales
Burkholderiales
Caulobacterales
Cytophagales
Opitutales
Planctomycetales
Sphingomonadales
Fungal Orders:
Unclassified Fungi
Other Fungi
Eurotiales
Glomerellales
Hypocreales
Magnaporthales
Ophiostomatales
Sordariales
CAZy Family Contains:
Endoglucanases
Ligninases or Auxilliary Enzymes
Scale
0.5
10
25
100
250
(Counts per million reads)

3.4 Discussion

A decade and a half after the initiation of the LTSP Study and harvesting, a clear gradient had formed in soil organic matter, temperature and moisture corresponding to the initial degree of OM removal. The existence of a corresponding gradient in the relative abundances of a number of bacterial and fungal taxa demonstrated the long-term influence of these environmental factors. Though the majority of cosmopolitan, and also most abundant, taxa appeared unaffected by harvesting, significant compositional changes were observed in both upper organic soil layers and in deeper mineral layers. In mineral soil, the absence of differences in organic content proved that the quantity and quality of organic matter were not the sole factors driving changes. The expansion of radiation, desiccation and heat-tolerant organisms reflected the increased dryness and temperature in the decades post-harvesting. These compositional shifts were shared among biogeographically distinct forest soils, demonstrating the generalizability of long-term selection pressures on certain taxa. Yet, local differences in community structure, and the corresponding ecozone-specific effects of timber harvesting, have the potential to influence how harvesting impacts microbial processes during forest regeneration. This discussion will weigh considerations of biogeography, the relative influence of OM removal on biotic versus abiotic parameters and highlight where results confirm and expand upon previous observations of long-term harvesting effects on saprotrophic, methanotrophic and ectomycorrhizal communities.

To date, the LTSP Study has shown that rates of forest regeneration are highly variable and dependent on local conditions and, overall, that the effects of varying degrees of OM removal on primary productivity appear minor (Keenan and Kimmins, 1993; Page-Dumroese *et al.,* 2006; Sanchez *et al.,* 2006; Powers *et al.,* 2005; Fleming *et al.,* 2006; Thiffault *et al.,* 2011; Ponder *et al.,* 2012; Holub *et al.,* 2013). Where harvesting impacted soil microbial communities, the effect was greatest at the highest level of organic matter removal (OM3), while the effects of

intermediate levels (OM1 and OM2) were generally indistinguishable from one another, but distinct from unharvested controls (Hartmann *et al.,* 2012; Cardenas *et al.,* 2015; Leung *et al.,* 2015). The present findings are in accordance with these reports, where patterns of expanding or declining abundances were clearest between REF and OM3, while OM1 and OM2 abundances were typically interchangeable and midway in-between. The most pronounced shifts in relative abundance were observed in taxa adapted to, or susceptible to, warmer and drier conditions or, in the case of EM fungi, the loss of tree hosts. Yet, a unique set of taxa had been selected for at intermediate harvesting treatments, and these populations have greater likelihood of responding to the trophic effects of retaining OM (i.e. nutritional), opposed to OM mitigating abiotic factors.

The claim that organic matter retention was a significant factor in the long-term of distinct communities was supported by the number of uniquely overlapping OTUs (i.e. not present in REF or OM3) in OM1 and OM2 and a number of taxa indicative of intermediate harvesting intensities. Taxa indicative of OM1 and OM2 were largely associated with the organic layer, where differences in organic content were most pronounced. The predominance of saprotrophic groups and other strict wood-rot fungi in OM1 (and to a lesser extent OM2) reflected the influence of retaining coarse woody debris. Yet, despite the fact a large number of OTUs indicative of intermediate OM removal exhibited strong signals, their abundance patterns were less consistent across ecozones. These localized responses may have ecological meaning, since certain populations may be adapted to local sources of organic matter (Ayres *et al.,* 2009; Prescott, 2010; Freschet *et al.,* 2012). Yet, the sheer volume of individual, ecozone-specific cases that result made reporting and interpretation difficult, especially given the lack of knowledge regarding the ecological roles of the vast majority of taxa identified.

The hypothesis that OM retention might stimulate decomposition by selecting for saprotrophs was a major motivation for characterizing the impact on cellulolytic populations

with SIP. The well-documented adaptation of wood rot fungi to colonize coarse woody debris (Folman *et al.,* 2008) led to the hypothesis that OM retention would select for higher abundances and diversity of cellulolytic fungi. However, contrary to these expectations, both organic matter rich OM1 and REF soils exhibited predominantly bacterial cellulolytic activity relative to OM2 and OM3, which were dominated by fungi. These differences were attributed to shifts in the relative abundance of members of *Actinobacteria* (*Streptomycetaceae*) and *Sordariomycetes* (*Chaetomiaceae*). The abundances of both *Actinobacteria* (positively) and *Sordariomycetes* (negatively) have previously been correlated to C:N ratio in litter and soil samples (Lauber *et al.,* 2008; Strickland *et al.,* 2009), revealing how OM quality can influence the decomposer community. Yet, in SIP and *in situ* pyrotag libraries, both *Sordariomycetes* and *Actinobacteria* populations were negatively correlated with C:N ratio, and C:N ratio did not significantly differ among treatments. Therefore, it is unlikely that differences in organic matter quality were driving the abundance of these groups and overall differences in cellulolytic activity.

The more plausible cause for the expansion of cellulolytic fungi according to the OM removal gradient, were long-term changes in abiotic condition such as soil temperature and moisture. The fungi which expanded were members of *Chaetomiaceae*, either *Chaetomium* (ITS classification) or *Myceliopthora* (draft genome classification). Members of both groups are thermophilic, cellulolytic 'dark septate' (melanized) fungi reportedly abundant in hot, arid environments (Berka *et al.,* 2011; Powell *et al.,* 2012) and also possess the ability to decompose furans produced by forest fire (Rajulu *et al.,* 2014). Though the decline in *Streptomycetaceae* in harvested plots was a robust trend in both SIP-cellulose and *in situ* pyrotag libraries (in B.C., PP_{CA} and LP_{TX}), it was unclear from the present data what the underlying cause might be. *Streptomycetaceae* are predominantly mesophilic organisms, with some thermophilic species (Kämpfer *et al.,* 2014), and their sensitivity to desiccation could not be established in the

literature. *Streptomycetaceae* were not the only family of Actinobacteria to exhibit declining populations in harvested plots, others included *Actinospicaceae*, *Micromonosporaceae*, *Solirubrobacteraceae*, *Streptosporangiaceae*, *Thermomonosporaceae*. Future studies of these groups may provide an explanation, but from the data gathered here, the role of OM quality or abiotic factors are unclear.

The inclusion of OM3 treatment, where organic layer soil was removed and where abiotic changes were most pronounced, led to the identification of the strongest populations trends. Yet, the forestry practice mimicked by OM3, to remove the seed bank fast-growing shrubs that outcompete seedlings, was rare even in the early 1990's when the LTSP was initiated. It was included as an extreme example of possible changes resulting from organic matter removal. Despite the limited comparison to current harvesting strategies, OM3 proved useful in identifying the rise in radiation, desiccation and heat-tolerant organisms, occurring even in OM1 and OM2. This phenomenon has largely gone unnoticed in previous surveys of harvesting impacts on soil communities (Hartmann *et al.,* 2009; Hartmann *et al.,* 2012; Holden *et al.,* 2013a; Leung *et al.,* 2016). However, the pronounced effects of OM3 may give the appearance that abiotic factors are of greater importance than biotic changes, as they were artificially most pronounced in the removal of topsoil. A closer examination of the character and size of effect between OM1 and OM2 may yield additional insights to the effects of harvesting, especially given that OM1 and OM2 treatments represent the most realistic management decisions regarding OM removal.

The extent of ecological change brought about by harvesting was reflected in the consistent expansion of sequences corresponding to mitochondrial rRNA genes classified to mosses (*Polytrichum* and *Trichostomum*) and algae (*Pavlova* and *Exanthemachrysis*) as well as the expansion of plastid rRNA genes classified to a variety of higher plants in harvested plots

(Appendix B; Figure B.1). The decline of fungi from the genus *Lecanicillium*, across all ecozones, further illustrated the scale of impact traceable through soil pyrotag libraries (Figure B.1). *Lecanicillium* are commonly entomopathic fungi with many nematophagous species (Goettel *et al.,* 2008). Their decline suggests changes occurring at higher trophic levels, possibly related to the decline of nematode populations that has been observed in the years following harvest (Persiani *et al.,* 1998; Forge and Simard, 2001). These observations reflect the broad differences in environmental conditions in harvested plots relating to a greater exposure to radiation, wind erosion, moisture loss, soil compaction, elevated temperatures, diurnal fluctuation and changes in soil chemistry/nutrients. Soil temperatures throughout the top 20-30 cm were 2 °C warmer between REF and the lowest organic matter removal treatment (OM1) and became progressively warmer with OM3 plot. Greater fluctuations in daily soil temperature were observed and soil moisture was correspondingly drier in all harvested plots. The rise of taxa from the 'Terrabacteria group,' well-regarded for their tolerance to desiccation, radiation and heat (Battistuzi *et al.,* 2009; Sghaier *et al.,* 2016), was evidence for the significant changes in microbial community brought about by the altered physical regime post-harvesting.

The expansion of Terrabacteria phyla, specifically *Actinobacteria*, *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria* and *Firmicutes*, has been reported following forest fire (Xiang *et al.,* 2014; Tas *et al.,* 2014) and in other exposed soil environments such as glacial forefelds (Rime *et al.,* 2015). Populations of members within Terrabacteria broadly increased in harvested plots, including populations of endolithic, desert-dwelling *Actinobacteria* from the family *Geodermatophilaceae* (Sghaier *et al.,* 2016). The response of all groups within Terrabacteria was not coherent, as noted with *Streptomycetaceae*, but the scale of expansion of groups that did respond was readily detectable even aggregated at the superphylum level. The increase in sequences recovered from both fungal (*Cladonia sp.* and *Lecanorales*) and cyanobacterial

substituent of lichen, and lichenolous fungi, and desert or rock-inhabiting fungi further indicated the shift in harvested plots towards desiccation, radiation and heat-tolerant organism. Many taxa flourishing in harvested plots resembled those found in glacial forefelds, where mostly barren soil is exposed to high levels of UV radiation, temperature and moisture fluctuation, with even similarities in pH, total carbon and nitrogen (Rime *et al.*, 2015). These taxa included *Clostridium*, *Cupriavidus*, *Geobacter*, and *Massilia* (bacteria) and *Cryptococcus*, *Cladophialophora* and *Tetracladium* (fungi).

Shifts in the functional character of soil communities of stress-tolerant taxa has the potential to impact soil chemistry during the decades of forest renewal. This phenomenon has possibility to compound over multiple harvesting cycles given the capacity of hardier species to persist in soil. Sampling for this research occurred just prior to canopy closure, a moment during forest regeneration where conditions selecting for stress-tolerant taxa may begin to wane, but following a significant period of time during which populations could influence soil chemistry. The expansion of thermophilic sulfate reducers *Syntrophobacteraceae* (Kuever *et al.*, 2014) was a major feature in BC ecozones, while populations of metal-reducing bacterial genera, *Geothrix* and *Geobacter*, expanded in the majority of all ecozones to sizeable population (~0.2% of total reads). Long-term changes in bulk soil chemistry may even result from differences in the quality of microbial biomass. Stress-tolerant taxa typically have unique cell envelope structures, such as Gram-positive bacteria, which persist longer in soils than their Gram-negative counterparts (Throckmorton *et al.*, 2012).

The functional character of cellulolytic populations was altered by the dramatic shift in relative abundance of *Chaetomiaceae* and *Streptomycetaceae* in OM2 and OM3, leading to reduced cellulolytic activity. Similar long-term changes in relative abundance of saprotrophic fungi and Gram-positive bacteria were observed in regenerating forests seven (Lewandowski *et*

al., 2015), fifteen (Hartmann *et al.,* 2009; Hartmann *et al.,* 2012) and forty years after timber harvesting (Chatterjee *et al.,* 2008) and following forest fire (Xiang *et al.,* 2014). Populations of *Sordariales*, including the family *Chaetomiaceae* expanded in logged forests in Southeast Asian tropical forests (McGuire *et al.,* 2014), as well as following large-scale tree die back due to insect herbivory (Šturs ová *et al.,* 2014), revealing these trends may be broadly applicable to forest disturbance. The conclusion that an increase in relative abundance of cellulolytic fungi resulted in decreased cellulolytic activity is at odds with the conventional view that fungi predominate the breakdown of recalcitrant plant polymers. However, there are a number of cases in which soil properties, nutrient availability and litter quality influence whether decomposition is predominantly fungal or bacterial. The following studies demonstrated bacterial decomposition can predominate with changes in the N:P ratio (Güsewell and Gessner 2009), minimization of disturbance (Jastrow *et al.,* 2007), and at higher soil pH (Strickland and Rousk 2010). The inverse relationship between cellulolytic activity of *Streptomycetaceae* and *Chaetomiaceae* (family of *Sordariomycetes*) populations, and an overall reduction in respiration, reported here was not without precedent. Strickland *et al.,* (2009) demonstrated that the abundance of *Sordariomycetes* was negatively correlated with net respiration during litter decomposition, while the reverse was true for *Actinobacteria*. These observations suggest that the underlying cause of the reduction in respiration and rate of decomposition commonly found in harvested plots (Whitford *et al.,* 1981; Yin *et al.,* 1989; Prescott *et al.,* 2000; Webster *et al.,* 2016), may result from biological changes, not solely abiotic constraints.

The decline of more susceptible taxa may also alter the functional character of soil communities. Ectomycorrhiza were the most prominent group of fungi, occupying between 10 to 50% of ITS libraries, and were also one of the populations most clearly impacted by harvesting. The decreased relative abundance and diversity of EM populations in harvested plots was

consistent with expectations, given the removal of tree hosts and previous characterizations of harvesting impacts at B.C. LTSP sites (Hartmann *et al.,* 2012). So, too, was the strong expansion of a small minority of EM taxa, namely *Rhizopogon*, *Suillus*, *Wilcoxina*, *Tomentella* and *Thelephora* that are commonly observed as early-colonizers of young conifer stands (Visser, 1995) and in the years and decades following forest fire (Buscardo *et al.*, 2015; Glassman *et al.*, 2015; Oliver *et al.,* 2015) and timber harvesting (Hartmann *et al.*, 2012; McGuire *et al.*, 2014). The success of these EM fungi may be due to their ability to outcompete other EM species during root colonization (Cairney and Chambers 1999) or their resistance to high temperatures (Horton *et al.*, 1998; Baar *et al.*, 1999; Peay *et al.,* 2009). One EM species of *Thelephora* was consistently more abundant at OM1 sites, lending evidence to the claim that some EM may function as facultative decomposers (Lindahl and Tunlid, 2015). Yet, the recent re-classification of a number of *Thelephora sp.* as *Odontia*, a non-EM, putatively saprobic group (Tedersoo et al., 2014), suggests that the phylogenetic delineations of saprobic and EM lifestyles between *Thelephora* and *Odontia* are not yet clear. *Thelephora* represent an interesting lifestyle, documented as cryptic, epiphytic by Ramírez-López *et al.,* (2013). Compositional shifts in EM community have clear implications for the long-term ecology of forest plantations, especially given that, of all EM genera observed to expand, only *Rhizopogon* are known to stimulate host growth (Cairney and Chambers 1999). Yet, mycorrhizal communities demonstrate successional changes according to stand maturity (Visser, 1995; Twieg *et al.,* 2007), and early-stage EM may fulfill nutritional needs of young plantations (Kranabetter, 2004), exemplified by the nitrogen fixing activity in *Suillus tomentosus* tubers (Paul *et al.,* 2012). Thus, the potential long-term consequences of changes in EM abundance and diversity need to be studied in greater detail and in relation to planned harvesting cycles.

The decline of a number of rhizospheric bacteria also has the potential to alter the functional character of regenerating forest soils. All but one of the indicator taxa classified as *Alphaproteobacteria* and *Gammaproteobacteria* were indicative of population decline and nearly all of the genera identified (*Ancylobacter, Caulobacter, Labrys*, *Luteibacter, Rhodanobacter* and *Sphingobium*) have isolates characterized from the rhizosphere. These taxa were consistently more abundant in organic layer soils, revealing that they are exposed the greatest changes in terms of exposure to heat and desiccation. Yet, similar to the phenomenon observed with EM fungi, the population of a common rhizospheric genus, *Methylobacterium* (*Alphaproteobacteria*), was drastically expanded in organic layer soils from harvested plots across all ecozones (26-fold in PP_{CA}). Some members of *Methylobacterium* possess extreme radiation and desiccation tolerance, such as *Methylobacterium radiotolerans* (Noguiera *et al.,* 1998; Rokitko *et al.*, 2003). Members of *Methylobacterium* also possess plant-growth promoting (Abanda-Nkpwatt *et al.*, 2006; Kutschera *et al.*, 2007) and nitrogen fixing capabilities (Renier *et al.*, 2008), revealing that shifts in the composition of rhizospheric bacteria have the possibility to be neutral or even have positive effects on plant regeneration. A previous LTSP study found no differences in the rhizospheric bacterial populations of lodgepole pine seedlings in a comparison of OM2 and OM3 (Chow *et al.*, 2002), though this study may have missed the larger effect between unharvested and harvested plots. Future study of the impacts of harvesting on EM fungi may be complemented by considerations of the potential impacts on rhizospheric bacteria.

Given the scale of forestry operations in North America and threat of greenhouse gas-induced climate change, one of the most serious consequences of timber harvesting may be the sizeable reduction of methane oxidation by soil-borne methanotrophs. Temperate forest soils are significant atmospheric methane sinks due to these populations (Henckel *et al.*, 2000; Kolb *et al.*, 2005) and harvesting has been widely reported to decrease methane uptake over the short (Castro

et al., 2000; Zerva and Mencuccini, 2005; Takakai *et al.,* 2008; Kulama *et al.,* 2014) and long term (Wu *et al.,* 2011; Nazaries *et al.,* 2011). In one case, this phenomenon was attributable to declining abundances of *Methylocapsa*-related, type-II methanotrophs (Nazaries *et al.,* 2011), which was a major trend in harvested plots across all ecozones in the present research. No explanation for this phenomenon has yet been proposed, though analysis performed for this thesis revealed that *Methylocapsa* were more abundant in upper organic layer soil and negatively correlated with pH ($r=-0.39$, $p < 0.001$). It is reasonable to suspect that these populations may be adversely affected by exposure to the warmer, drier soils and compounded by the slight increase of pH found at harvested plots. Notably, in two ecozones, PP_{CA} and JP_{ON}, the retention of woody debris in OM1 had a net positive effect on population sizes, and, overall, retention was seen to mitigate the decline of *Methylocapsa*. Further research is needed to understand what is driving the decline of *Methylocapsa* and whether biomass retention may be a viable mitigation strategy.

The long-term rise of stress-tolerant groups in harvested plots, reported here, resembled the compositional shifts found in the decades following forest fire. Both types of disturbance did not alter overall diversity, as the expansion of heat-tolerant taxa did not dominate communities, nor did susceptible taxa disappear altogether, as evidenced in repeated burnings of forests plots (Oliver *et al.,* 2015). Further, the decline in relative abundance of *Basidiomycota* in favour of *Ascomycota* was consistent with long-term changes in fire-affected soils (Buscardo *et al.,* 2015; Holden *et al.,* 2013b). The expansion of arbuscular mycorrhiza, from phylum *Glomeromycota*, in BS_{ON} (undetected in REF and ~1% of total reads in OM3) was consistent with their rapid rebound post-fire (Xiang *et al.,* 2015). The decrease in *Verrucomicrobia* (*Spartobacteria* & *Opitutus*) observed among a majority of ecozones was consistent with their decreased abundances in fire-affect soils upwards of seven-years post-fire (Tas *et al.,* 2014; Weber *et al.,* 2014). One of the most notable similarities between the two disturbances was the sizeable

expansion of populations of candidate phylum *AD3*, which quadrupled in OM3 of JP_{ON} (~ 8% of the total library) and was a major finding of Tas *et al.,* (2014) where *AD3* populations had expanded by ~ 68% seven years after forest fire. Nothing is known about the ecology or physiology of AD3, but this research suggests they may possess adaptations to post-disturbance conditions or may be associated with the rhizosphere of ruderal plant species, given the similar pattern of expansion in plant mitochondrial and chloroplast DNA in JP_{ON} (Figure B.1).

Soil microbial diversity decreased in forested land converted to agricultural use (Rodrigues *et al.,* 2013; Paula *et al.,* 2014), yet comparisons between primary and secondary forests typically note no discernible changes in diversity (Lauber *et al.,* 2008; Paula *et al.,* 2014; Štursová *et al.,* 2014; Oliver *et al.,* 2015). The impacts of timber harvesting on microbial diversity reported here were also not striking. The only consistent trend was the increased diversity of fungal populations in mineral soils at all harvesting intensities for five out of six ecozones. In every case, the increase in diversity was driven by an increase in both species richness and the evenness of populations. McGuire *et al.,* (2014) found a similar, though slight, increase in fungal diversity in a 50-year-old secondary forest relative to unharvested controls, but there is a paucity of data on this potential phenomenon and no proposed explanation as of yet. Globally, biodiversity is strongly correlated with temperature purportedly due to the kinetics of biochemical reactions (Allen *et al.,* 2002). In one study of fungal populations, higher mean annual temperature was the main driver of increased diversity in forest soils along an altitudinal gradient (Bahram *et al.,* 2011). It is possible that the increased diversity in mineral soil fungal populations may be driven by increased temperature, though it is unclear why organic layer populations do not exhibit a similar trend. Hartmann *et al.,* (2012) hypothesized that increased fungal evenness in harvested plots (diversity was not described) may relate to a loss of functional organization, due to the loss of EM and expansion of more diverse saprotrophic populations. Yet,

the two ecozones exhibiting the greatest increase in overall fungal diversity in mineral soils (SBS_{BC} and JP_{ON}) did not exhibit significant or exceptionally strong changes in EM abundance or diversity among harvested plots. In cellulose incubations, the decreased richness of ^{13}C-enriched fatty acids with increasing organic matter removal was another indication that harvesting can reduce the diversity of other sub-populations than just EM fungi. However, this trend may or may not reflect the actual diversity of cellulolytic taxa given that lower richness of ^{13}C-labeled PLFAs could also result from a reduced interdependency of organisms feeding on cellulolytic organisms or their by-products, suggesting, rather, a degree of decreased trophic complexity. Trends in diversity have unclear implications on the long-term impacts of timber harvesting, though diversity has been linked to the robustness of soil processes (Griffiths and Philippot, 2013), and may be of secondary importance to studying the nature of change brought about by shifts in specific taxa.

The distinct composition of microbial communities among ecozones was a source of ecozone-specific responses to harvesting. Localized responses were mainly the result of differences in the relative abundance, even presence or absence, of taxa among ecozones. The irregular distribution of many taxa was expected given the large differences in edaphic factors, historical soil development and plant overstory among ecozones. A recent biogeographical survey of *Streptomyces*, part of the same family as *Kitasatospora*, found species distributions were affected by latitude, which was attributed to the last glaciation (Choudoir *et al.*, 2016). This study reveals geography can play a major role in species distribution across the continent, a feature which was evident in our data. *Kitasatospora* exhibited variation in species and response to harvesting according to east and west populations, perhaps resulting from the boundary presented by the Columbian mountain range.

The impacts of harvesting on any given population may also vary depending on changes in local environmental conditions (climate, soil type etc.) brought about from harvesting. For example, populations of *Boletales* showed stark increased in harvested plots in northern ecozones, yet declined in southern locations. A plausible explanation for this pattern might be that soil warming in northern climes had a positive effect on organisms with higher temperature growth optima, but temperature extrema rose above tolerance thresholds in southern sites.

Differences in response to harvesting between closely-related species suggests that ecotypic variation may be factor in localized responses. Ecotypic variation in genome content may confer different tolerance traits and has been commonly observed among organisms sharing highly similar (Rocap *et al.*, 2003) or even identical copies of the 16S rRNA gene (VanInsberghe *et al.*, 2015). However, the impacts of such variation are rarely considered in broad characterizations of disturbance on microbial communities. Youngblut *et al.*, (2012) found that species and strain level responses to disturbance played an important role in the potential ecological impacts of disturbance. Timber harvesting produced different responses among a number of closely related genera (*Pseudotomentella spp.* and *Tomentella spp.*) and species (within *Kitasatospora, Rhizopogon* and *Suillus*), demonstrating the necessity of both fine-scale and broad characterizations of community structure. Focusing attention on resolving fine-scale phylogenetic has the secondary benefit of identifying novel phenotypes, aiding both our understanding of the physiology of forest soil microorganisms and disturbance ecology.

The degree to which biogeographical differences may limit generalizations about the long-term effects of harvesting was evident in the absence of many common trends in LP_{TX}. No remarkable differences in the abundance or diversity of EM fungi or relative abundances of *Basidiomycota* and *Ascomycota* were observed in LP_{TX}, which were major features of all other ecozones. LP_{TX} was the only ecozone in which harvesting treatments were insignificant in

explaining variation in fungal populations. LP_{TX} was an outlier in terms of other broad trends such as the expansion of candidate phylum *AD3* and unclassified sequences as well as the decline of *Verrucomicrobia*. The distinctive lack of common trends observed in LP_{TX} mirrors the general distinctiveness of its community which had double the number of unique bacterial taxa (7,400) relative to other ecozones and the second highest number of unique fungal taxa. Lignocellulolytic populations, described in Chapter 4, were also largely unique in LP_{TX}. Conditions in LP_{TX} soils were markedly different, containing less than half the total C and total N found at other sites and had the most acidic of any mineral layer soil. The conditions, coupled with the fact LP_{TX} had the lowest soil moisture content and highest mean annual temperature, suggest that the microbial community may be composed of taxa already adapted to the harsher conditions caused by harvesting. Another critical consideration is that LP_{TX} REF plots were the only plots that had been previously harvested (~75 years prior to sampling), and still exhibited signs of past disturbance such as skid roads (Andrew Scott, USDA, personal communication). Historically, the LP_{TX} forests have been managed with fire for many hundreds of years by humans and are also prone to lightning fires (Andrew Scott, USDA, personal communication). The lack of clear distinction between REF plots and harvested plots in LP_{TX} may exemplify the equilibration of soil communities to long-term exposures to disturbance (i.e. a perpetual disturbed state). A third explanation, none of which are mutually exclusive, may be that microbial communities have rebounded more quickly to a pre-harvest conditions due to the faster forest regeneration observed at LP_{TX} sites (Ponder *et al.*, 2012), where trees reach maturity in approximately half the time (~ 25 year cycles). Thus, LP_{TX} exemplifies how biogeographical factors and land-use legacy can influence the potential impacts of harvesting. As a case study, LP_{TX} may help reveal which organisms and microbial process have the greatest effect on

regeneration by correlating differences in community composition with measures of forest productivity in perpetually disturbed and less disturbed sites.

One important caveat to consider when interpreting data presented in Chapter 3 is that OM removal affected the depth of organic layer soil development. Therefore, abundances in organic soils from harvested plots were unavoidably weighted towards surficially abundant taxa. This distortion would likely favour taxa adapted to radiation, desiccation and heat which would thrive in surficial soil. Yet, stress-tolerant taxa also flourished in mineral layer soils including a number of desert-dwelling taxa, such as *Ramlibacter*, *Lysobacter*, *Rubrobacter* and *Clostridium*, and thermophilic fungi. Soil temperature was elevated along the full depth profile and, presumably, so too was higher degree of dryness, though the mineral samples processed did not notably differ in moisture content. In future, this bias may be avoided by sampling more finely along the soil profile.

Substantiating the SIP method, the majority of taxa we identified (~75%) had previously documented cellulolytic activity, including well-characterized groups such as *Actinobacteria*, *Bacteroidetes*, *Cytophaga Myxococcales* and *Sordariomycetes*. The greatest number of novel cellulolytic taxa were associated with mineral layer soil, which, in our microcosms, demonstrated comparable cellulolytic activity to organic layer populations. These novel taxa included *Armatimonadetes* from candidate division *FBP*, with no representative genome or isolate, and members of the ubiquitous, yet poorly characterized phylum, *Candidatus Saccharibacter* (formerly TM7), of which we were able to recover a partial draft genome (~0.4 Mb). Other members of *Armatimonadetes*, as well as members of *Verrucomicrobia* and *Planctomycetes*, were also designated cellulolytic and associated with the mineral layer. These three groups have few cultured representatives, but at least one of each is known to degrade cellulose (Sangwan *et al.*, 2004; Dedysh *et al.*, 2013; Lee *et al.*, 2014). Populations of fungal of

cellulose-degraders were represented by largely well-known saprotrophic fungi, such as *Clitopilus*, *Humicola*, *Myceliopthora* and *Chaetomium*. The identification of members of *Sebacinaceae*, a group of saprobic and mycorrhizal fungi, as cellulolytic was novel, but consistent with circumstantial evidence that *Sebacinaceae* played a role in decomposition of maize lignocellulose in agricultural soils (Kuramae *et al.*, 2013).

Our research builds upon previous studies that demonstrated a consistent long-term impact of timber harvesting on forest soil microbial communities. Here, we provide evidence that organic matter removal during harvesting impacted soil populations, in particular at the extreme in OM3, and that changes in soil physical properties, such as temperature and moisture content, may be equal to or more important than changes in organic matter quality in mediating this impact. Canopy closure had occurred in the years immediately prior to sampling in most ecozones. Thus, sampling occurred at a time just after soils experienced the longest period of heightened exposure to solar radiation, soil drying and temperature. Outside of groups with known importance to forest ecology, such as EM fungi, the impacts of shifting microbial populations during these first decades of regeneration are unknown. In the case of stress-tolerant taxa, any impact will likely depend on their sustained activity as forests mature and whether they persist across harvesting cycles. The potential legacy effect could be strong. For example, in cellulolytic populations, the early colonization of decaying woody debris can influence succession and ultimately the quality of decomposition (Song *et al.*, 2015). Further, the totality of the changes brought about by timber harvesting are far from clear, exemplified by the expansion of unclassifiable sequences in harvested plots. As such, this work contributes a foundation upon which future research can build towards better understanding the impacts of forest disturbance and what soil processes underlie variation in forest regeneration across North America.

Chapter 4: Survey of Lignocellulolytic Populations of Forest Soils from Across North America with Stable Isotope Probing

4.1 Rationale

The following section describes a broad survey of hemicellulolytic, cellulolytic and lignolytic populations from soil layers and ecozones previously described in Chapter 3. Samples were selected to maximize the diversity of lignocellulolytic taxa observed in order to i) attribute activity to an array of novel uncultured taxa, ii) assess the prevalence of previously characterized taxa in an environmental context; iii) determine if the overall rate of decomposition during incubations was correlated with community composition; and iv) study the biogeography of the decomposer communities. The contributions of bacterial and fungal degraders were contrasted given the recent evidence that bacterial lignin-degrading activity may be underestimated (Bugg *et al.*, 2011). All three major polymers of lignocellulose were tested in hope of identifying taxonomic groups that possess specialization for the degradation of recalcitrant plant matter.

4.2 Results

SIP-microcosms amended with ^{13}C-labeled bacterial cellulose (99 atom % ^{13}C) were performed on samples from all ecozones except SBS_{BC}, while SIP-lignin (DHP-lignin; 75 atom % ^{13}C) was performed only on PP_{CA}, BS_{ON} and LP_{TX} and SIP-hemicellulose only on IDF_{BC} and PP_{CA}. All datasets utilized in Chapter 4 are summarized in Table E.4. In brief, DNA from SIP-cellulose and SIP-lignin microcosms was sequenced to produce: i) 59 cellulose and 64 lignin pyrotag libraries (~ half ^{12}C-controls) averaging 8,400 high-quality 250-bp reads per sample; ii) 100 corresponding PLFA profiles (including ^{12}C-controls); iii) 38 cellulose and 46 lignin shotgun metagenomic libraries with an average of 86 million and 56 million quality filtered 100 bp reads per library, respectively, and iv) eight shotgun metagenomic libraries from mineral soil incubated

with ^{13}C-lignin with or without fungicide, averaging 63 million reads per library. All raw sequencing libraries can be found at the European Sequencing Archive under the project accession PRJEB12502. Pyrotag libraries were supplemented with 50 SIP-hemicellulose libraries (~ half ^{12}C-controls) from Leung *et al.,* (2016) and 94 '*in situ*' libraries from corresponding reference field samples from Chapter 3.

4.2.1 Characterization of ^{13}C-Enrichment by Substrate, Soil Layer and Ecozone

Time-course experiments revealed low-level enrichment as early as four days into incubations with labeled cellulose and lignin (Figure 3.26). The enrichment of microbial biomass (DNA and PLFA) was comparable between organic and mineral layers in cellulose (~ 15% atom C% in DNA), while enrichment occurred more gradually from lignin and was lower in the modified organic soils than in mineral soils, at 8 and 11 atom C% DNA, respectively (Figure 4.1). Measures of enrichment in DNA and PLFA were in close agreement, demonstrating that organisms assimilating ^{13}C were growing and replicating. Improved assembly of metagenomes derived from ^{13}C-enriched DNA demonstrated a reduced diversity of genomic content, indicative of a selection for sub-populations (Figure 4.2).

The ^{13}C-enrichment of microbial biomass was comparable among ecozones except for a few stand-out cases. The assimilation of cellulose was considerably greater in IDF_{BC} than in any other organic layer soil. Cellulolytic and lignolytic taxa from LP_{TX} mineral soil had the highest activities of any lignocellulolytic populations. Notably, the quality of assembly for these stand-out samples was lower than other samples (Figure 4.2), possibly resulting from the higher proportion of fungi found at these sites whose larger, more complex genomes were less readily assembled (see section 4.2.4).

Of the initial eighty-four microcosms incubated with ^{13}C-lignin, approximately ¼ of lignin samples did not yield sufficient DNA or sufficiently clean DNA for PCR amplification and approximately half of metagenomic library preparations failed. Texan soils, in particular, contained high levels of inhibitory compounds which were partly remedied for PCR by diluting template DNA. However, this was not an option in preparing metagenomic libraries due to concentration requirements. A polyvinylpyrrolidone treatment was attempted, but did not help.

Despite comparable levels of enrichment in soil DNA extracts from both cellulose and lignin microcosms, the net recovery of ^{13}C-enriched DNA from lignin was far lower than from cellulose (Figure 4.3). Low DNA yields for lignin samples necessitated the use of Nextera XT library preparation which required only 1 ng of DNA. A comparison between the Nextera and Nextera XT prep kits demonstrated the two methods were comparable and that the ability to pool fewer fractions, afforded by the XT kit, resulted in greater differentiation between ^{12}C- and ^{13}C-libraries (Figure 4.4).

The assimilation of ^{13}C-carbon by non-functional populations (i.e. cross-feeding) was not apparent in preliminary time-course experiments. Assuming that PLFAs lacking enrichment at Day 4, when the active community had already become slightly enriched, represent the non-functional community (also likely the slow growing functional community), the total enrichment of 'non-functional' PLFAs was less than 5% by the end of the time course. Select PLFAs showed increasing enrichment over-time, while a broad pattern of progressive enrichment, what might be indicative of cross-feeding, was not observed (Figure 4.5). However, in experimentation with fungicide, the co-decline of fungal and bacterial enrichment suggested cross-feeding (see section 4.2.4 for more details).

Figure 4.1. Overview of the ^{13}C-enrichment of microbial biomass according to substrate, ecozone and soil layer in delta-^{13}C in PLFA (A) and % atom ^{13}C in soil DNA extract (B). In panel A, control microcosms incubated with ^{12}C-compounds are shaded pink.

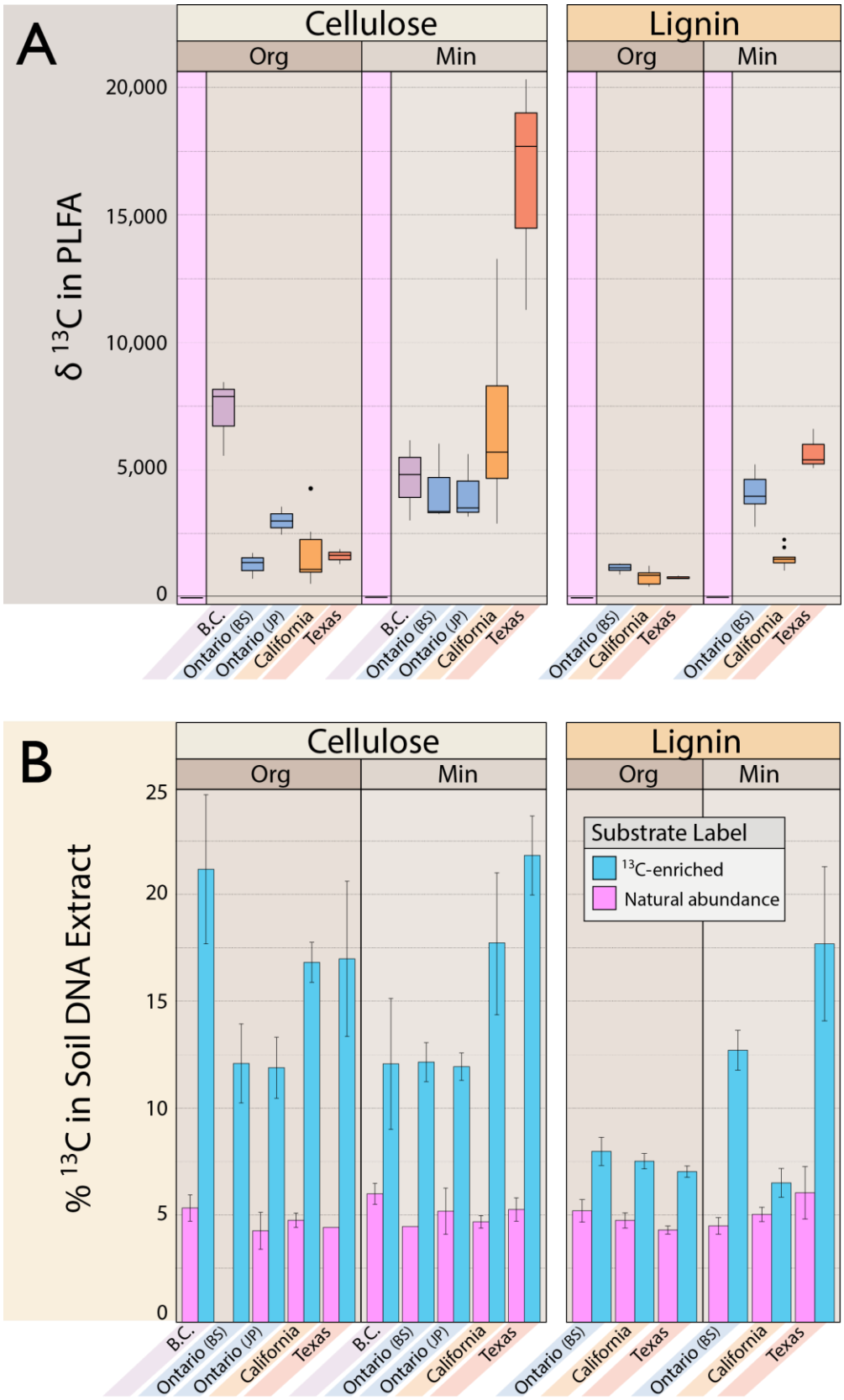


Figure 4.2. Comparison of metagenome assembly according to substrate, ecozone and soil layer as evidence for the selection of lignocellulolytic subpopulations and decrease in overall complexity in SIP metagenomes.

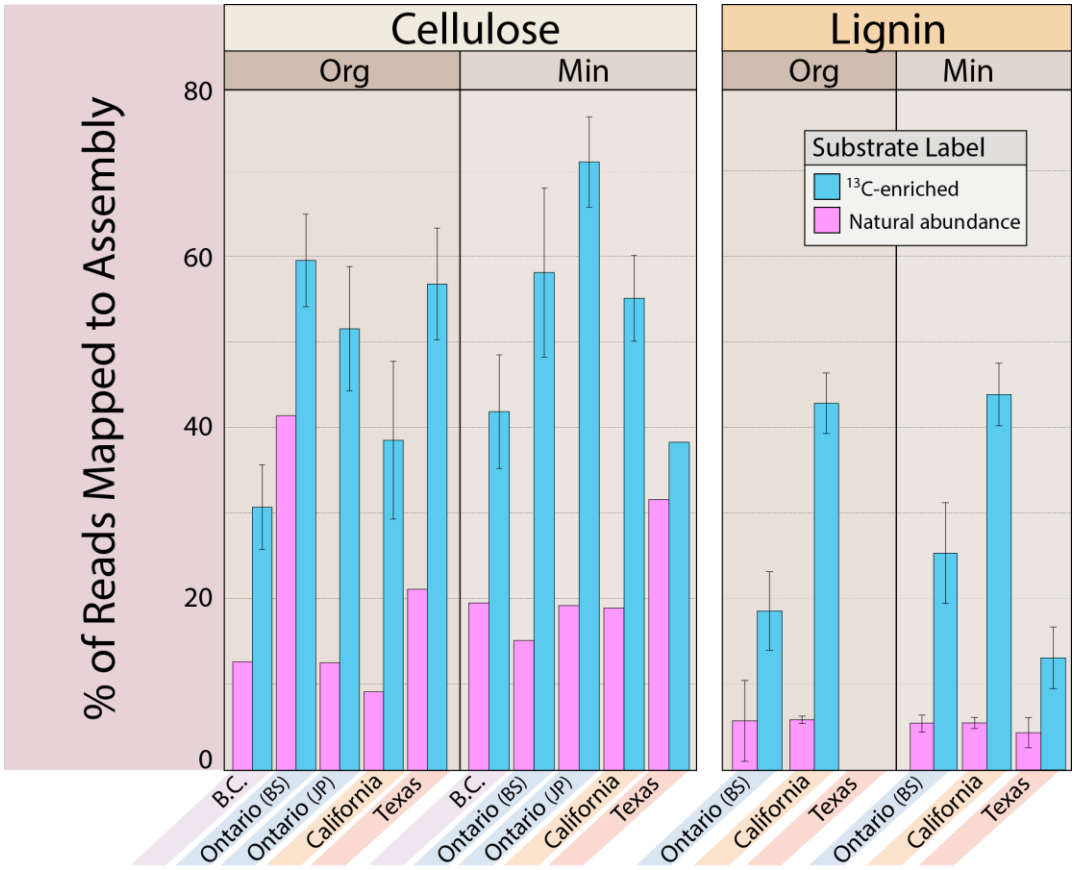


Figure 4.3. Average mass of DNA recovered from the densest CsCl gradient fractions according to substrate. Identical patterns were observed in both mineral and organic soil layers. Pyrotag and metagenomic libraries were created from DNA recovered from $F_{1\text{-}7}$, though additional fractions had to be pooled to recover sufficient DNA for the occasional ^{12}C-control library.

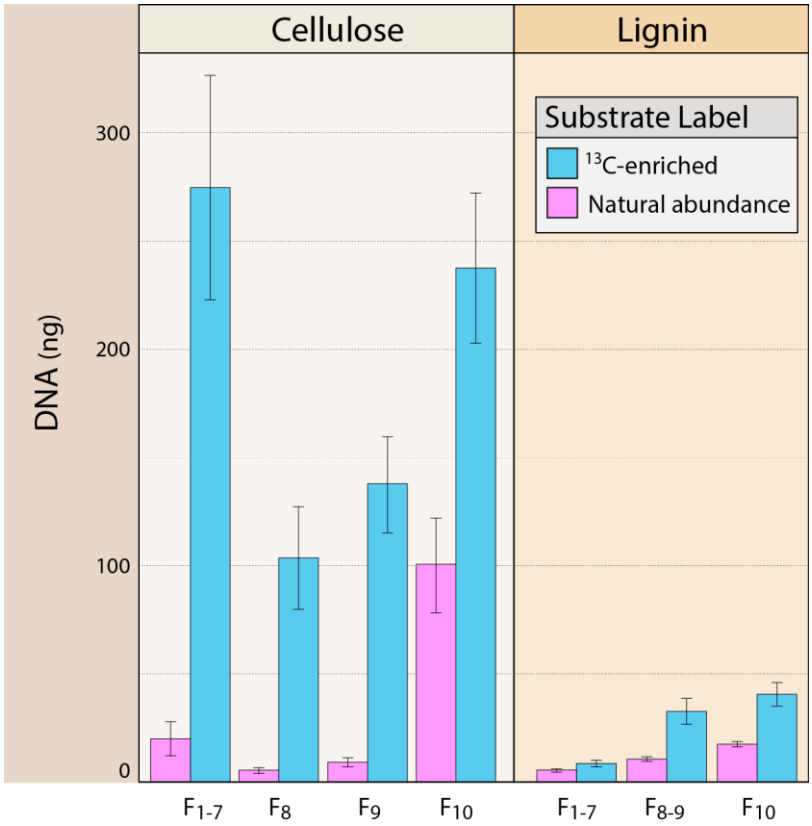


Figure 4.4. Comparison of metagenomic libraries prepared using either 'Nextera' (50 ng DNA) or Nextera 'XT' (1 ng DNA) kits based on LCA classification of unassembled reads at the rank order. Two samples incubated with lignin were arbitrarily selected and fractions heavy fractions were sequenced from ^{12}C and ^{13}C-pairs. In order to recover 50 ng of DNA for 'Nextera' prep, fractions F_1-F_9 were pooled, while fractions F_1-F_7 were used for 'XT'. Only taxa occupying greater than 0.5% of total library are shown. Taxa found in both ^{12}C and ^{13}C-libraries are coloured solid, while taxa only found in ^{13}C-libraries in black and white pattern.

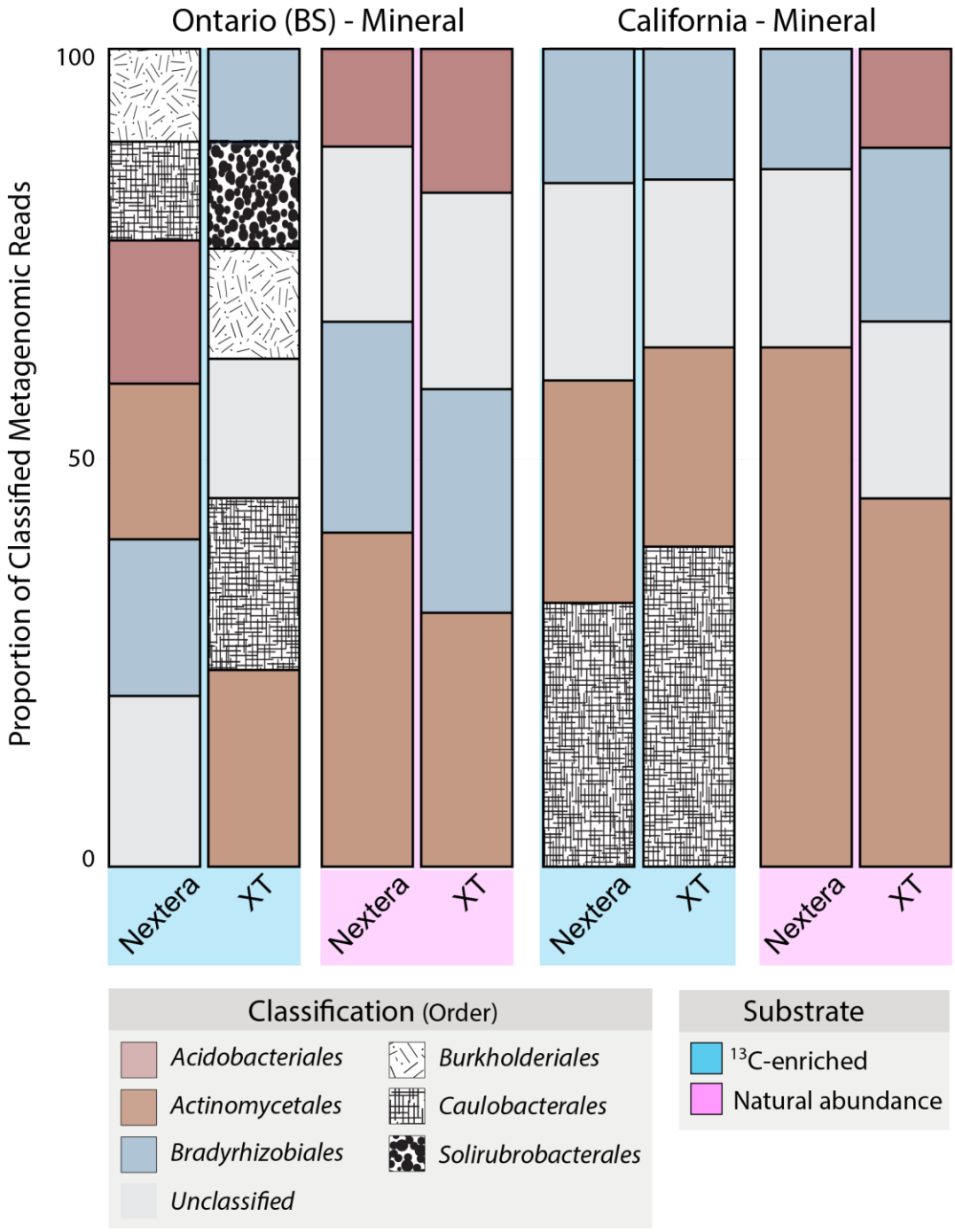
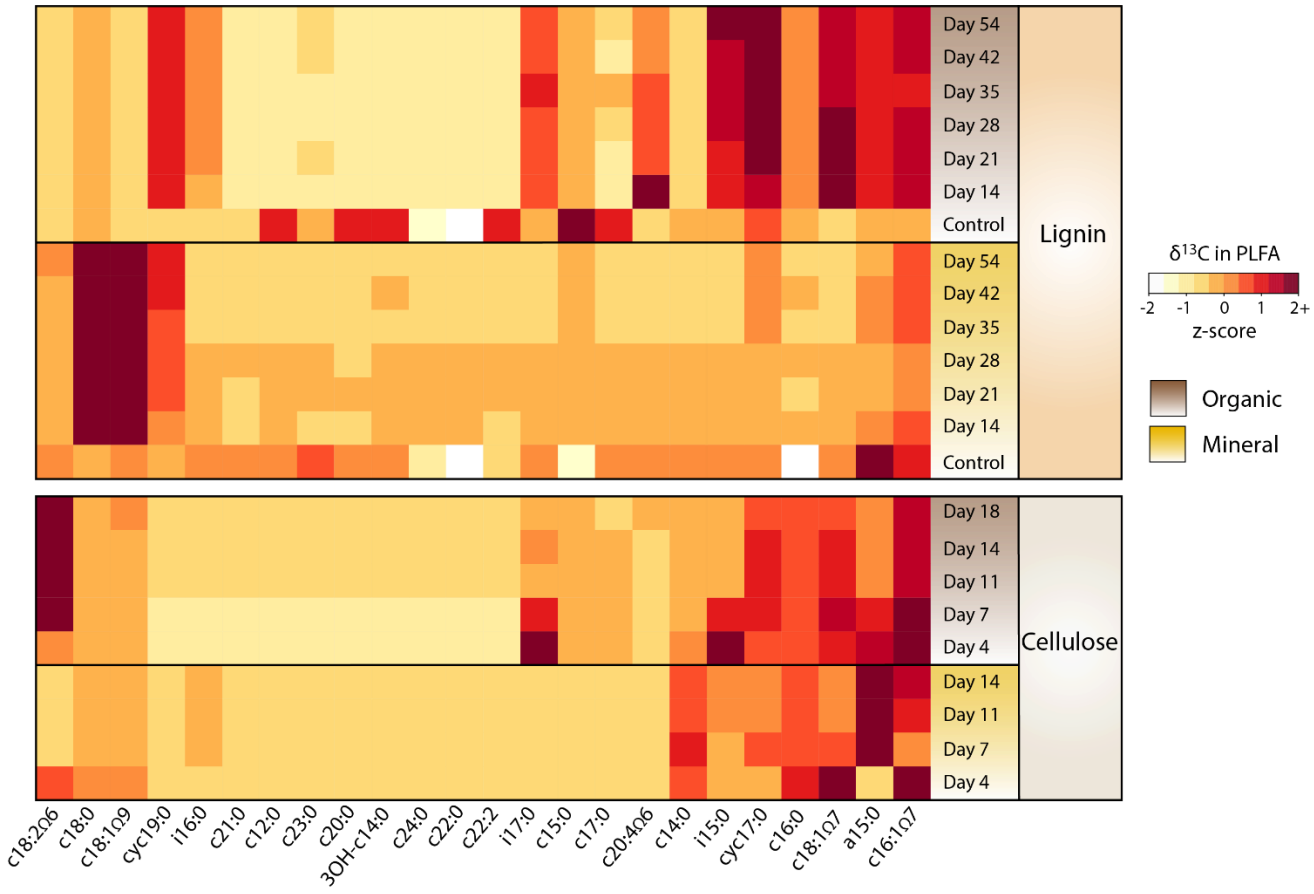


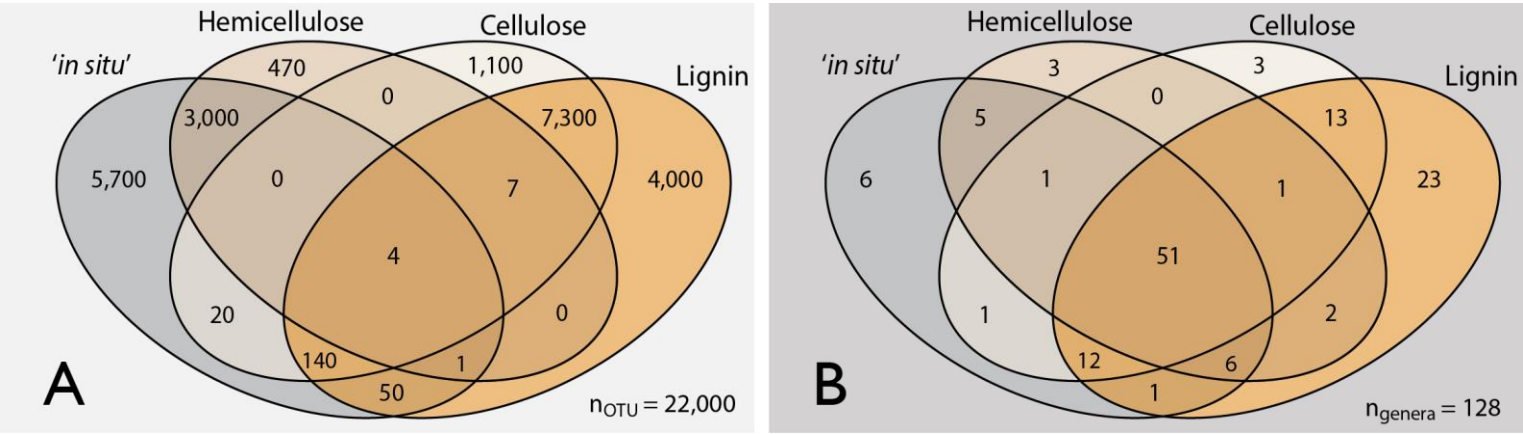
Figure 4.5. Heat map of the normalized delta-^{13}C enrichment of PLFAs across time-course experiments for both soil layers and substrates. Each time-point is represented by a single sample. Samples were sacrificed, not repeatedly sampled.



4.2.2 Comparison of Hemicellulolytic, Cellulolytic and Lignolytic Taxa

Among 16S rRNA gene pyrotag libraries, ^{13}C-cellulose and ^{13}C-lignin libraries shared few common OTUs with *in situ* libraries and even fewer with ^{13}C-hemicellulose libraries based on clustering at 0.01% (Figure 4.6A). Conversely, *in situ* and ^{13}C-hemicellulose libraries shared substantial overlap. More overlap was observed based on taxonomic classifications of genera (Figure 4.6B). The alignment of all pyrotag libraries was manually validated and, while sequencing libraries were prepared by different people, the same methods, primer stocks, and sequencing facility were used and raw files were processed concurrently in Mothur. Differences may be explicable given the substantially shorter incubation period used for hemicellulose (2 days) versus cellulose (~14 days) versus lignin (~60 days), as well as the possibility that SIP substrates enriched for largely different functional populations.

Figure 4.6. Venn diagrams depicting overlapping OTUs (A) and genera (B) among ^{13}C- and *in situ* pyrotag libraries for all lignocellulose substrates.



Differences existed between populations decomposing lignin and cellulose polymers based on profiles of PLFA ^{13}C-enrichment (Figure 4.7; hemicellulose data not available). The most discriminating feature between cellulose and lignin-degrading populations was the

enrichment of fungal PLFAs (c18:1Ω9 and c18:2Ω6) and a number of longer chain PLFAs associated with eukaryotes (likely fungal) in cellulose microcosms. The prominence of fungi in SIP-cellulose samples, and relative absence in SIP-lignin samples, was supported by metagenomic data (Figure 4.8). Markers for Gram-positive bacteria (branched PLFAs i16:0 and i17:0) were also correlated to ^{13}C-cellulose PLFA profiles, whereas one Gram-negative PLFA (3OH-c14:0) was correlated to ^{13}C-lignin profiles in organic soils. These differences in the utilization of cellulose and lignin were reflected in the prevalence of *Actinobacteria* (Gram-positives) in ^{13}C-cellulose pyrotag libraries and *Betaproteobacteria* and *Alphaproteobacteria* (Gram-negatives) in ^{13}C-lignin pyrotag libraries (Figure 4.9). Cellulolytic and lignolytic populations were also discriminated by unidentifiable PLFAs ('UF'; i.e. PLFAs not present in the standard used for peak identification). Lignin-degrading taxa from LP_{TX} soils were substantially different than degraders from other ecozones, consistent with observations throughout this thesis of the unique community therein.

NMS of pyrotag libraries reinforced a number of differences between cellulolytic and lignolytic populations and helped further identify which groups were driving differences (Figure 4.10). ^{13}C-cellulose libraries demonstrated greater clustering and diverged more clear from their paired ^{12}C-libraries in contrast to lignin libraries. *Burkholderiales* and *Caulobacterales* were clearly associated with, and more abundant in, both ^{13}C-cellulose and ^{13}C-lignin libraries. Other groups that clustered with ^{13}C-cellulose libraries included *Cytophagales*, *Opitutales* (*Verrucomicrobia*), *FW68* (*Armatimonadetes*), *BD7-3* (*Planctomycetes*), *Myxococcales* (*Deltaproteobacteria*) and *Actinomycetales*. Groups that clustered with ^{13}C-lignin libraries (left side and bottom of Figure 4.10) included *Elusimicrobiales* and *FAC88* (*Elusimicrobia*), *Solibacterales* and *iii1-15* (*Acidobacteria*), *Gaiellales* and *Solirubrobacterales* (*Actinobacteria*) and *Sphingomonadales* (*Alphaproteobacteria*).

Figure 4.7. Principal components analysis of delta-^{13}C enrichment of PLFAs for cellulose and lignin and both soil layers. All included samples were amended with a ^{13}C-labeled substrate (i.e. controls not included). Vectors were fitted for individual PLFAs to illustrate the relative contributions to the differentiation of enrichment profiles.

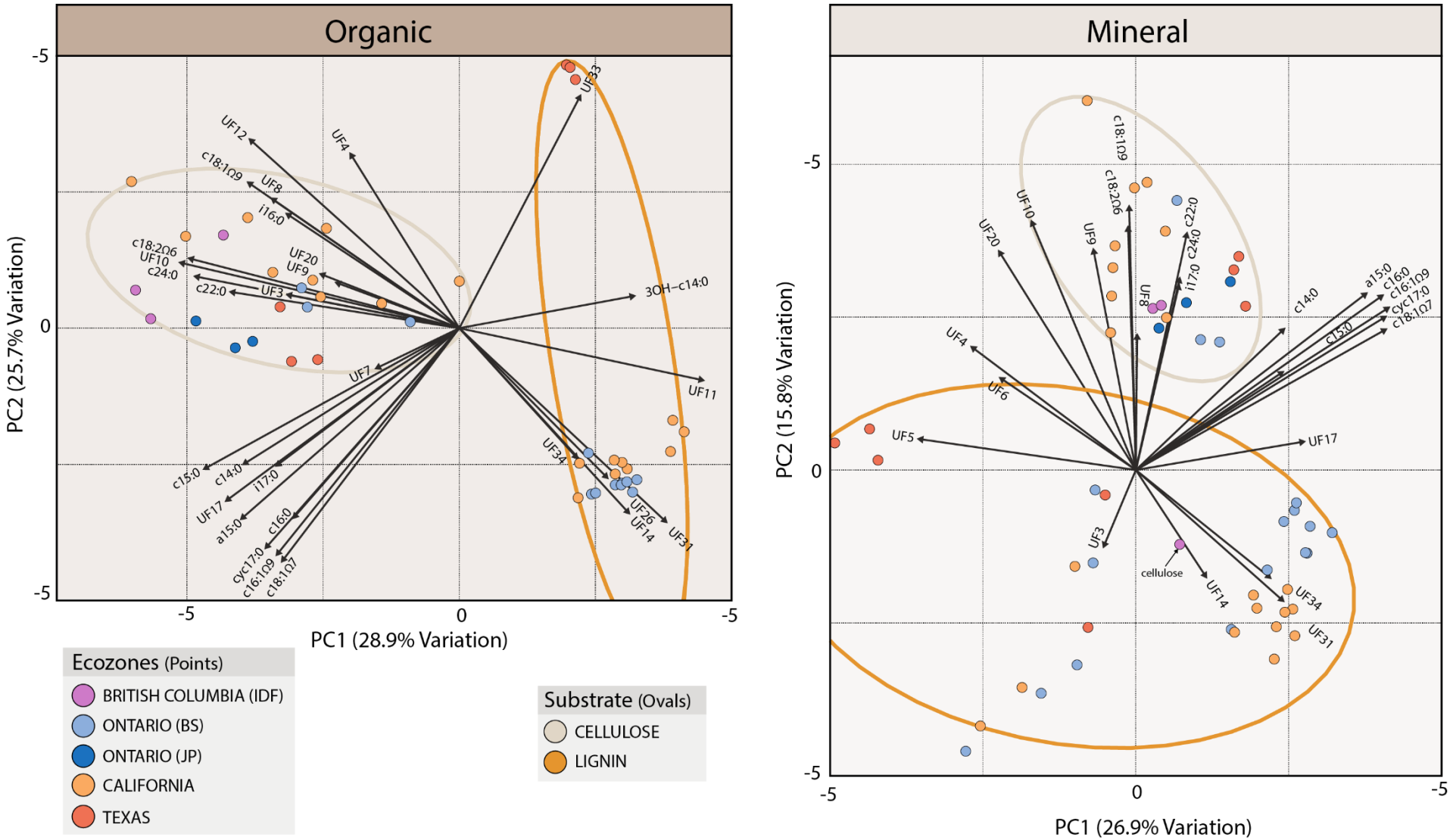


Figure 4.8. Abundances of *Ascomycota* and *Basidiomycota* in metagenomic libraries. Error bars correspond to one standard error of the mean.

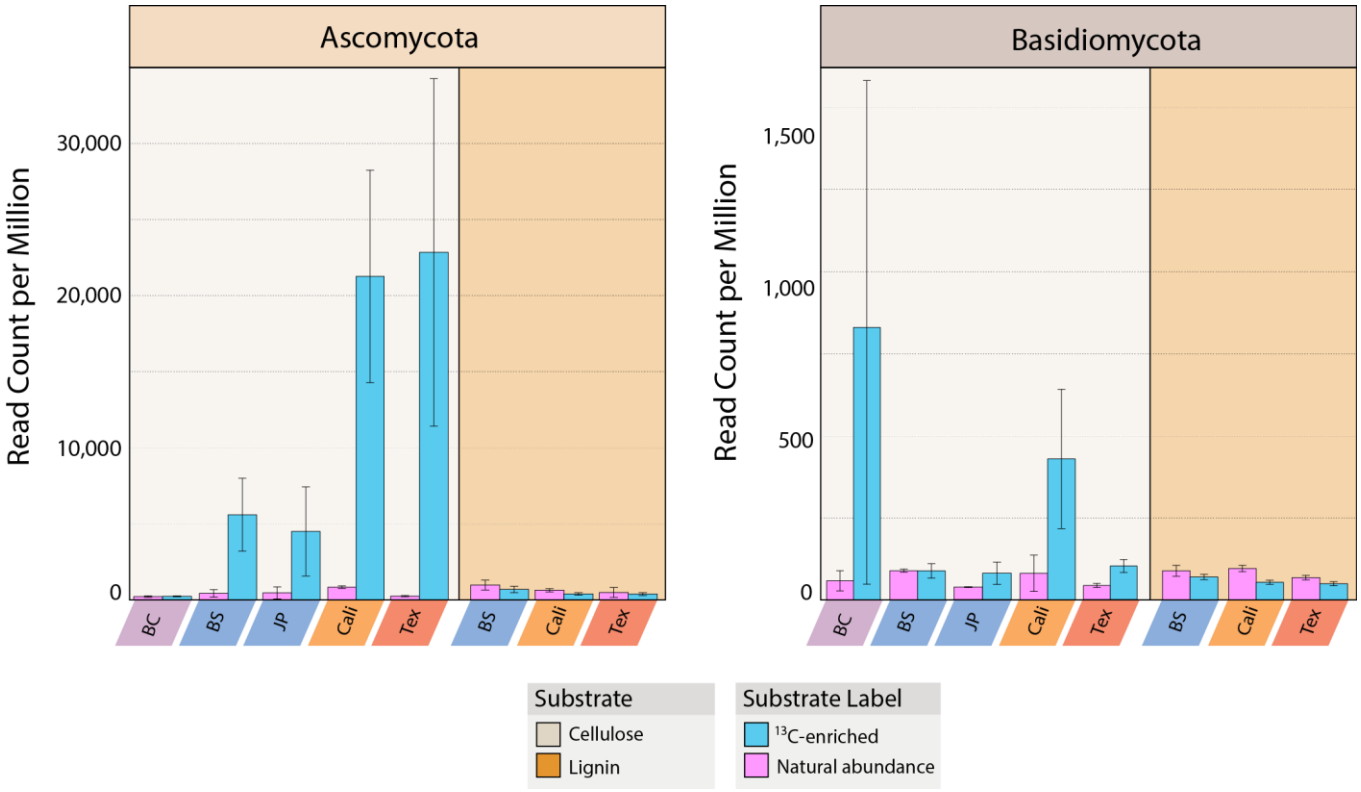


Figure 4.9. Abundances of prominent bacterial phyla (classes) according to differential abundance in ^{12}C- and ^{13}C-pyrotag libraries for hemicellulose, cellulose and lignin among ecozones. Error bars correspond to one standard error of the mean.

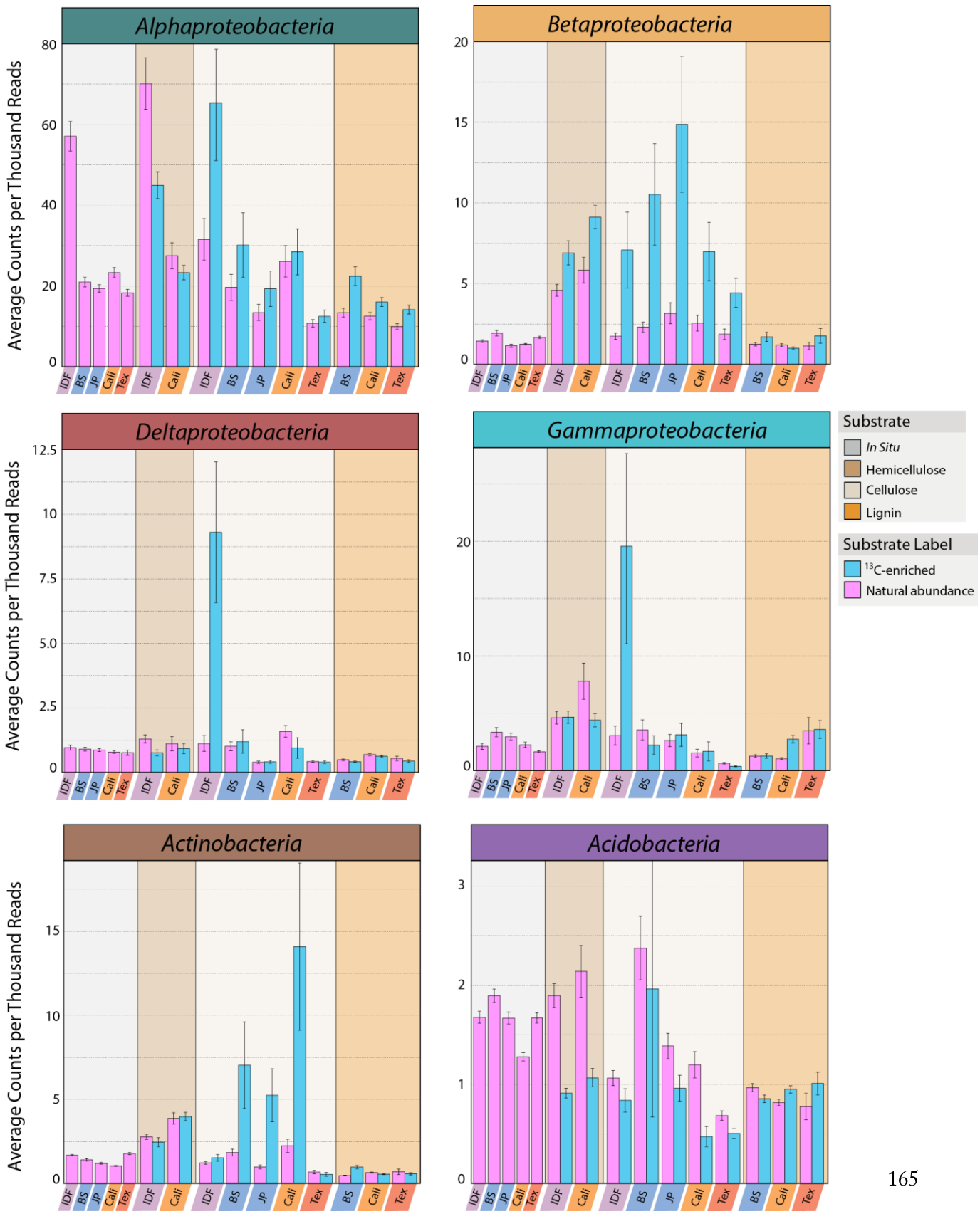


Figure 4.9 *continued…*

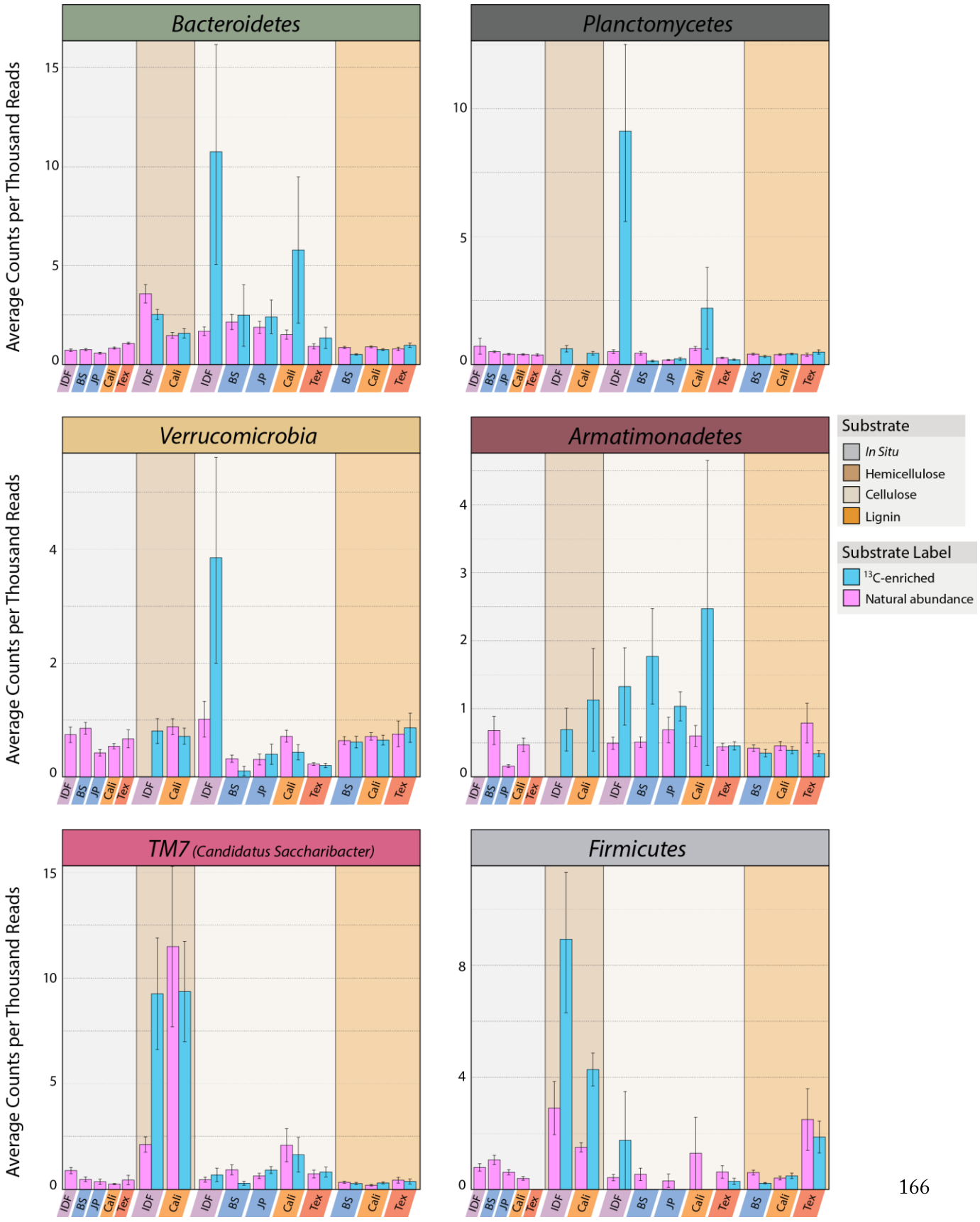
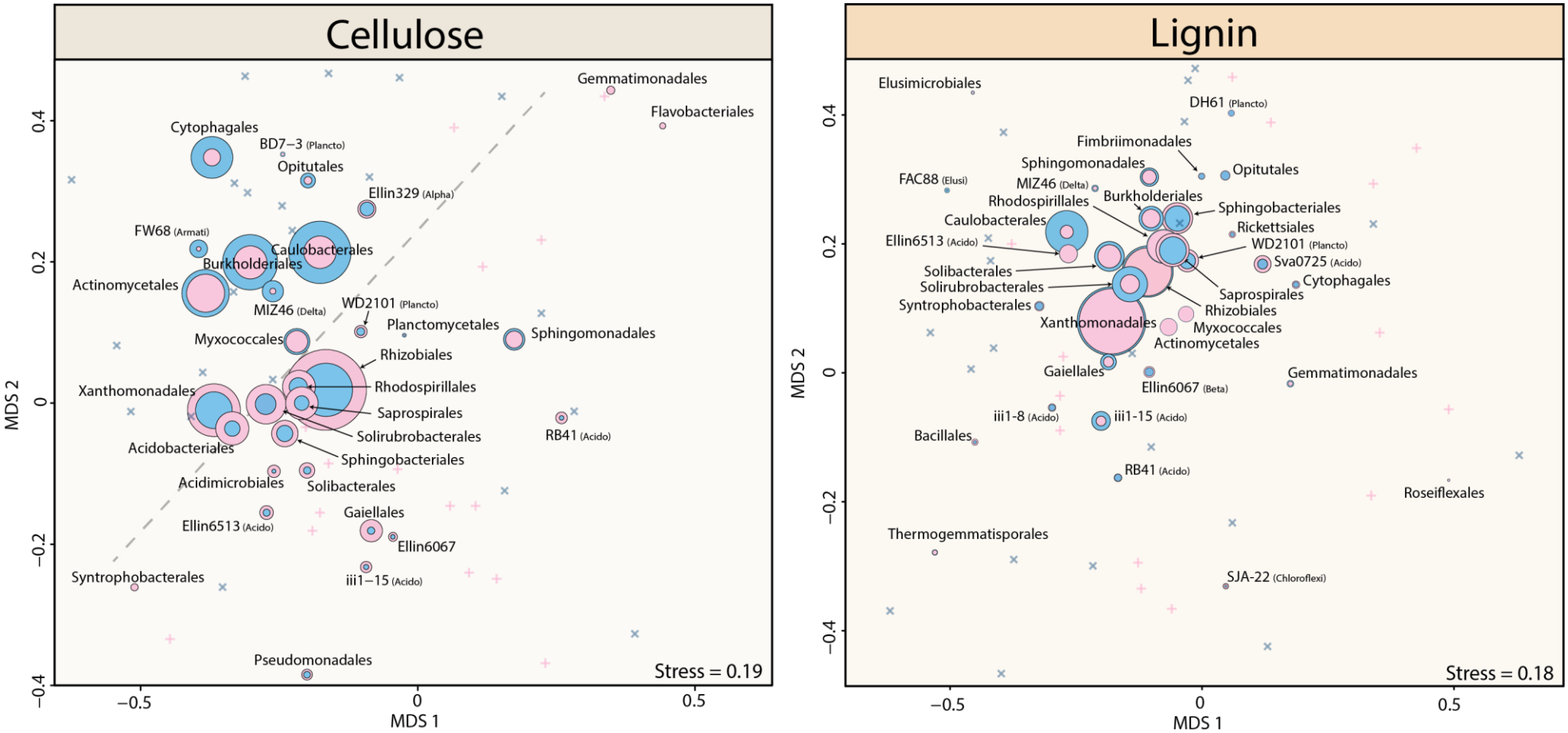


Figure 4.10. Non-parametric multidimensional scaling plots depicting differences in composition of ^{12}C- and ^{13}C- pyrotag libraries for cellulose and lignin. Bray-Curtis dissimilarities among samples were calculated from OTU count matrices and plotted with coloured x's corresponding to samples (blue: ^{13}C; pink ^{12}C) and with taxa corresponding to circles scaled to average abundances in ^{12}C (pink) or ^{13}C (blue) libraries. ^{12}C- and ^{13}C-cellulose libraries were delineated by a single dotted line.



All ^{13}C-pyrotag libraries were enriched in a number of bacterial classes not common in ^{12}C-libraries with notable differences among these groups according to substrate and soil layer (Figure 4.11). Yet, a large number of putatively lignocellulolytic bacterial classes were common in both ^{12}C- and ^{13}C- libraries and differed only at lower taxonomic ranks. For example, the differential abundance of certain families of *Alphaproteobacteria*, such as *Caulobacteraceae*, was obscured by the widespread abundance of *Bradyrhizobiaceae* in all samples, a group which was not implicated in decomposition of any lignocellulosic substrate. A combination of DESeq2 and indicator species analysis was used to assign putative lignocellulolytic function to differentially abundant taxonomic groups in ^{13}C-pyrotag and metagenomic libraries (Table 4.1).

Members of *Burkholderiales* and family *Caulobacteraceae* exhibited marked assimilation of ^{13}C-label from all three substrates. All three major families of *Burkholderiales*, namely *Burkholderiaceae*, *Comamonadaceae* and *Oxalobacteraceae*, possessed taxa differentially abundant in ^{13}C-pyrotag libraries (Appendix Figure C.6). In the family *Caulobacteraceae*, the genus *Asticcacaulis* exhibited activity on both hemicellulose and cellulose, while *Caulobacter* demonstrated the capacity to degrade all three (Figure 4.12). Putatively cellulolytic OTUs were spread throughout the *Asticcacaulis* clade (not shown), whereas OTUs assigned to *Caulobacter* and other unclassified *Caulobacteraceae* formed relatively cohesive clades (Figure 4.13). Lignolytic *Caulobacteraceae* grouped into four clades (‘L1’ - ‘L4’), while one cluster of cellulose and hemicellulose-degrading *Caulobacter* was identified (‘CH’). Lignolytic clades were more abundant in mineral soils (μ_{org}=6.0, μ_{min}=21.5; p=0.02), whereas their outgroups (i.e. closely related clusters of OTUs that did not show any or, in some cases mixed, enrichment) were more abundant in organic soils (μ_{org}=3.0, μ_{min}=1.4; $p < 0.001$).

Figure 4.11. Abundant classes of bacteria in pyrotag libraries according to substrate and soil layer. Taxa are organized into groups based on whether they were common to ^{12}C-libraries (muted, solid colour), common to ^{13}C-libraries (patterned), or specific to either organic and mineral horizon (solid colour). Only classes occupying more than 0.5% of the total library are displayed.

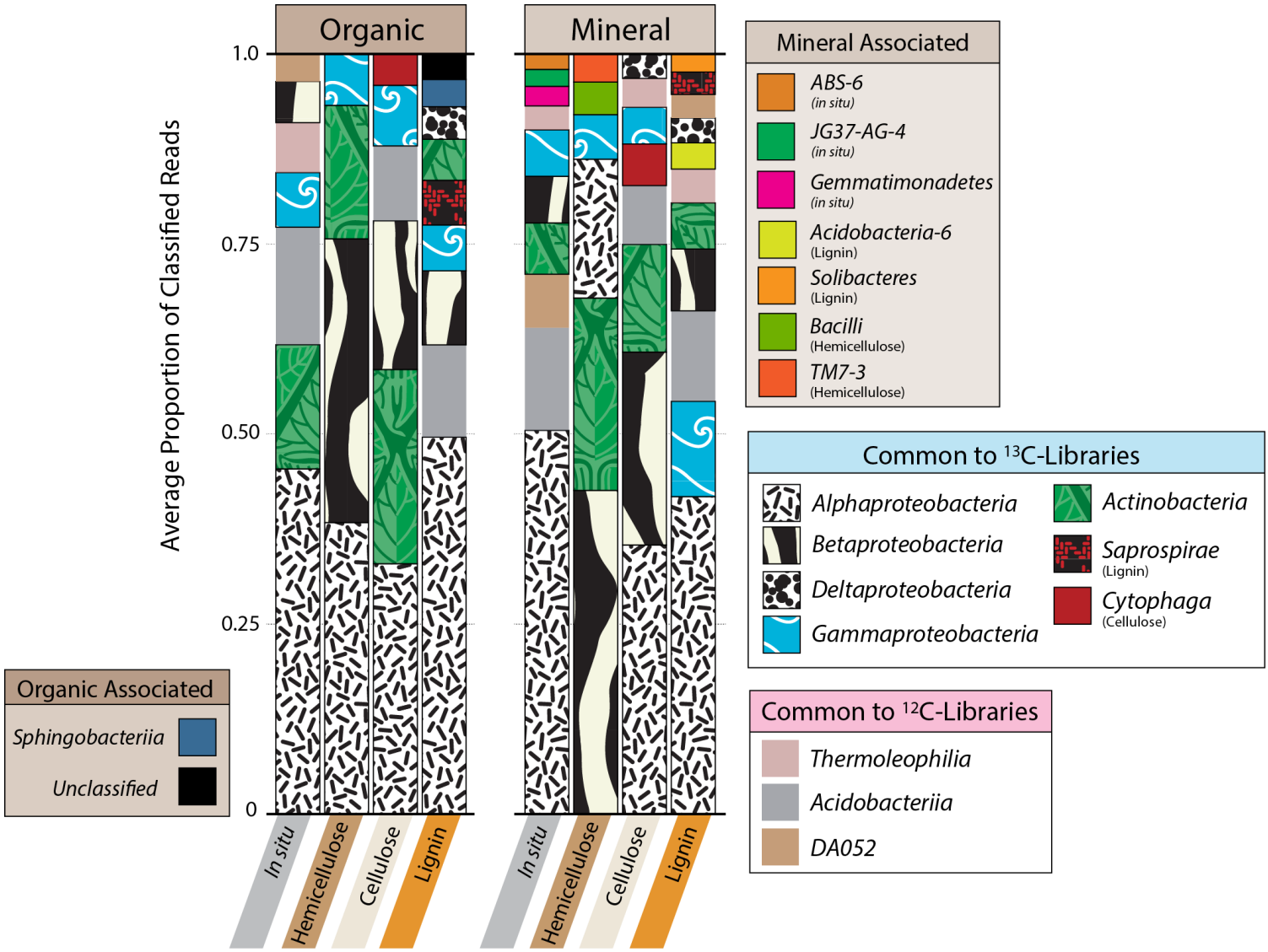


Table 4.1. All putatively hemicellulolytic, cellulolytic and lignolytic taxa based on differential abundance between ^{13}C and ^{12}C-pyrotag or metagenomic libraries. Best classifications have been given with the prefix corresponding to taxonomic rank. The *in situ* abundance of each taxon is represented by a scaled circle which has been coloured according to whether the taxon was differentially abundant for a given substrate in that ecozone. White circles indicate data was not gathered for that ecozone (both SIP-hemicellulose and SIP-lignin experiments use a subset of ecozones) and grey circles indicate data existed, but the taxon was not enriched in ^{13}C-libraries from microcosms in that ecozone. The total number of enriched OTUs are given as well as the averaged ratio of abundance in ^{13}C- by ^{12}C-libraries for each taxon (not each OTU) and each substrate where differentially abundance was observed. Additional information can be found in Table E.12.

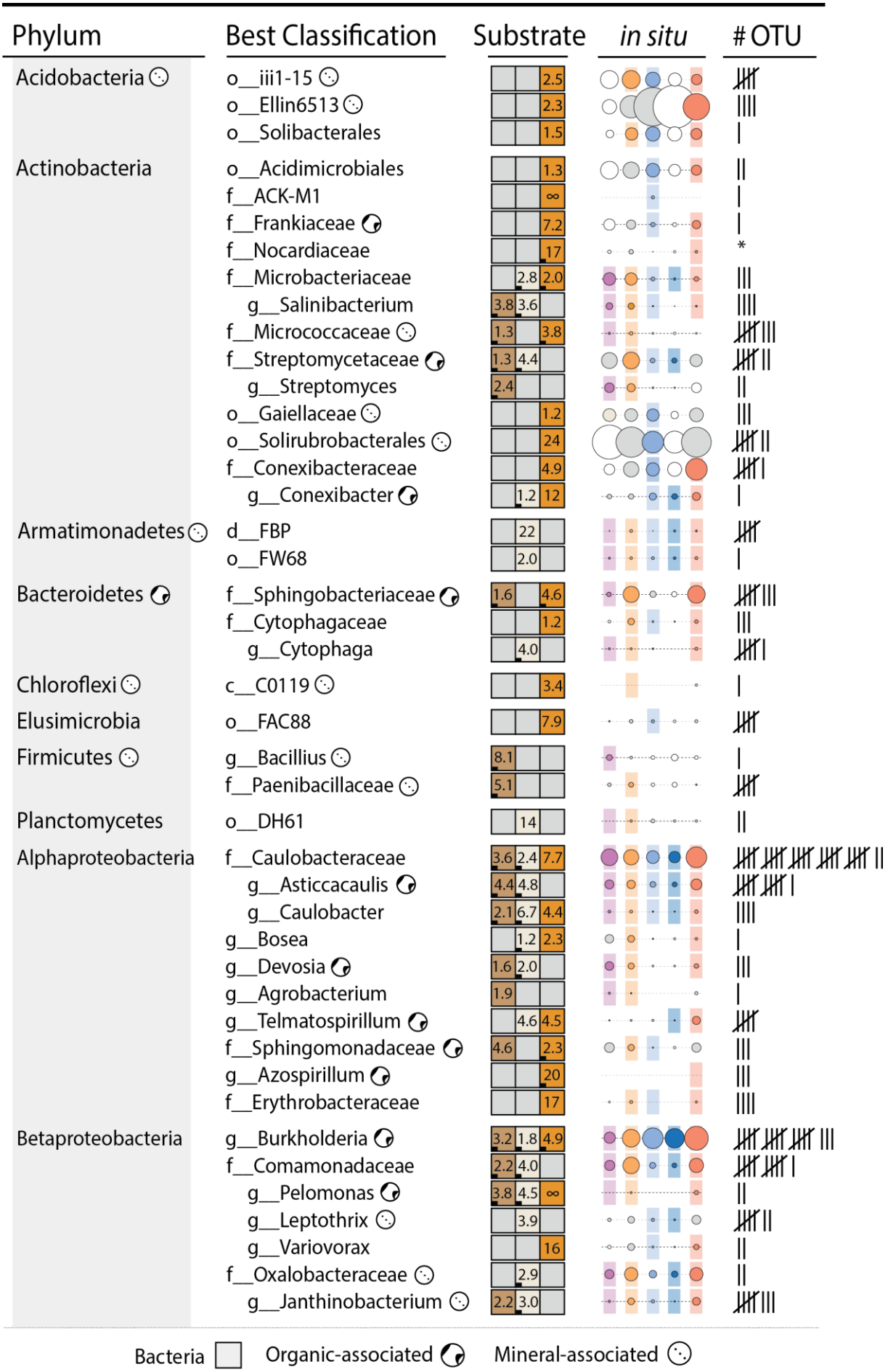

Phylum
Best Classification
Substrate
in situ
OTU
Acidobacteria
o__iii1-15
o__Ellin6513
o__Solibacterales
Actinobacteria
o__Acidimicrobiales
f__ACK-M1
f__Frankiaceae
f__Nocardiaceae
f__Microbacteriaceae
g__Salinibacterium
f__Micrococcaceae
f__Streptomycetaceae
g__Streptomyces
o__Gaiellaceae
o__Solirubrobacterales
f__Conexibacteraceae
g__Conexibacter
Armatimonadetes
d__FBP
o__FW68
Bacteroidetes
f__Sphingobacteriaceae
f__Cytophagaceae
g__Cytophaga
Chloroflexi
c__C0119
Elusimicrobia
o__FAC88
Firmicutes
g__Bacillus
f__Paenibacillaceae
Planctomycetes
o__DH61
Alphaproteobacteria
f__Caulobacteraceae
g__Asticcacaulis
g__Caulobacter
g__Bosea
g__Devosia
g__Agrobacterium
g__Telmatospirillum
f__Sphingomonadaceae
g__Azospirillum
f__Erythrobacteraceae
Betaproteobacteria
g__Burkholderia
f__Comamonadaceae
g__Pelomonas
g__Leptothrix
g__Variovorax
f__Oxalobacteraceae
g__Janthinobacterium
Bacteria
Organic-associated
Mineral-associated
Previously reported activity by members of group
Continued...

Table 4.1 *continued…*

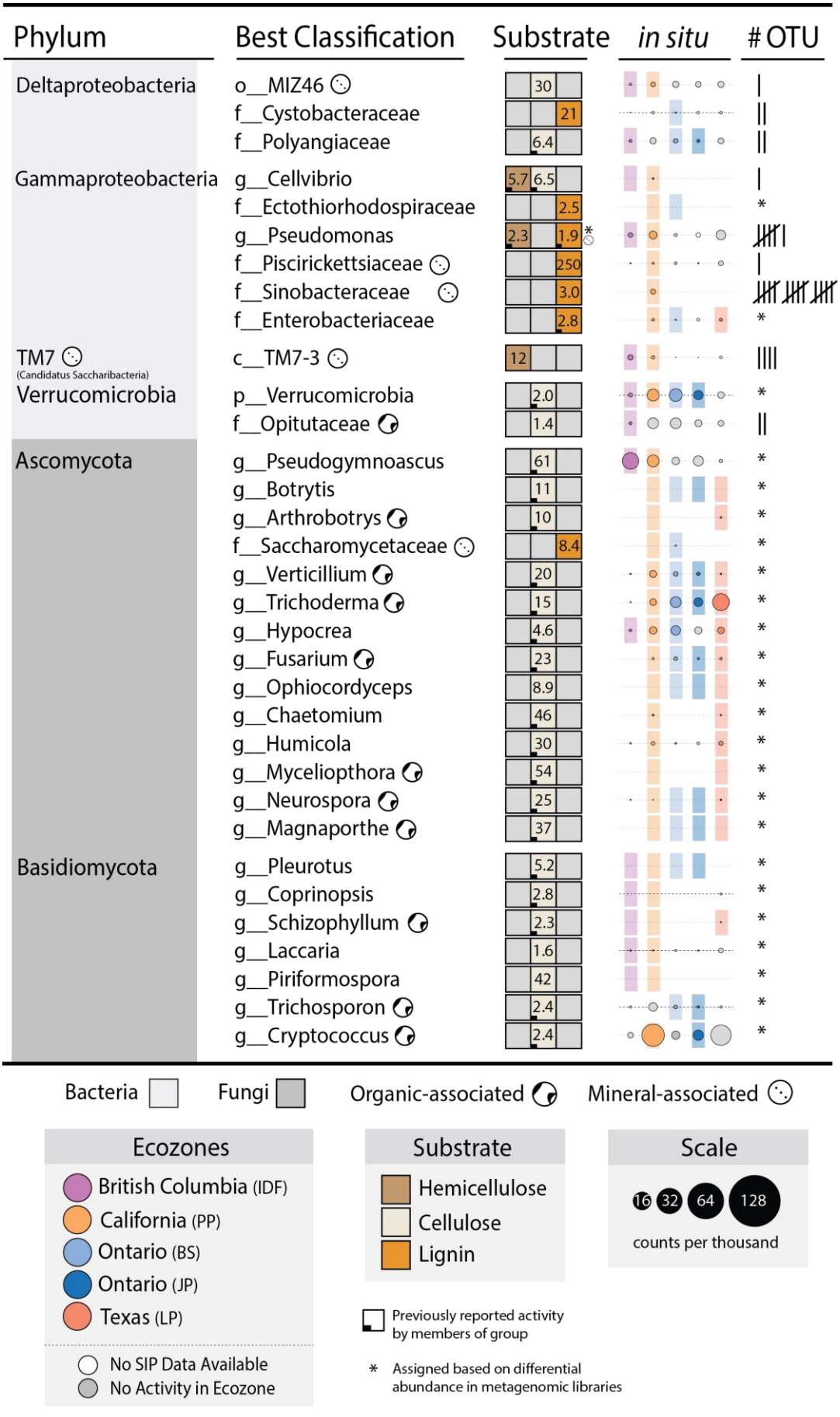
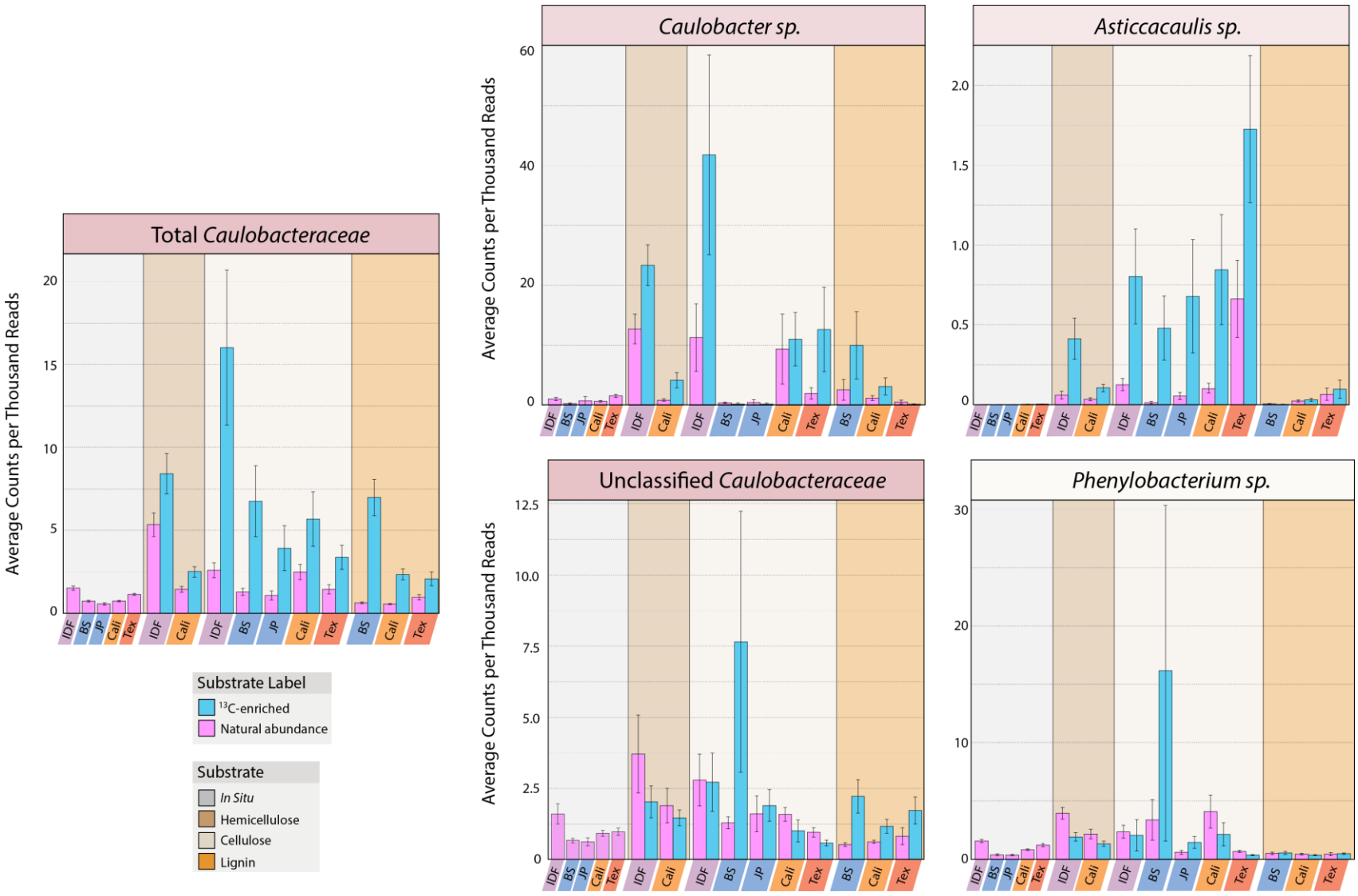


Figure 4.12. Abundances of all prominently hemicellulolytic, cellulolytic and lignolytic genera of *Caulobacteraceae* based on differential abundance between ^{12}C- and ^{13}C-pyrotag libraries.



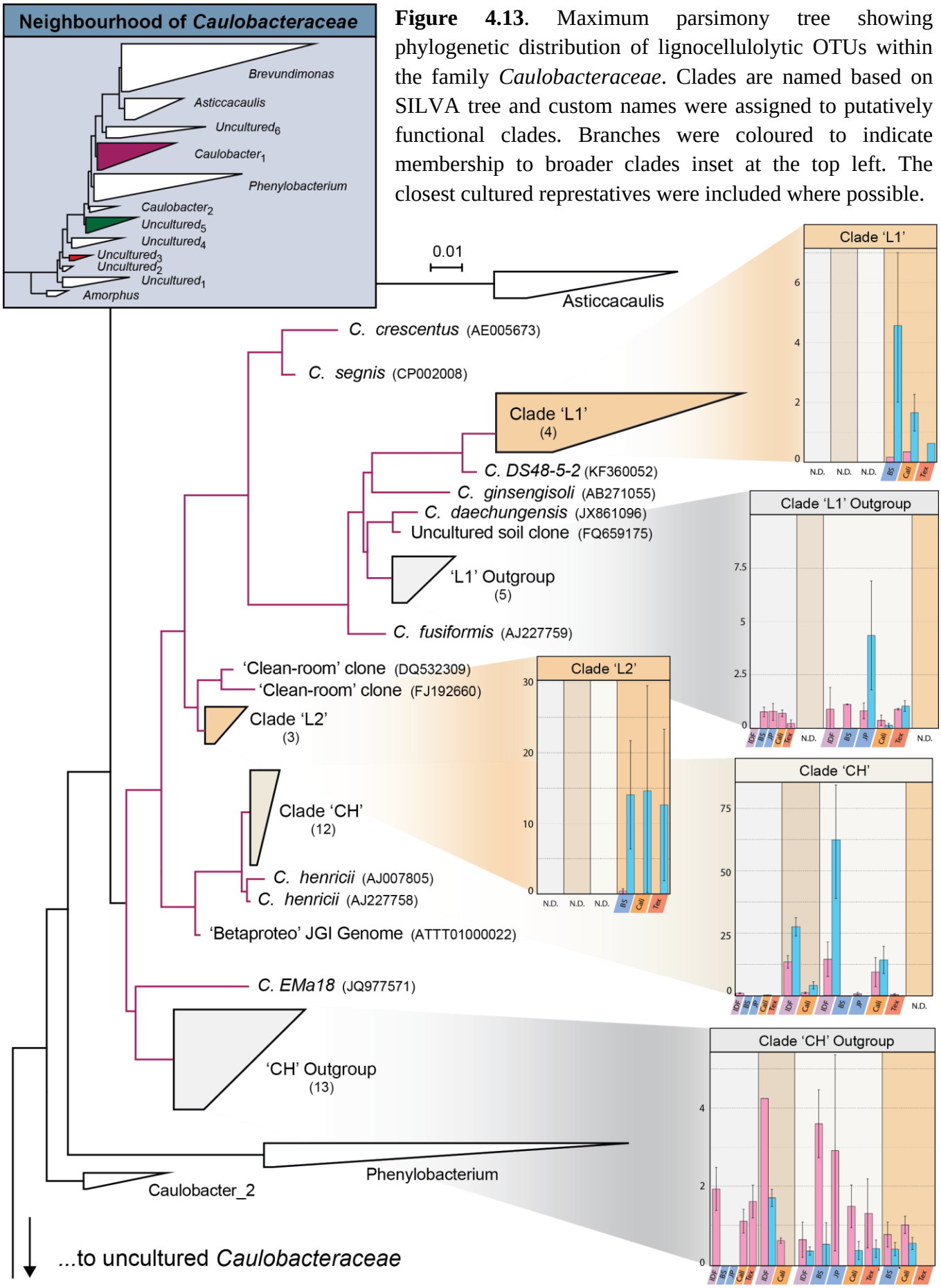
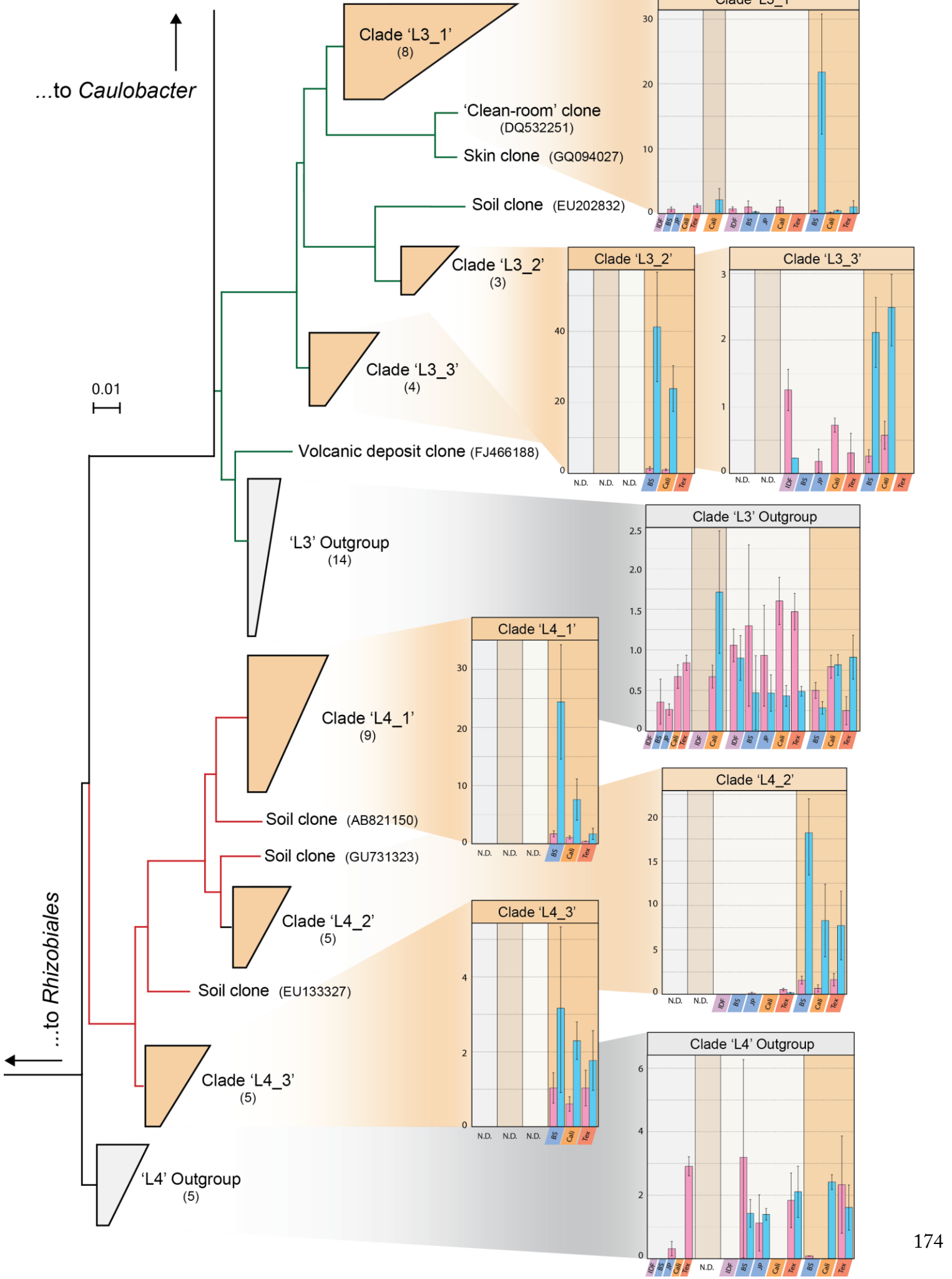


Figure 4.13. Maximum parsimony tree showing phylogenetic distribution of lignocellulolytic OTUs within the family *Caulobacteraceae*. Clades are named based on SILVA tree and custom names were assigned to putatively functional clades. Branches were coloured to indicate membership to broader clades inset at the top left. The closest cultured represtatives were included where possible.


...to Caulobacter
Clade 'L3_1'
(8)
'Clean-room' clone
(DQ532251)
Skin clone (GQ094027)
Soil clone (EU202832)
Clade 'L3_2'
(3)
Clade 'L3_3'
(4)
0.01
Volcanic deposit clone (FJ466188)
'L3' Outgroup
(14)
Clade 'L4_1'
(9)
Soil clone (AB821150)
Soil clone (GU731323)
Clade 'L4_2'
(5)
Soil clone (EU133327)
Clade 'L4_3'
(5)
'L4' Outgroup
(5)
...to Rhizobiales
Clade 'L3_1'
Clade 'L3_2'
Clade 'L3_3'
Clade 'L3' Outgroup
Clade 'L4_1'
Clade 'L4_2'
Clade 'L4_3'
Clade 'L4' Outgroup
N.D.
IDF
BS
JP
Cali
Tex

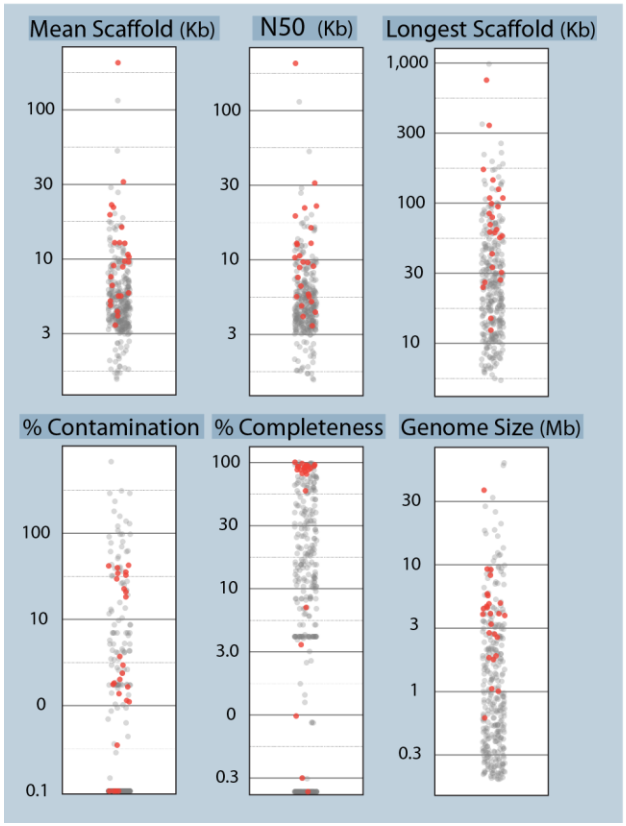
Partial draft genomes classified to *Caulobacteraceae* accounted for over half of all drafts recovered from ^{13}C-lignin and approximately a quarter from ^{13}C-cellulose libraries (Figure 4.14). Of the drafts recovered for cellulolytic *Caulobacteraceae*, 40% were classified as *Asticcacaulis*, 53% as *Caulobacter*, while drafts recovered from lignolytic taxa were 95% *Caulobacter*. Similar differences between cellulolytic and lignolytic *Caulobacteraceae* were found based on CAZy content (section 4.2.3).

The bacterial genera with the greatest enrichment in ^{13}C-hemicellulose libraries were *Agrobacterium*, *Bacillus*, *Paenibacillus*, *TM7-3* and *Streptomyces* and included taxa also active on cellulose, such as *Cellvibrio*, *Janthinobacterium*, *Cytophagaceae* and *Salinibacterium*. The most notable cellulose-degraders were from candidate division *FBP* and order *FW68* within *Armatimonadetes*, which were highly enriched across all ecozones, though were low abundance *in situ*. Novel putatively lignolytic bacteria were classified to poorly characterized taxonomic groups, such as acidobacterial groups 2 (*Ellin6531*), 3 (*Solibacterales*) and 6 (*iii1-15*), *Elusimicrobia*, and *Sinobacteraceae*, as well as to a number of taxa available in culture collections, such *Bradyrhizobium canariense* (‘*Bosea*’ in Table 4.1), *Altererythrobacter dongtanensis* (‘*Erythrobacteraceae’*), *Aquaspirillum polymorphum* (‘*Telmatospirillum’*), and *Cystobacter gracilis* (‘*Cystobacteraceae’*). Descriptions and detailed phylogenetic affiliations of lignocellulolytic taxa can be found in Appendix C.

No single OTU possessed activity on all three substrates, which reflects the low degree of overlap with hemicellulolytic libraries. A custom search for OTUs differentially abundant for multiple substrates within a given ecozone yielded a total of five OTUs putatively active on lignin and cellulose, which were related to *Simplicispira* (*Burkholderiaceae*), *Aquincola* (*Comamonadaceae*), *Caulobacteraceae spp.* (clade ‘LH3_1’) and two *Sinobacteraceae spp.* (Figure 4.15).

Figure 4.14. Overview of information on top quality draft genomes recovered from ^{13}C-cellulose and lignin libraries, including a list of all top quality genomes recovered (< 40% 'contamination' and >80% 'completeness'), an overview of bin characteristics derived from CheckM, and the LCA taxonomic classifications of all draft genome bins. Points shaded red in the scatterplots correspond to the listed top quality drafts. The following colours were used to code substrate, ecozone and horizon: off-white (cellulose), dark gold (lignin); purple (BC_{IDF}), blue (O.N. ecozones), yellow (PP_{CA}), orange (LP_{TX}); dark brown (organic layer) and light brown (mineral layer). [Figure Appears on Next Page]

Phylum	Classification	Substrate	Ecozone	Horizon	Complete	Genome Size	Longest Scaffold	n50 (Scaffold)
α - Proteo;	g__Asticcacaulis (87%)					4.1 Mb	43 Kb	10.9 Kb
α - Proteo;	g__Asticcacaulis (94%)					4.7	109	29.0
NA	d__Unclassified					2.9	357	119
Actinobacteria;	g__Conexibacter (44%)					3.4	56	10.6
Actinobacteria;	g__Conexibacter (55%)					2.7	174	36.7
α - Proteo;	g__Caulobacter (50%)					4.5	145	42.1
Actinobacteria;	g__Leifsonia (42%)					4.6	93	15.3
β - Proteo;	g__Herbaspirillum (41%)					4.0	109	7.6
Verrucomicrobia;	g__Chthoniobacter (51%)					9.3	98	19.2
Verrucomicrobia;	f__Verrucomicrobiaceae (48%)					8.3	755	435
Actinobacteria;	g__Arthrobacter (78%)					4.9	70	12.6
Actinobacteria;	g__Conexibacter (56%)					2.8	84	34.6
δ - Proteo;	g__Sorangium (67%)					9.2	27	5.7
Verrucomicrobia;	g__Opitutus (65%)					4.1	61	18.4
α - Proteo;	g__Magnetospirillum (28%)					1.9	62	23.4
β - Proteo;	g__Collimonas (46%)					5.9	79	14.7
α - Proteo;	g__Caulobacter (44%)					5.7	34	6.5
β - Proteo;	g__Comamonadaceae (75%)					4.9	64	12.6
α - Proteo;	g__Caulobacter (58%)					4.1	58	16.8
Ascomycota;	g__Chaetomium (21%)					1.7	32	4.4
Ascomycota;	g__Pseudogymnoascus (91%)					0.62	12.3	6.1
NA	d__Unclassified					1.0	25	6.3
Ascomycota;	g__Neurospora (22%)					1.0	28	6.4
Ascomycota;	g__Chaetomium (48%)					38.6	125	14.6
Ascomycota;	g__Neurospora (21%)					1.8	15	3.5



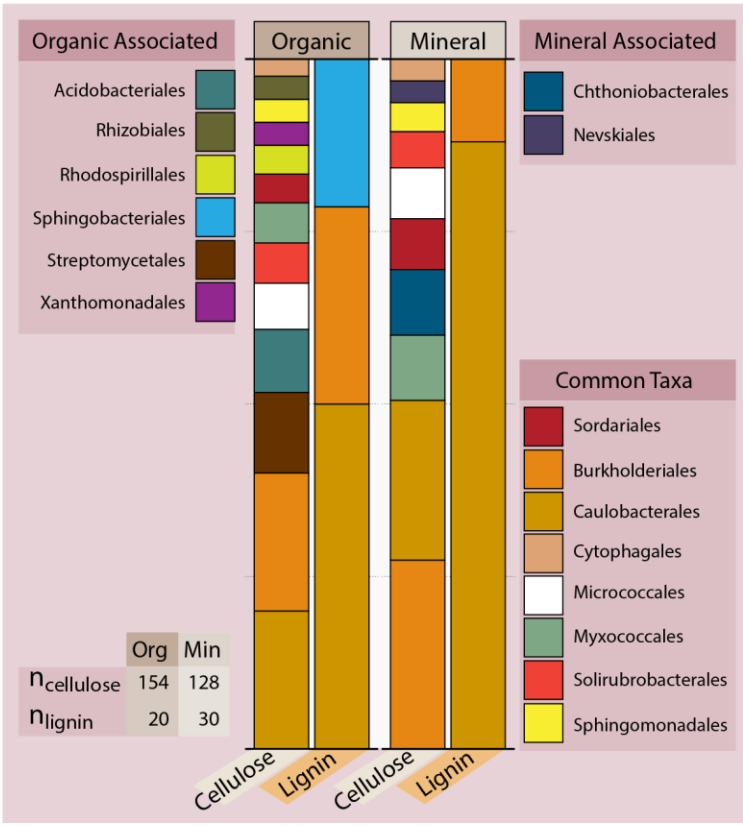
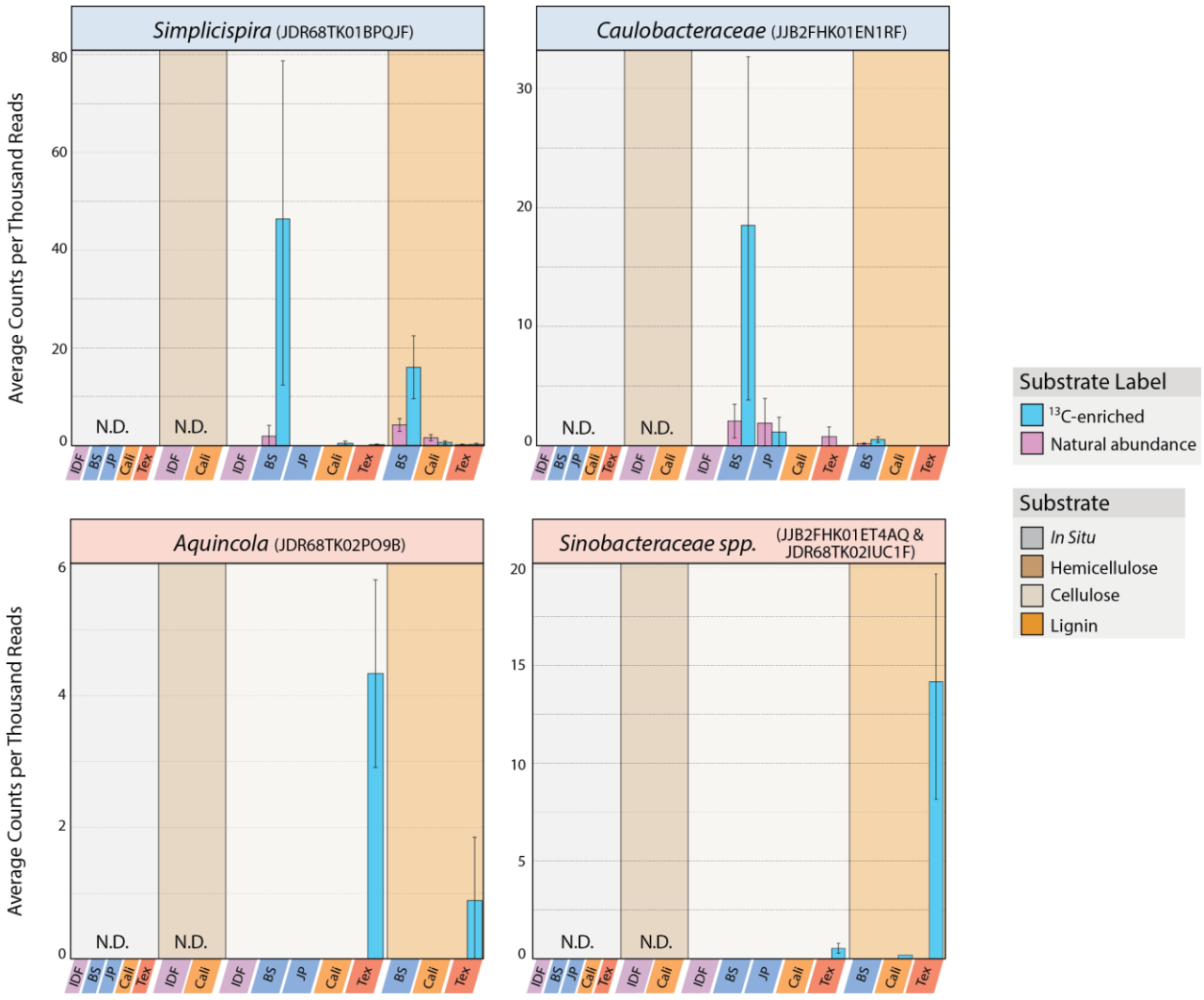
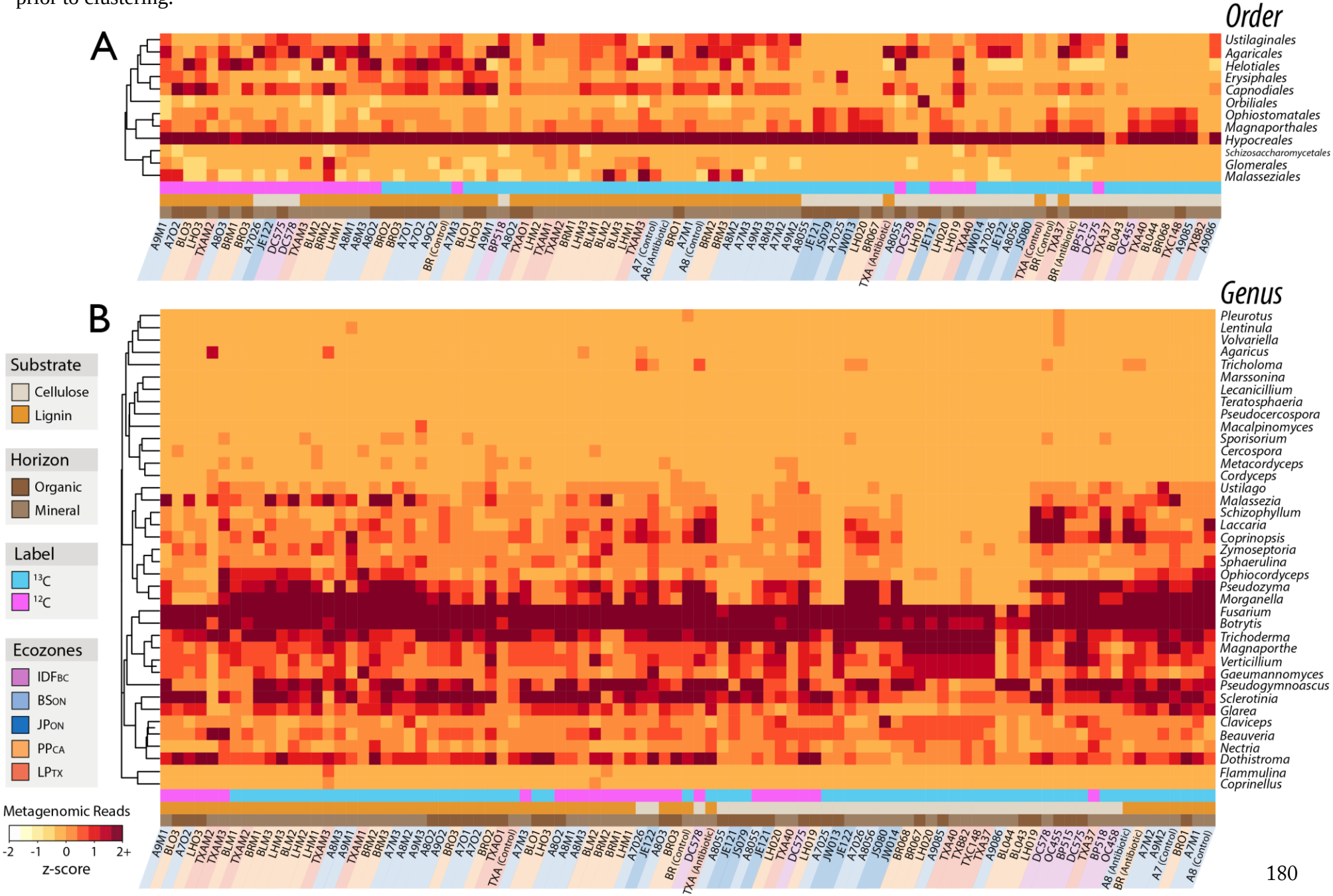


Figure 4.15. Abundances plots of OTUs enriched in both lignin and cellulose ^{13}C-pyrotag libraries within the same ecozone. Plots are labeled with the lowest taxonomic classification supported and with representative read names. Where two OTUs were combined, both independently exhibited the trend in multiple substrate use.



Fungal taxa were characterized using metagenomic libraries (ITS pyrotag libraries were no prepared) based on LCA classification. Distinct clustering of ^{12}C- and ^{13}C-libraries as well as cellulose- and lignin-degrading taxa occurred based on taxonomic profiles at both the rank order and genus (Figure 4.16). *Ophiostomatales* and *Magnaporthales* (*Ascomycota*) and *Agaricales* (*Basidiomycota*) were enriched in ^{13}C-cellulose libraries, while *Malasseziales* and *Ustilaginales* (both *Basidiomycota* from class *Ustilaginomycotina*) were enriched for in ^{13}C-lignin libraries. Members of the order *Hypocreales* were abundant in both ^{12}C- and ^{13}C-libraries (0.04% and 0.2% of total metagenomes, respectively), but genera within *Hypocreales*, such as *Trichoderma*, *Verticillium*, *Ophiocordyceps* and *Fusarium*, were differentially abundant in ^{13}C-cellulose libraries. *Ascomycota* were highly abundant in 13C-cellulolytic libraries, including members of the *Sordariales*, such as *Chaetomium*, *Myceliophthora* and *Neurospora*, as well as other groups, such as *Arthrobotrys* and *Cladophialophora* (Table 4.1). While *Ascomycota* were far more differentially abundant than *Basidiomycota* in ^{13}C-cellulose libraries, a number of putatively cellulolytic *Basidiomycota* were identified, namely: *Piriformospora*, *Trichosporon*, *Cryptococcus*, *Coprinopsis*, *Schizophyllum* and *Laccaria*. The only fungal family differentially abundant in ^{13}C-lignin libraries was *Saccharomycetaceae* (Appendix C; Figure C.3). *Saccharomycetaceae* were not differentially abundant in cellulose libraries and were the only fungal group to be associated with mineral soil (~2-fold more abundant therein). A necessary caveat regarding LCA-based classifications is that reads originating from a single genome may be artificially classified to separate genera due to the lack of specificity of BLAST-based homology searches used in LCA classification. This phenomenon was apparent in closely related genera (i.e. within a family) which frequently had nearly identical abundance patterns across ecozones and between ^{12}C- and ^{13}C-libraries, making it difficult to positively identify to which genus the reads actually belong (Appendix C; Figure C.5).

Figure 4.16. Heat map of the relative abundance of the most populous fungal orders (A) and genera (B) based on LCA classification of unassembled metagenomic libraries. Clustering was performed on Bray-Curtis dissimilarity of samples (x-axis) and taxa (y-axis). Read counts were normalized prior to clustering.



4.2.3 Carbohydrate Active Enzymes in Cellulolytic and Lignolytic Populations

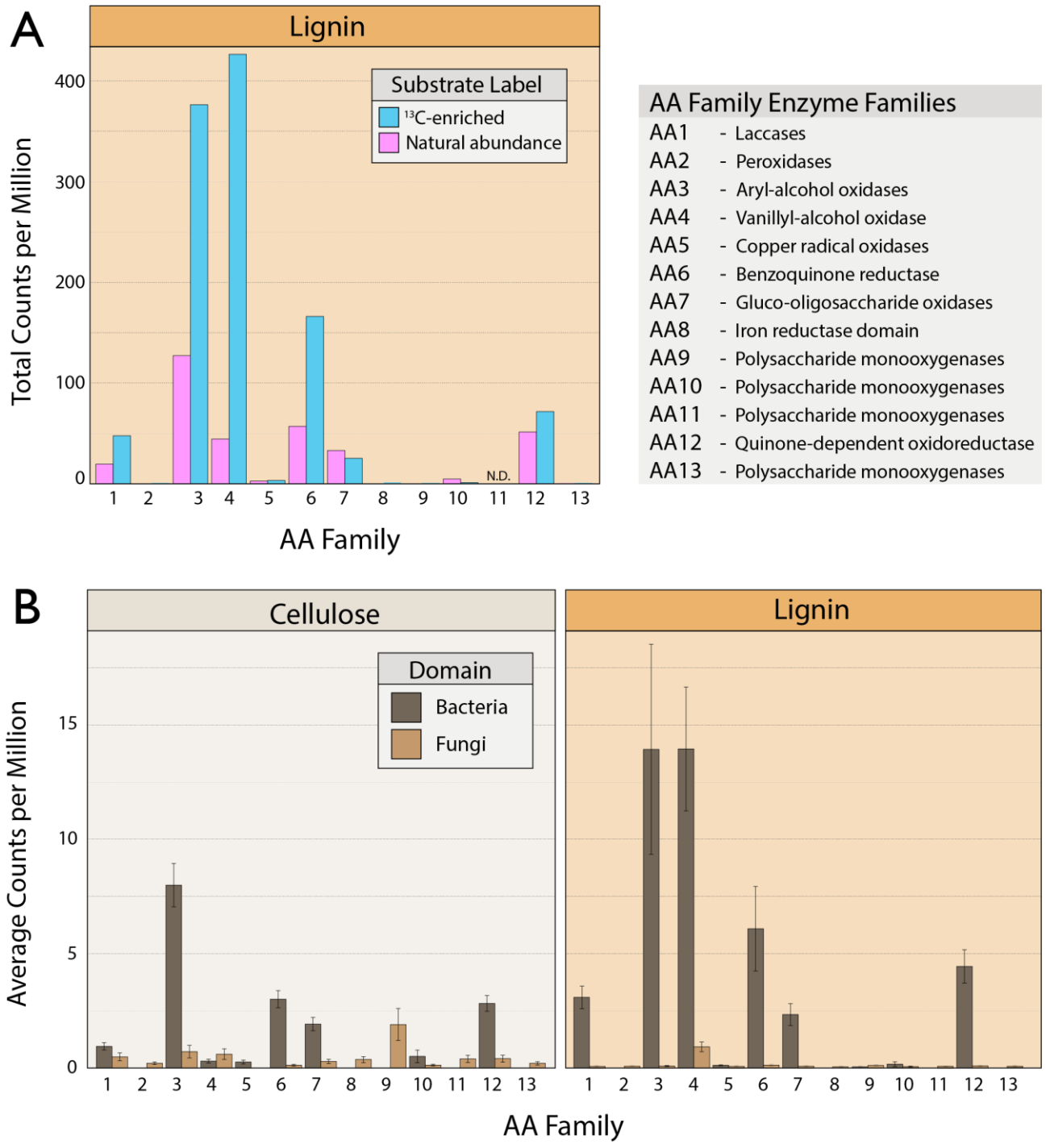
CAZyme genes related to cellulose and lignin degradation were expanded in unassembled ^{13}C-metagenomes. All endoglucanase containing GH families were between 1.3 and 32-fold more abundant in ^{13}C- than in ^{12}C-cellulose libraries (Table 4.2). A substantial number of 'Auxiliary Activity' families containing lignin-modifying enzymes were also enriched in ^{13}C-cellulose libraries, such as iron reductase domains (AA8) and lytic polysaccharide monooxygenases (AA9, AA10, A11, & AA13). For DHP-lignin libraries, the majority of all endoglucanases and AA families were actually depleted in ^{13}C- relative to ^{12}C-libraries, with genes from only three endoglucanases (GH7, GH12 & GH26), and vanillyl-alcohol oxidases (AA4) more abundant in ^{13}C-libraries (Table 4.2). However, when read sets annotated to AA families were subsetted to contain only families previously designated as lignolytic (Table 4.1), a number of AA families were more abundant in ^{13}C-libraries (Figure 4.17). Laccases (AA1), oxidases (AA3, AA4 & AA7) and quinone-dependent oxidoreductases (AA6 & AA12) were largely bacterial, while peroxidases, iron reductase domains and most lytic polysaccharide monooxygenase families were fungal (Figure 4.17).

CAZy family abundance profiles differed between ^{13}C-cellulose and ^{13}C-lignin metagenomic libraries (ANOSIM: R=0.08, p=0.004), but not between soil layers or among ecozones. CAZy composition did not significantly covary with taxonomic composition according to a Mantel test on Bray-Curtis dissimilarities (r=-0.07; p-value=0.91). However, a variety of samples formed distinct groups based on substrate, ecozone and horizon when samples were clustered (Figure 4.18a). The clustering revealed the co-occurrence of a number of CAZymes within certain ecozones (see 'hotspots' in Figure 4.18b) indicative of the prevalence of certain functional taxa present there.

Table 4.2. Overview of abundances of GH families containing characterized endoglucanases and 'Auxiliary Activity' enzymes with known lignin-modifying activity. Abundances correspond to the average counts per million reads (RCPM) in unassembled metagenomic libraries. The percentage of libraries in which a given GH or AA family were detected is given. The rounded ratio of counts in ^{13}C- relative to ^{12}C is provided.

CAZy Family	Cellulose					Lignin				
	^{12}C-lib	% lib	^{13}C-lib	% lib	^{13}C:^{12}C	^{12}C-lib	% lib	^{13}C-lib	% lib	^{13}C:^{12}C
AA1	0.96 ±0.3	100	1.33 ±0.2	100	1.0	7.22 ±1.4	100	3.21 ±0.5	100%	0.5
AA2	0.02 ±0.0	50	0.2 ±0.1	82	13.0	0.72 ±0.6	38	0.03 ±0.0	39%	0.04
AA3	4.57 ±0.6	100	9.3 ±1.2	100	2.0	15.27 ±2.4	100	14.84 ±4.6	100%	1.0
AA4	0.29 ±0.1	100	0.85 ±0.2	100	3.0	4.86 ±1.5	100	16.02 ±3.1	100%	3.3
AA5	0.24 ±0.1	100	0.37 ±0.1	93	1.6	0.39 ±0.1	75	0.13 ±0.1	84%	0.3
AA6	1.15 ±0.1	100	3.14 ±0.4	100	2.7	5.65 ±1.0	100	6.18 ±1.8	100%	1.0
AA7	3.05 ±0.3	100	2.53 ±0.4	100	0.8	8.73 ±1.8	100	2.72 ±0.6	97%	0.3
AA8	-	6	0.36 ±0.1	39	-	-	0	-	5%	-
AA9	0.04 ±0.0	40	1.93 ±0.7	75	55.0	0.88 ±0.8	31	0.03 ±0.0	32%	0.04
AA10	0.26 ±0.1	100	0.74 ±0.3	100	2.8	1.79 ±0.5	94	0.38 ±0.1	97%	0.2
AA11	-	10	0.4 ±0.2	57	38.7	1.19 ±1.6	13	0.03 ±0.0	11%	0.03
AA12	1.49 ±0.2	100	3.13 ±0.4	100	2.1	8.28 ±1.8	100	4.55 ±0.7	97%	0.6
AA13	-	0	0.21 ±0.1	43	-	0.13 ±0.1	19	0.02 ±0.0	5%	0.1
GH5	0.68 ±0.2	100	1.88 ±0.4	100	2.8	1.87 ±0.4	100	0.77 ±0.1	97%	0.4
GH6	0.62 ±0.1	100	1.66 ±0.6	96	2.7	1.23 ±0.3	94	0.35 ±0.1	92%	0.3
GH7	0.03 ±0.0	40	0.88 ±0.3	86	28.3	0.02 ±0.1	19	0.04 ±0.0	24%	1.6
GH8	0.45 ±0.1	100	1.55 ±0.3	100	3.4	3.67 ±2.3	88	3.19 ±2.7	100%	0.9
GH9	1.41 ±0.4	100	4.69 ±0.6	100	3.3	10.61 ±3.2	100	2.28 ±0.5	97%	0.2
GH12	0.16 +0.1	100	0.67 ±0.1	100	4.2	0.23 ±0.1	63	0.37 ±0.1	95%	1.6
GH26	0.65 ±0.1	100	0.83 ±0.2	100	1.3	2.18 ±0.6	94	4.02 ±3.7	100%	1.8
GH44	0.25 ±0.1	100	0.42 ±0.1	100	1.6	3.14 ±1.0	100	0.71 ±0.1	97%	0.2
GH45	-	10	0.11 ±0.1	75	10.4	0.34 ±0.4	13	0.01 ±0.0	11%	0.04
GH48	0.06 ±0.0	100	0.09 ±0.0	79	1.3	0.26 ±0.2	56	0.06 ±0.0	63%	0.2
GH51	2.84 ±0.8	100	7.54 ±0.9	100	2.7	20.31 ±4.3	100	5.68 ±0.8	97%	0.3
GH74	1.89 ±0.4	100	2.48 ±0.4	100	1.3	11.42 ±2.2	100	3.08 ±0.8	97%	0.3
GH81	0.19 ±0.1	100	1.0 ±0.4	100	5.4	0.42 ±0.1	81	0.05 ±0.0	66%	0.1
GH131	-	10	0.33 ±0.1	61	32.7	-	0	-	0%	-

Figure 4.17. Characterization of metagenomic reads annotated as lignin-modifying, 'Auxiliary Activity' (AA) families illustrating (A) the differential abundance of AA families summed for all putatively lignolytic families identified in pyrotag analysis (Table 4.1) and (B) the largely bacterial classification of all AA genes found in SIP-cellulose or SIP-lignin metagenomes. [Note: In Panel A, lignin families AA2, AA8, AA9 and AA13 are present at low abundances and exclusively in ^{13}C-libraries.]



The taxonomic profiles of CAZymes clustered in 'hot spot 1', namely CBM41, CBM12, GH6 (a family of strict endoglucanases) and CBM16, were consistently assigned to *Actinomycetales* (*Streptomycetaceae*) in Californian samples BR067, BR068 and LH020 (Figure 4.18b). In contrast, these families were classified to groups of *Proteobacteria* in samples comprising 'hotspot 2.' However, not all CAZymes that clustered within a given hotspot were taxonomically uniform, such as CBM18 and CBM1 ('hotspot 1'), which were largely from *Ascomycota* (Figure 4.18b). The BS_{ON} sample treated with fungicidal compounds, "A7 (Antibiotic)" had a distinct CAZy profile (far right Figure 4.18a) from all others and was comprised nearly exclusively of sequences from *Burkholderiaceae* (see Figure 4.39; section 4.2.4). The major AA enzymes identified in this sample were 'benzoquinone reductases' (AA6), which were one of the AA families frequently more abundant in ^{13}C-lignin libraries (highlighted row in Figure 4.18a).

The search for clusters of three or more CAZy genes on a scaffold recovered a total of ~11,500 clusters containing ~51,000 putative CAZymes. A number of longer scaffolds containing over 50 CAZymes were found and were largely classified to *Verrucomicrobiales* (Figure 4.19A). Longer scaffolds typically had lower clustering density of CAZymes, in particular large scaffolds which stretched the notion of a 'cluster.' However, scaffolds containing between 10 – 15 CAZymes exhibited an unexpected increased clustering density (Figure 4.19B), which were fungal in origin. A number of CAZy clusters contained lignin-modifying genes (Figure 4.20). Clusters containing lignin-modifying genes were predominantly bacterial in origin and all corresponded to taxa previously identified as putatively lignolytic with *Burkholderiales*, *Caulobacterales* and *Sordariales* exhibiting characteristic predominance.

Figure 4.18a. Heat map of CAZy family profiles for metagenomic libraries including only the most discriminating GH, PL, CBM and AA families (>1 sample with z-score > 1.5). Clustering was based on Bray-Curtis dissimilarity of samples (x-axis) and CAZy subfamilies (y-axis).

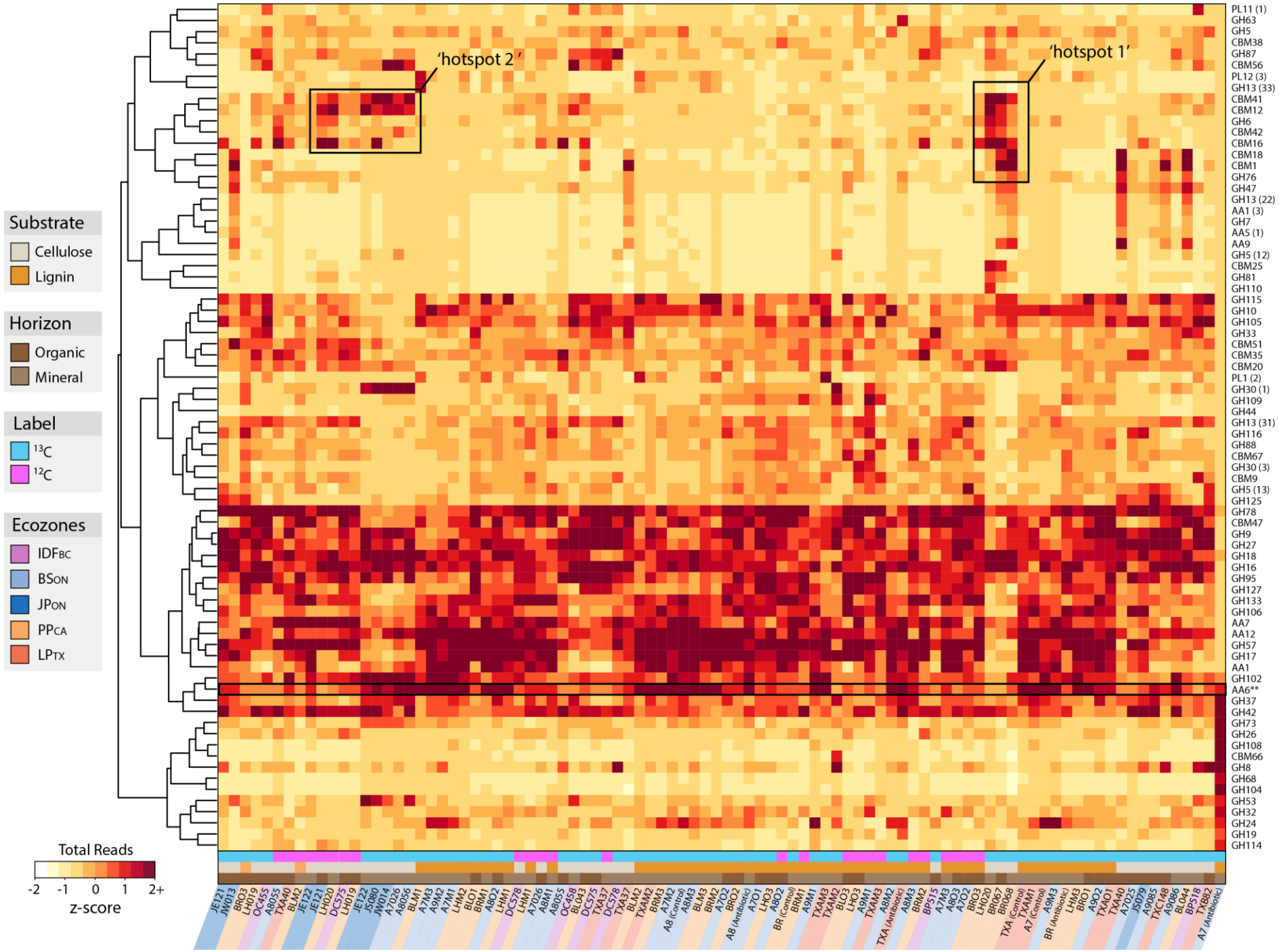


Figure 4.18b. Taxonomic profiles of CAZymes grouping in 'hotspots' in Figure 4.18a displayed as heat maps. Backgrounds are coloured according to the taxonomic affiliation of California samples BR067, BR068 and LH020 (^{13}C-cellulose) classified as either *Actinomycetales* (turquoise) or *Ascomycota* (pale orange). Differences among ecozone are also evident.

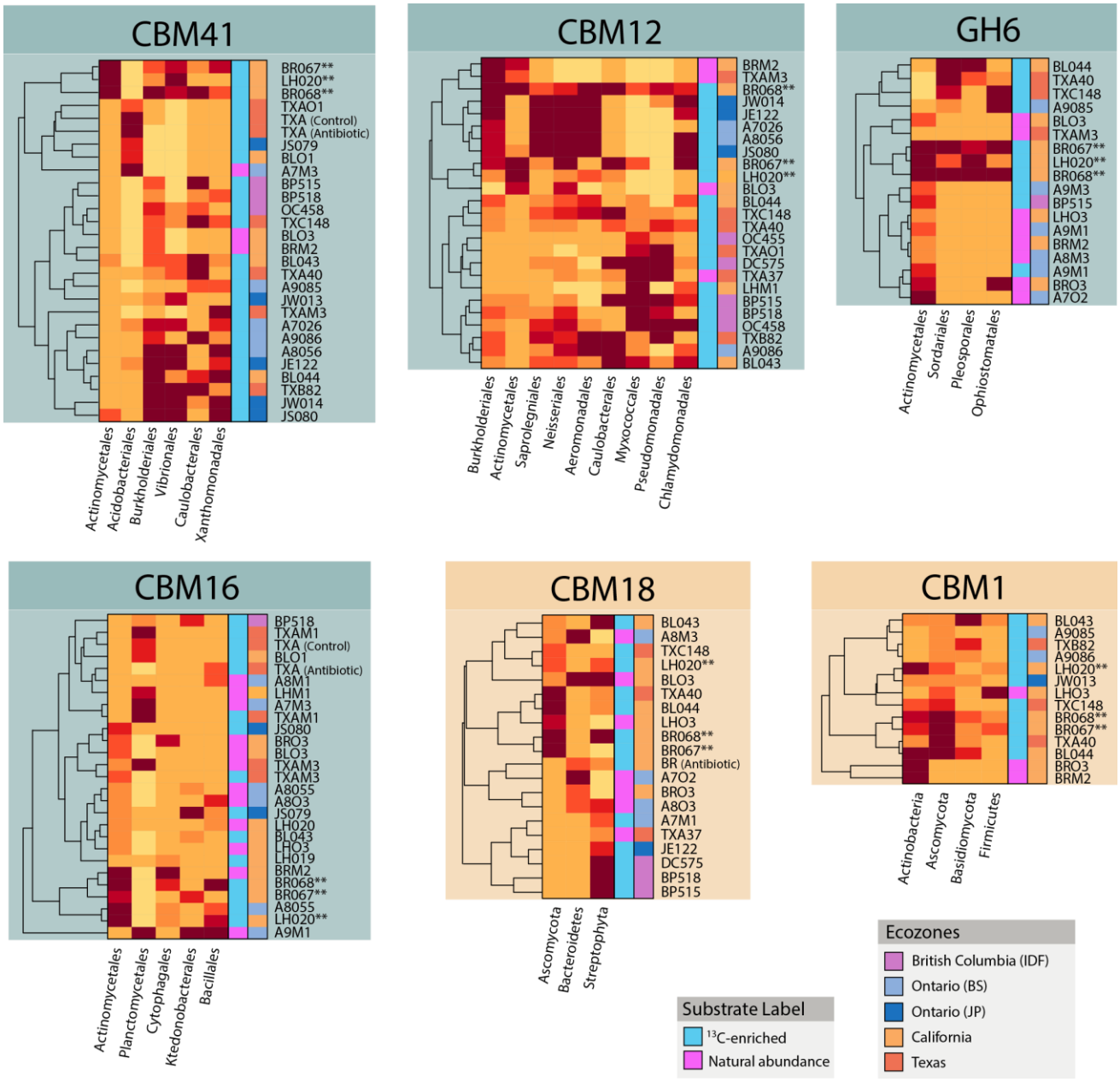


Figure 4.19. Characterization of scaffolds containing clusters of three or more putative CAZymes based on rank abundance of CAZy cluster size (Panel A) and CAZyme cluster density (scaffold length / number of CAZymes; Panel B). Clusters were derived from both cellulose and lignin ^{13}C-libraries.

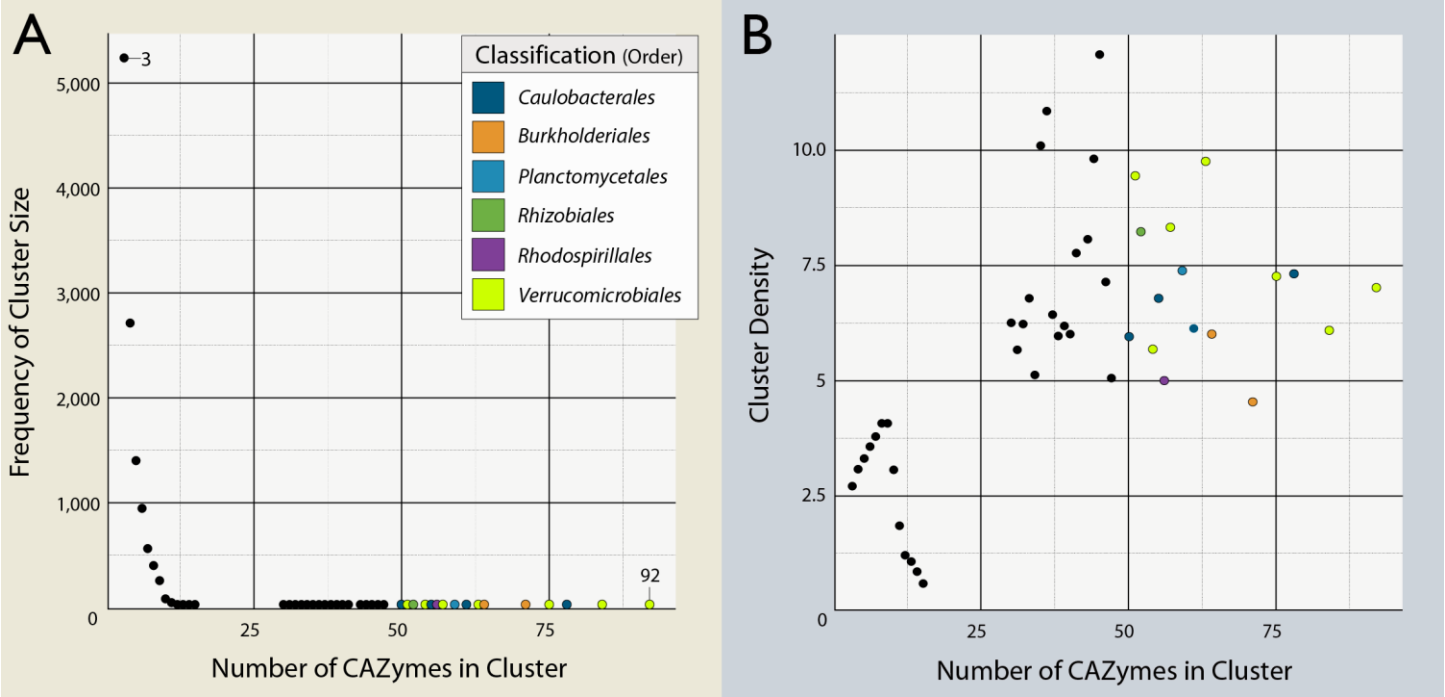
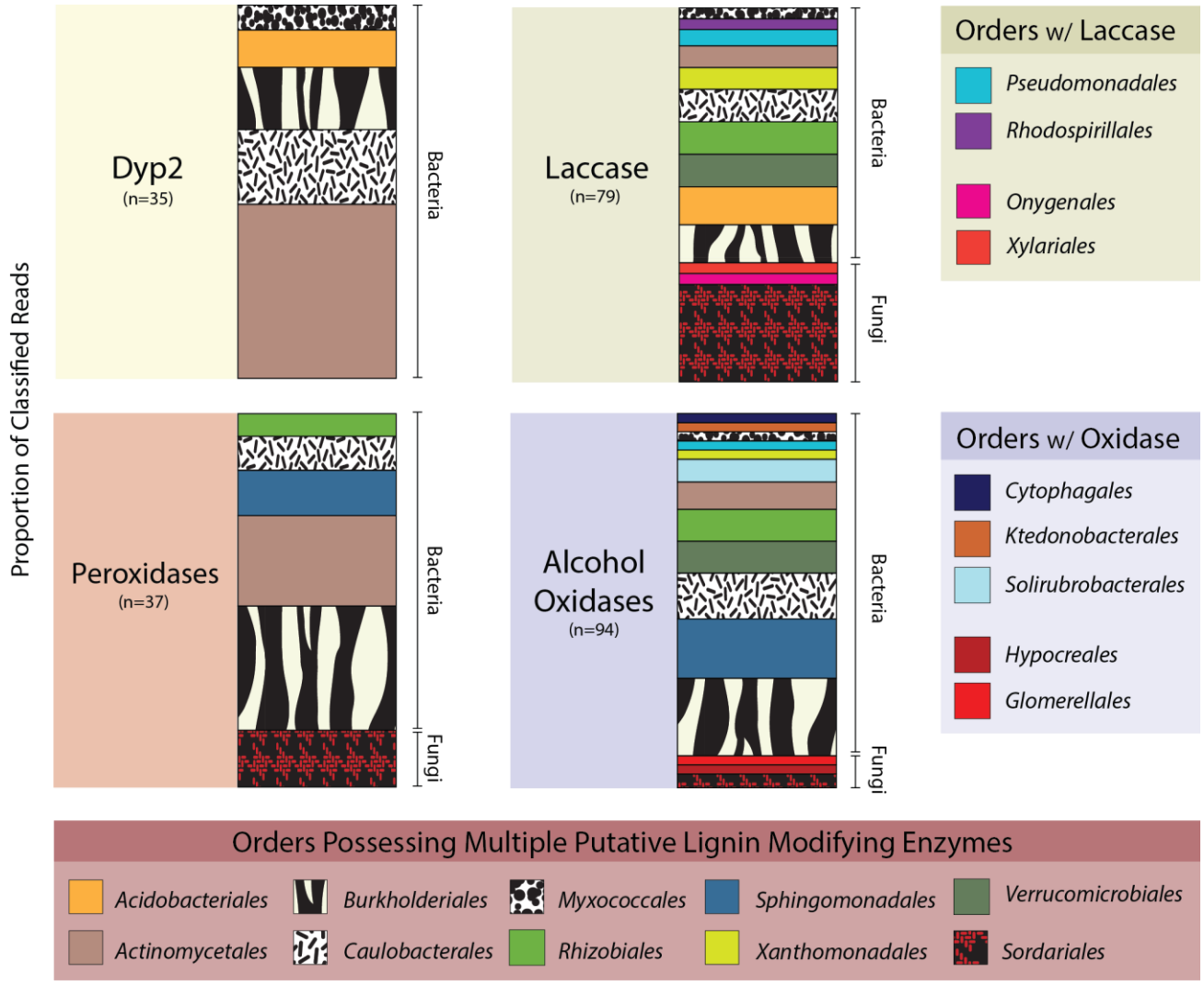


Figure 4.20. Taxonomic classification of CAZy clusters from ^{13}C-metagenomes which contain lignin-modifying enzymes: Dyp2, laccases, peroxidases and alcohol oxidases (aryl alcohol and vanillyl alcohol). Only orders which contained more than one CAZy cluster with a given lignin-modifying enzyme are displayed. Full details can be found in Table E.14.



The majority of putative bacterial peroxidases (AA2) were classified by PeroxiBase as Class I – catalase-peroxidases. One putative AA2 classified to *Actinomycetales* had 31% identity to a lignin peroxidase (Class II – catalase-peroxidase). All fungal AA2 genes were classified to hybrid ascorbate cytochrome C peroxidases. CAZy clusters encoding putatively lignin-modifying genes were recovered from all three families of *Burkholderiales* implicated in lignin-degradation with each containing at least one peroxidase (either DyP2 or AA2), laccase and oxidase (Table E.14). DyP2-like peroxidases were found in putatively novel lignolytic taxa, namely *Acidobacteriales*, *Ktedonobacterales* (*Chloroflexi*), and *Cystobacteraceae* (*Myxoccocales*). The recovery of *Nocardiaceae* clusters encoding Dyp2-like and vanillyl alcohol oxidase genes from LP_{TX} was consistent with their expanded populations following antibiotic treatment (Figure 4.29; section 4.2.4). Clusters containing Dyp genes were mainly actinobacterial: *Microbacteriaceae* (4 clusters), *Frankiaceae* (1) and *Streptomycetaceae* (5). Other novel lignolytic groups, such as *Chromatiales*, *Rhodospirillales* and *Rhodobacterales* possessed clusters containing laccase genes. One cluster, containing multiple laccase genes, was classified by LCA to *Enterobacter lignolyticus*, which possess characterized lignolytic capabilities. Clusters containing ligninase genes from *Streptomycetaceae*, *Pseudomonadaceae* and *Bradyrhizobiaceae* were unexpectedly abundant given the absence of differential abundance in pyrotag libraries. *Sphingobacteriaceae* were underrepresented in CAZy clusters with only a single cluster containing an aryl oxidase gene found. Forty-seven percent of CAZy clusters contained a putative CBM and, of those, ~ 1% contained a laccase, ~0.5% an aryl or vanillyl oxidase, and ~0.2% some form of peroxidase.

To assimilate ^{13}C from the ring-labeled DHP-lignin organisms would need to possess the capability to cleave and transform aromatic compounds into central metabolites. The paradigm for oxic degradation of aromatic compounds involves peripheral pathways which convert degradation products into protocatechuate or catechol intermediates which then feed into the β-ketoadipate pathway, though additional oxic and anoxic pathways have recently been reported (Fucks *et al.*,

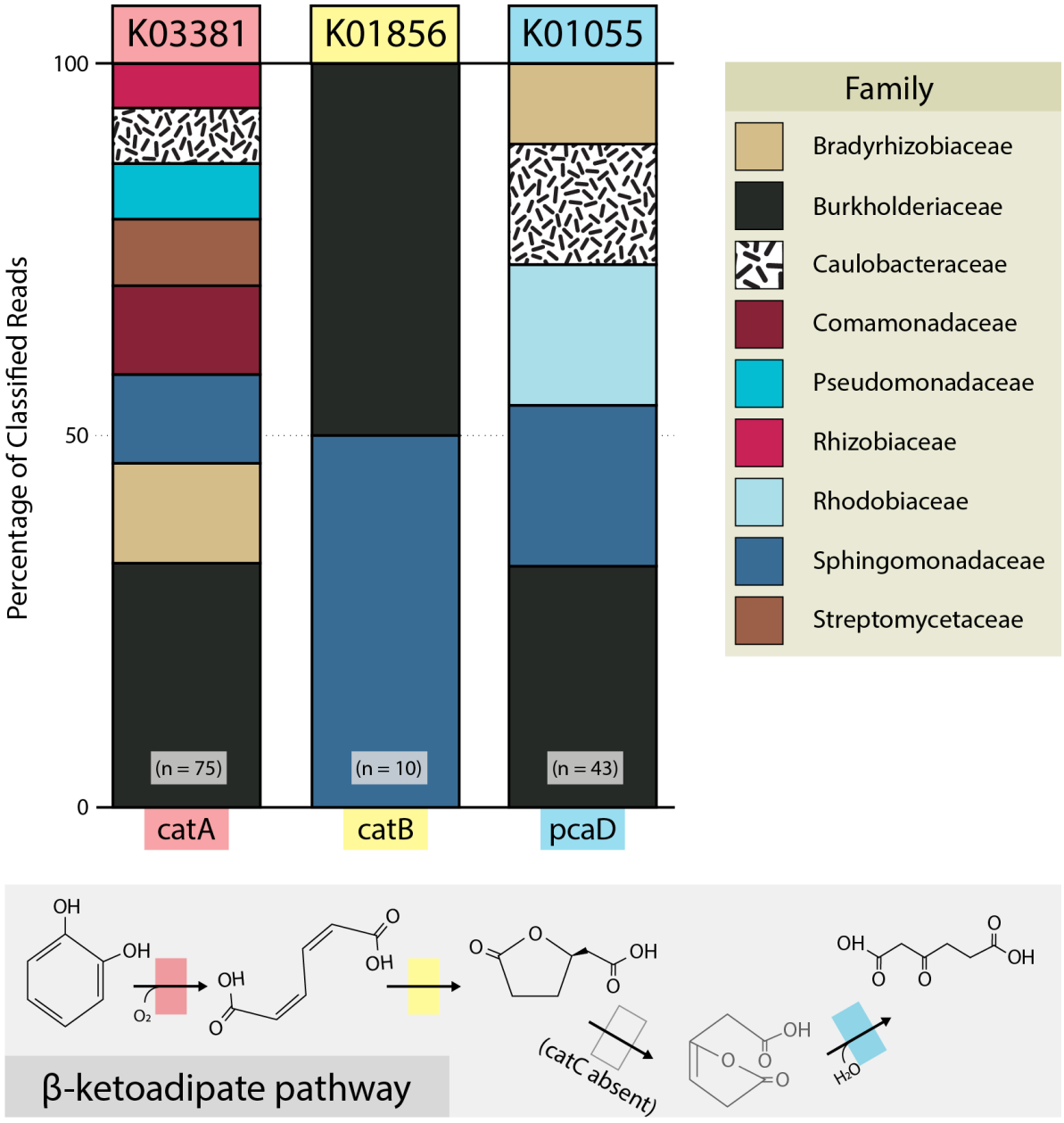
2011). The presence of genes in the KEGG Orthology (KO) β-ketoadipate module (M00568) was assessed in partial genome bins and in ^{13}C-lignin metagenome assemblies. Nine partial genomes contained homologous genes for the entire KO module and were classified as *Burkholderia*, *Sphingomonas*, *Caulobacter* and *Sorangium*. Upon closer examination, the specific scaffolds encoding the genes in the two *Caulobacter* bins were classified as *Sphingomonadaceae* (all other genome classifications matched their scaffold classifications). Assemblies from a diverse array of organisms possessed homologs of *catA*, encoding catechol 1,2-dioxygenase (Figure 4.21), including *Caulobacteraceae*. The *catC* gene, encoding muconolactone D-isomerase, was absent in all ^{13}C-lignin assemblies. Fourteen scaffolds contained neighbouring homologs for at least three of the four β-ketoadipate pathway genes and were classified to *Burkholderiaceae*, *Oxalobacteraceae*, *Enterobacteriaceae*, *Rhodobiaceae*, *Methylobacteriaceae*, *Rhizobiaceae* and *Sphingomonadaceae*.

CAZyme family profiles further substantiated differences in cellulolytic and lignolytic groups within *Caulobacteraceae*. Their CAZy profiles exhibited greater differentiation between cellulose and lignin libraries (ANOSIM; $R=0.202$; $p<0.001$) than overall metagenome profiles ($R=\sim 0.08$) and also grouped by ecozone (ANOSIM; $R=0.09$, $p=0.02$). The distinction between lignolytic and cellulolytic *Caulobacteraceae* was supported by PCA and by hierarchical clustering using Bray-Curtis dissimilarities (Figure 4.22). A number of CAZy families were differentially abundant in lignolytic *Caulobacteraceae* populations, including the oxidase family AA3, and in cellulolytic populations, such as the endoglucanase-containing family GH9 (Figure 4.22B). Taxonomic classifications also differed, with CAZymes classified as *Asticcacaulis* more abundant in ^{13}C-cellulose libraries and *Brevundimonas* and *Caulobacter* more abundant in ^{13}C-lignin libraries (Figure 4.22C), consistent with results from pyrotag analysis.

Fungi possessed far higher proportions of peroxidases (AA2), iron reductase domains (AA8) and lytic polysaccharide monooxygenases (AA9) in unassembled ^{13}C- libraries. These AA families were most abundant in southern ecozones, PP_{CA} and LP_{TX}, where members of the order

Sordariales were most abundant (Figure 4.23). LPMOs were also classified to *Sebacinales*, supporting their designation as cellulolytic in analysis of pyrotag libraries as well as their endemicity in western ecozones (Figure 4.23; details in section 4.2.5).

Figure 4.21. Taxonomic classifications of assembled scaffolds from ^{13}C-lignin metagenomes containing genes from the β-ketoadipate pathway: catechol 1,2-dioxygenase (*catA*; K03381), muconate cycloisomerase (*catB*; K01856) and 3-oxoadipate enol-lactonase (*pcaD*; K01055). Only families with >3 scaffolds are shown.



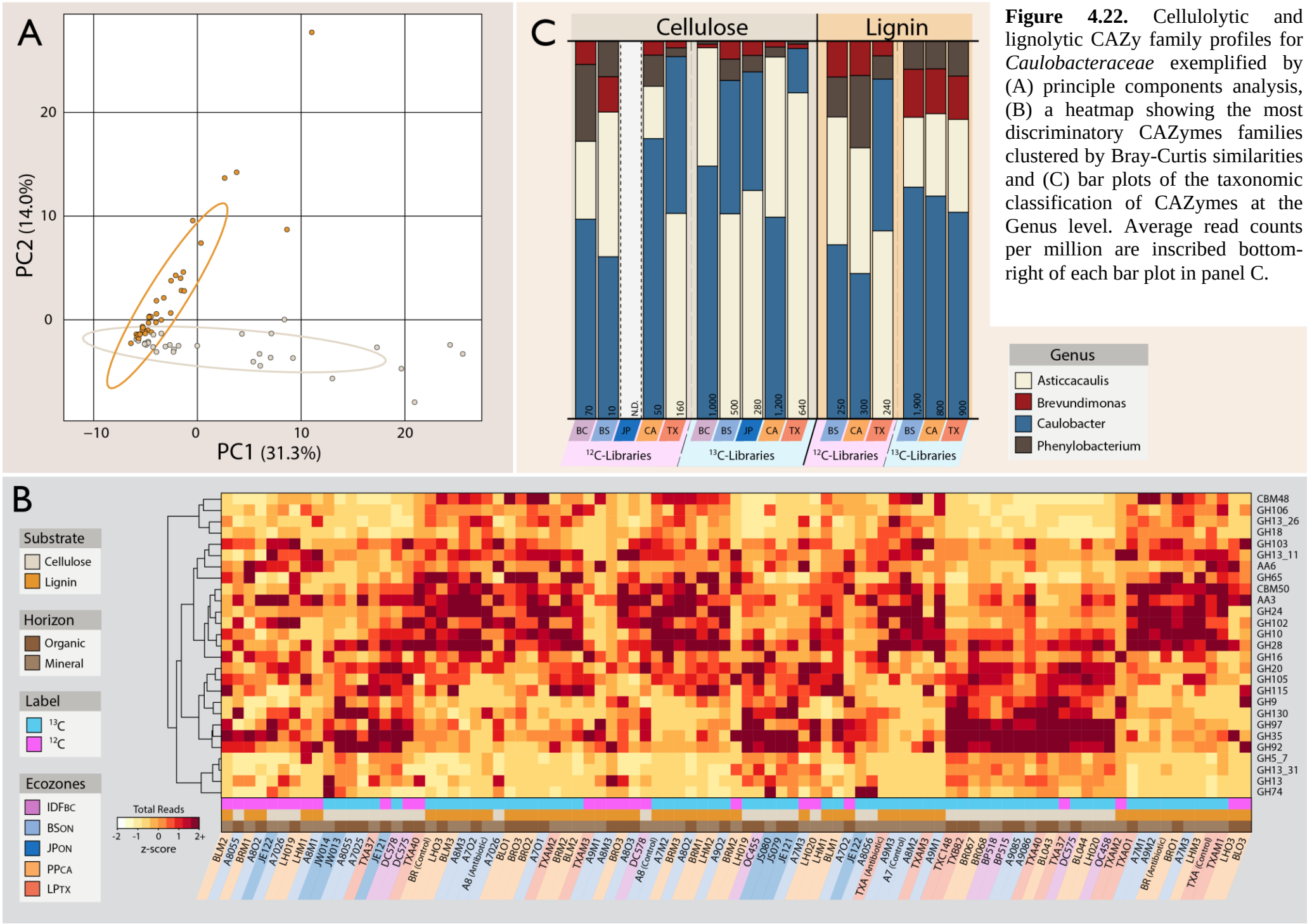
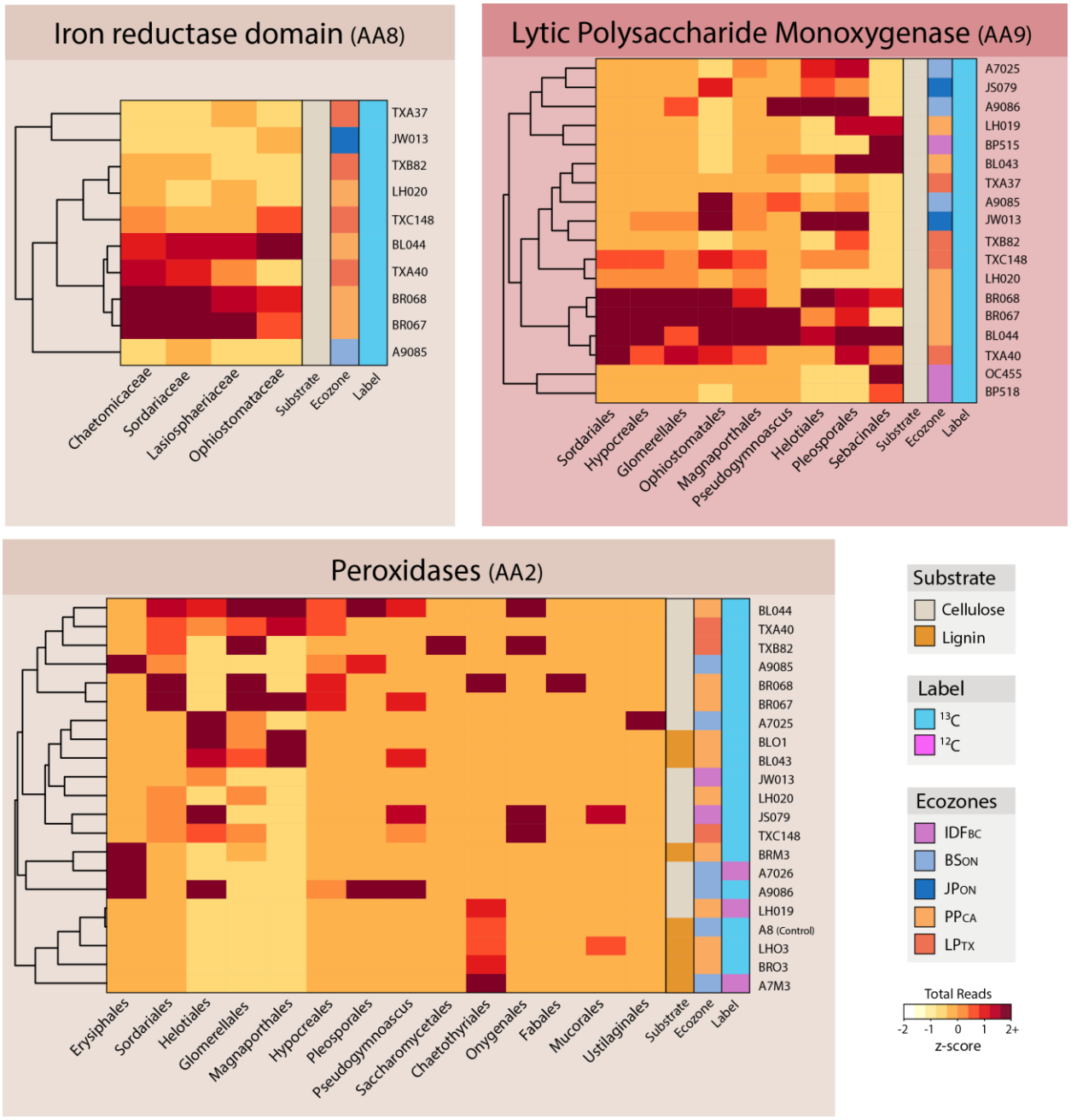


Figure 4.22. Cellulolytic and lignolytic CAZy family profiles for *Caulobacteraceae* exemplified by (A) principle components analysis, (B) a heatmap showing the most discriminatory CAZymes families clustered by Bray-Curtis similarities and (C) bar plots of the taxonomic classification of CAZymes at the Genus level. Average read counts per million are inscribed bottom-right of each bar plot in panel C.

Figure 4.23. Heat maps showing the relative abundance of fungal taxa in fungi-exclusive CAZyme families AA2, AA8 and AA9.



4.2.4 Relative Contributions of Lignocellulose-degrading Taxa

The importance of any given taxon in the rate of ^{13}C-assimilation from labeled substrate was assessed using 'Boruta' to test whether specific taxa were predictors of delta-^{13}C enrichment in both DNA and PLFA. A number of taxa were identified that were correlated with enrichment levels across all samples, namely *Caulobacteraceae* (r=0.49, p=0.006) and *Sphingomonadales* (r=0.44, p=0.02) for lignin, and *Opitutacaeae* (r=0.48; p=0.01) and *Planctomycetaceae* (r=0.53, p=0.004) for cellulose (Figure 4.24). Other lignolytic taxa were also selected, such as *Rhodospirillales* and *iii1-15*, though these groups exhibited a narrower distribution across samples. No fungal taxa were selected, but this could also be due to their inconsistent distribution among samples. Notably, the majority of samples with the highest average enrichment had greater proportions of fungi (Figure 4.25AB) from classes *Sebacinales*, *Helotiales*, *Sordariales*, *Orbilliales* and *Pseudogymnoascus* (Figure 4.25C).

Total bacterial incorporation of ^{13}C into PLFAs surpassed that of fungi for both cellulose and lignin substrates. Fungi and Gram-negative bacteria had comparable levels of enrichment on cellulose, whereas fungi had surprisingly low activity on lignin except in LP_{TX} (Figure 4.26). Fungal activity was more apparent when ^{13}C-enrichment was normalized to pre-existing biomass (Figure 4.27). Gram-negative bacteria had the highest delta-^{13}C enrichment from both substrates in 51 of 73 PLFA samples, while fungi and Gram-positive bacteria were comparable at 18 and 14, respectively. The total enrichment in samples dominated by fungi was not significantly greater than those dominated by Gram-negative bacteria (t-test; p=0.89), nor was ^{13}C-enrichment of PLFA or DNA correlated to the total number of bacterial or fungal metagenomic reads. The enrichment of fungal PLFAs had greater overall variability, between 2 and 10-fold greater variance, relative to Gram-negative and Gram-positive bacteria, respectively.

Figure 4.24. Linear regression of ^{13}C-enrichment of DNA and abundance for taxa selected by Boruta analysis as indicators of overall enrichment on either cellulose (off-white) or lignin (brown-gold). Spearman's rank correlation coefficients and p-values derived from randomization are given.

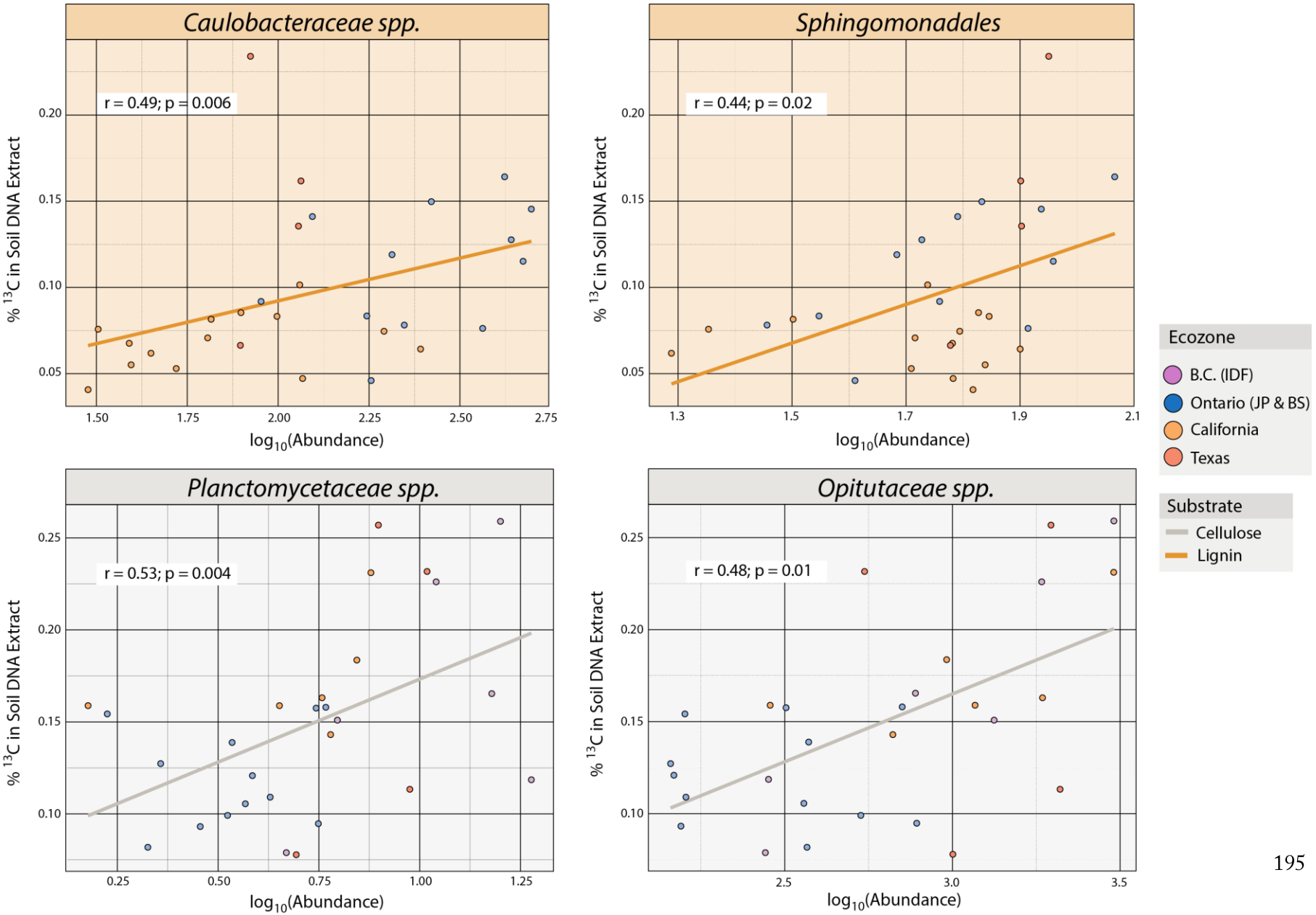


Figure 4.25. Total ^{13}C-enrichment of DNA (A) and corresponding ratio of Fungi:Bacteria (B) and overall taxonomic composition of metagenomic libraries (C) among ecozones and soil horizons. Barplots correspond to the proportion of unassembled metagenomic reads classified at the domain (B) and order (C) level. Taxa occupying fewer than 0.5% of reads were not shown.

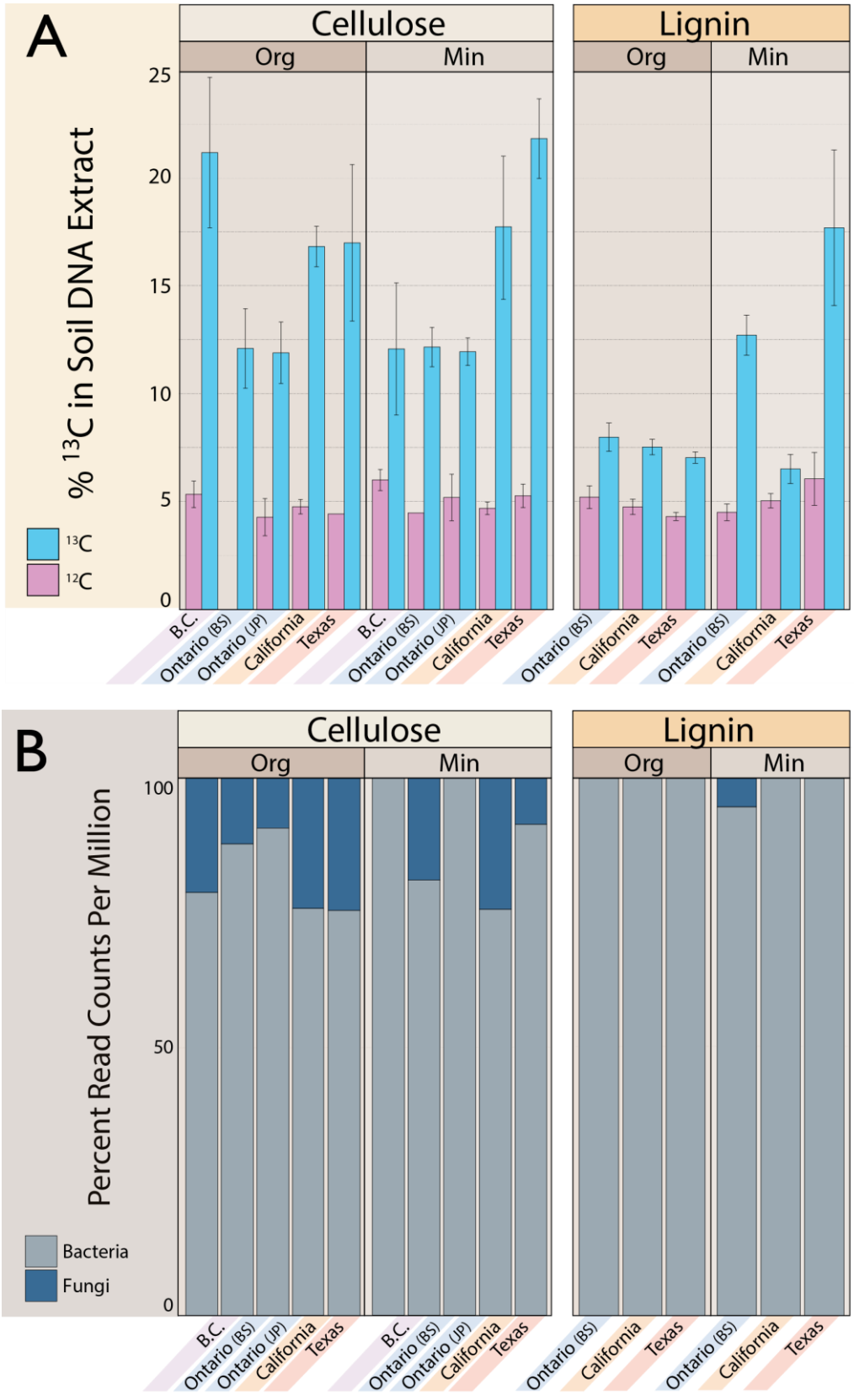
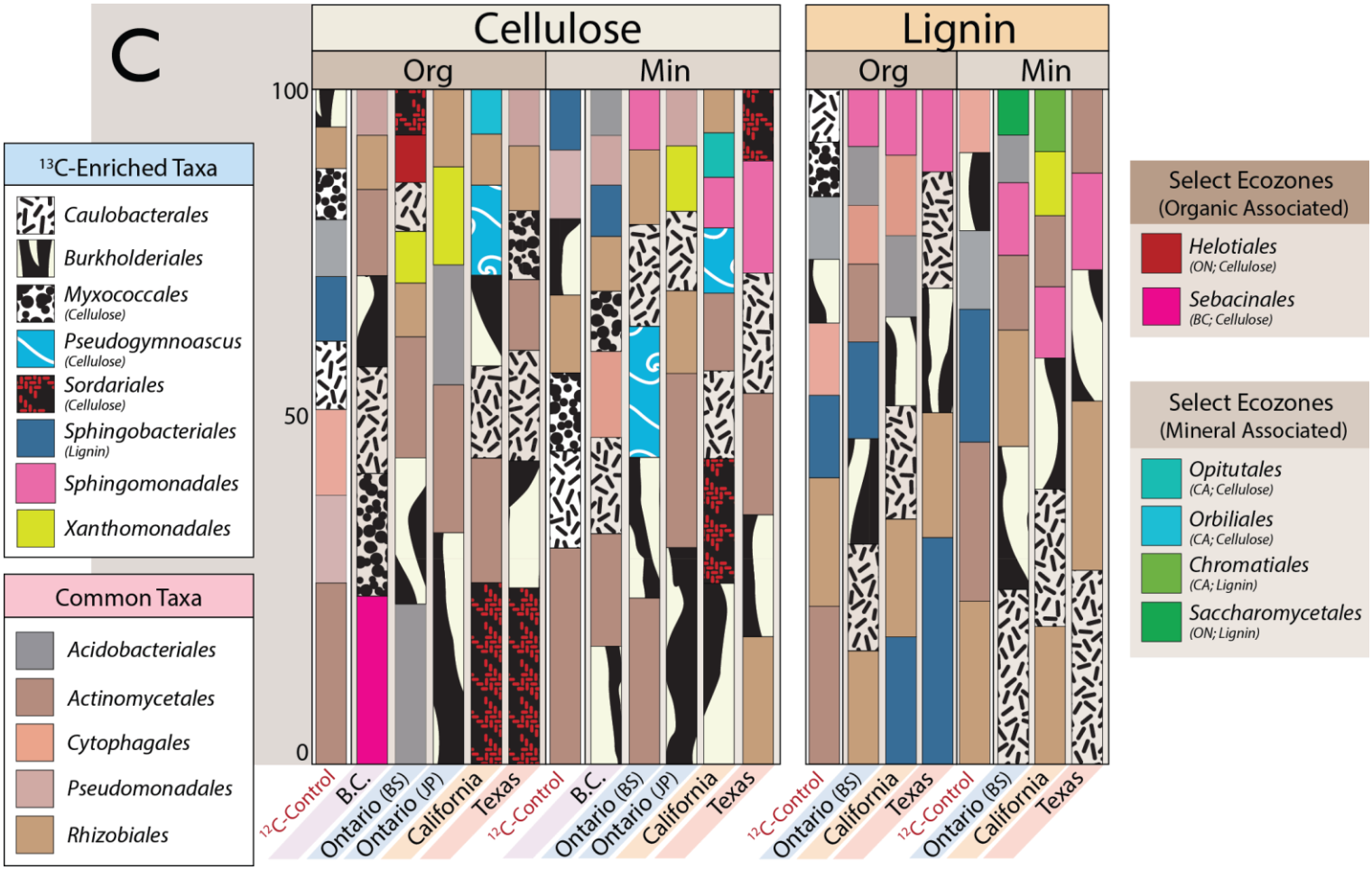


Figure 4.25… *continued*



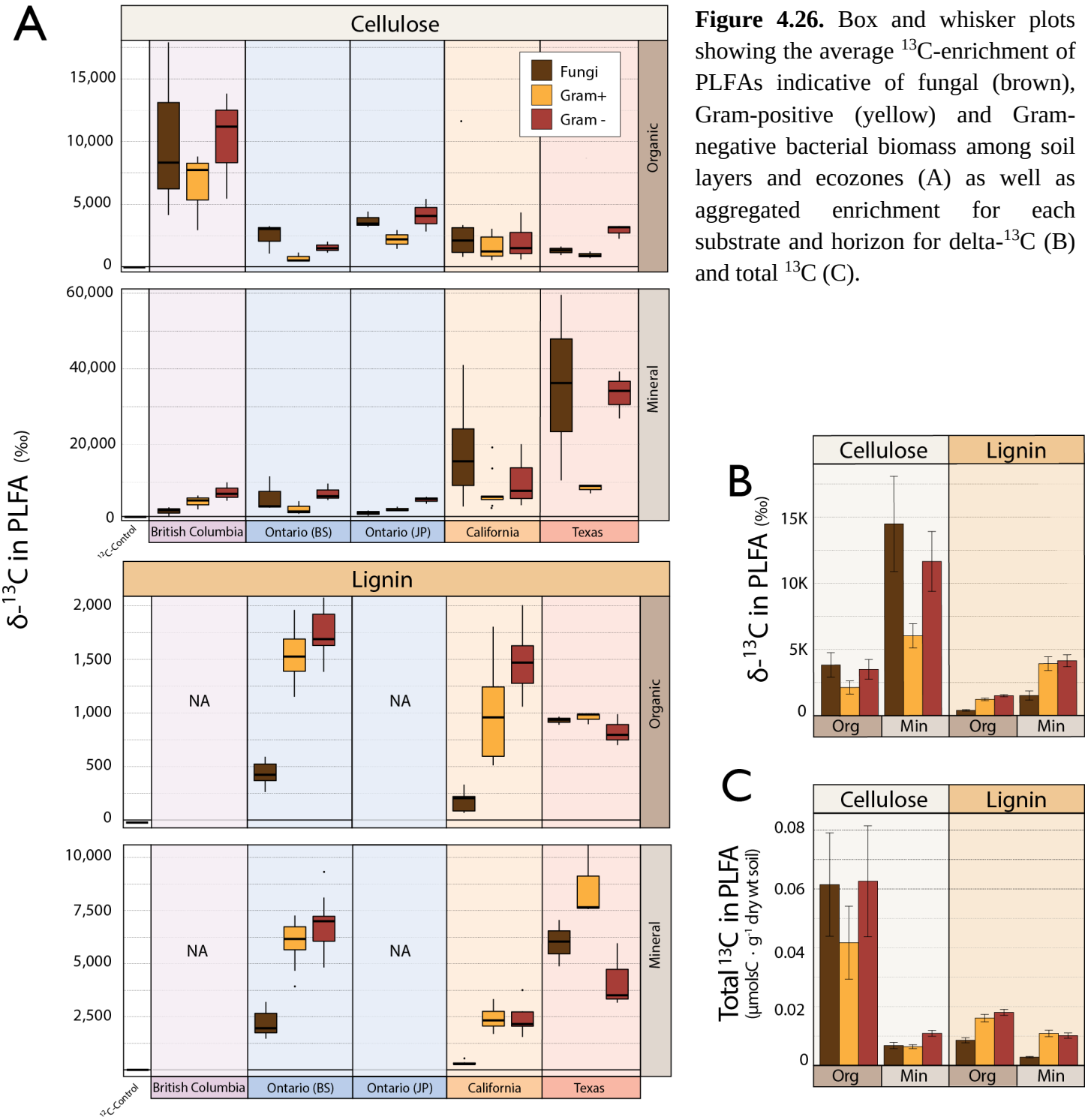
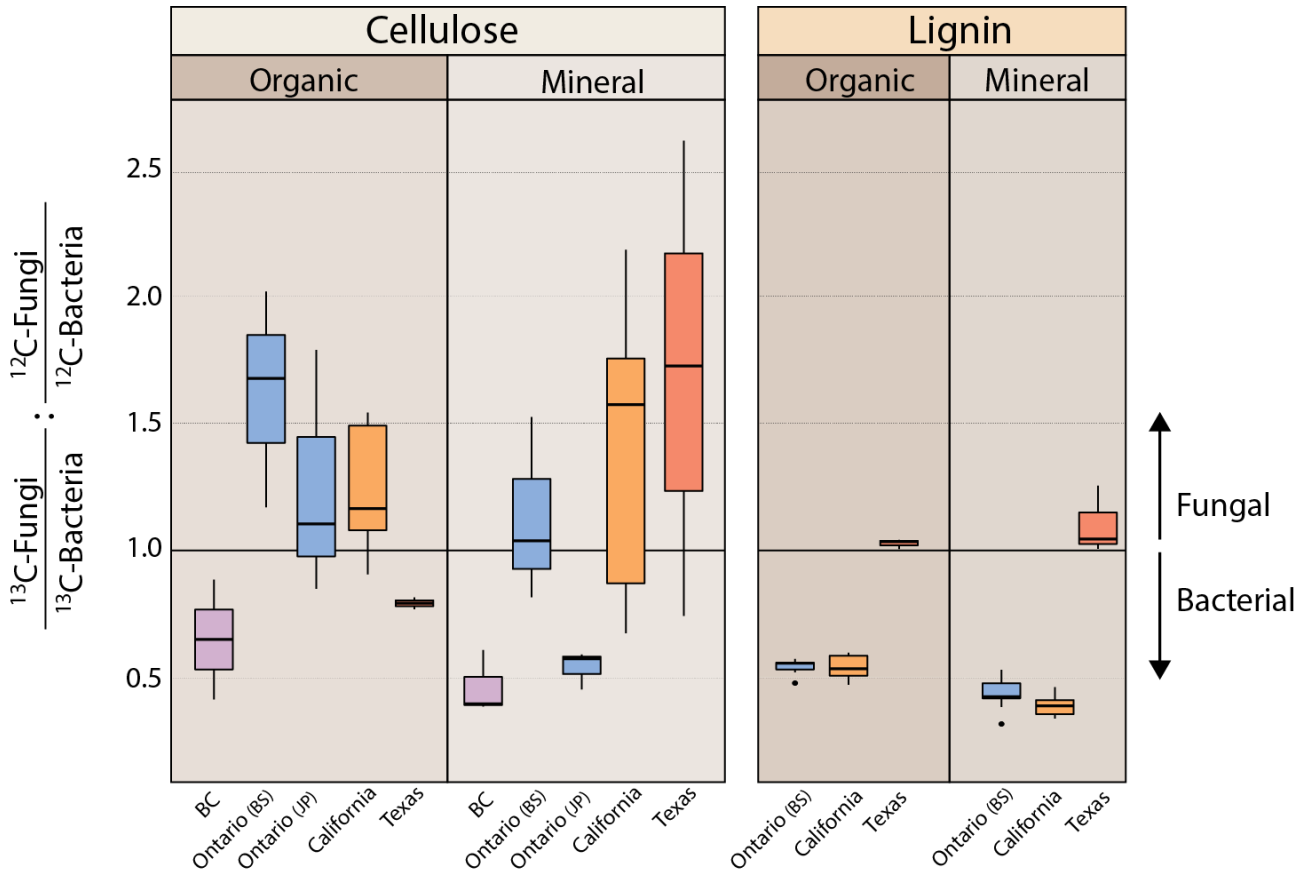


Figure 4.26. Box and whisker plots showing the average ^{13}C-enrichment of PLFAs indicative of fungal (brown), Gram-positive (yellow) and Gram-negative bacterial biomass among soil layers and ecozones (A) as well as aggregated enrichment for each substrate and horizon for delta-^{13}C (B) and total ^{13}C (C).

Figure 4.27. Box and whisker plots showing the relative enrichment of fungal relative to bacterial PLFAs (^{13}C-fungi:^{13}C-bacteria) normalized to pre-existing PLFA biomass (^{12}C-fungi:^{12}C-bacteria).



Fungicide treatment reduced the overall ^{13}C-enrichment of PLFAs by 70% and produced a steep decline in the enrichment of both fungi and Gram-positive bacteria during incubations with labeled lignin. Fungicide reduced ^{13}C-enrichment of fungal PLFAs by ~90%, reducing fungal enrichment in PP_{CA} to levels comparable to incubations with unlabeled substrate, -9 ‰ versus -28 ‰ delta-^{13}C, respectively (Figure 4.28). The enrichment of Gram-positive and Gram-negative bacteria was also reduced by fungicide treatment, ~90 and ~50%, respectively, but by only 2% for Gram-negative bacteria in soils from LP_{TX}. The major taxonomic groups active in fungicide treated microcosms were distinct from untreated incubations (Figure 4.29). The sustained activity of Gram-negative groups was evident in the increased relative abundance of families from *Burkholderiales*. In the case of BS_{ON} (site A7), the entire metagenomic library was classified as *Burkholderiaceae* (99%), demonstrating unprecedented recovery of DNA from functional subpopulations from a SIP experiment. Notably, *Burkholderiaceae spp.* showed the highest differential abundance in BS_{ON} between ^{13}C- and ^{12}C-lignin pyrotag libraries (Appendix Figure C.6). In contrast *Burkholderiaceae* populations were unaffected by fungicide treatment in PP_{CA} and were also not differentially abundant in pyrotag libraries. Despite the reduction in Gram-positive PLFA ^{13}C-enrichment, the relative abundance of *Actinobacteria* increased in metagenomes from fungicide treatments (Figure 4.30). Certain families of *Actinobacteria* were negatively impacted by fungicidal treatment, such as *Acidimicrobiales* and *Conexibacteraceae*, while others increased in abundance, such as *Nocardiaceae Microbacteriaceae* and *Micrococcaceae*.

Figure 4.28. Enrichment of PLFAs in soil samples incubated with ^{13}C-lignin with or without the addition of anti-fungal cocktail. Each bar represents a single sample, except for in BSON where n=2.

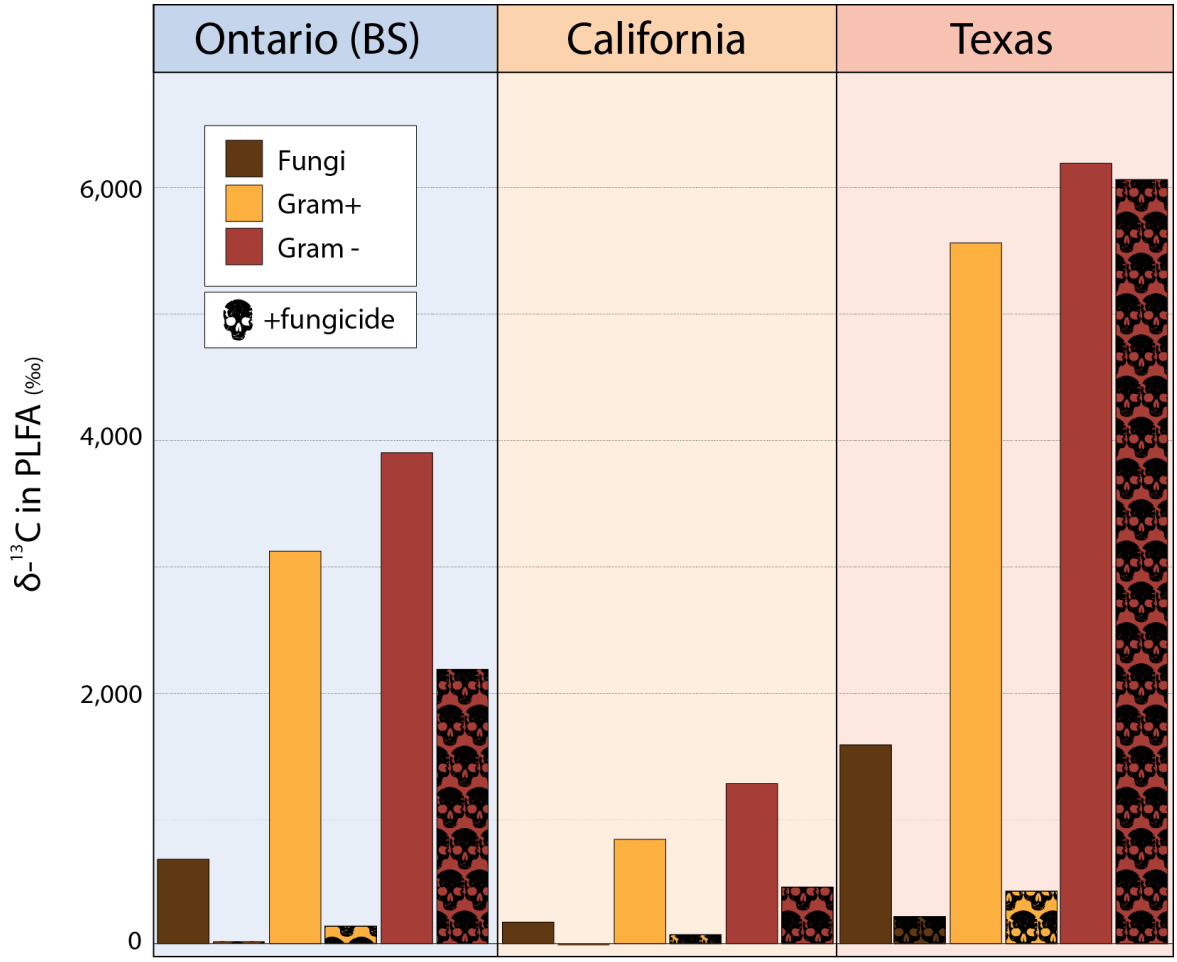


Figure 4.29. Abundances of predominant families in unassembled metagenomic libraries derived from ^{13}C-lignin incubations treated with 'fungicide' and without ('paired'). The averaged abundances of ^{12}C- and ^{13}C-lignin metagenomes from separate incubations were also included.

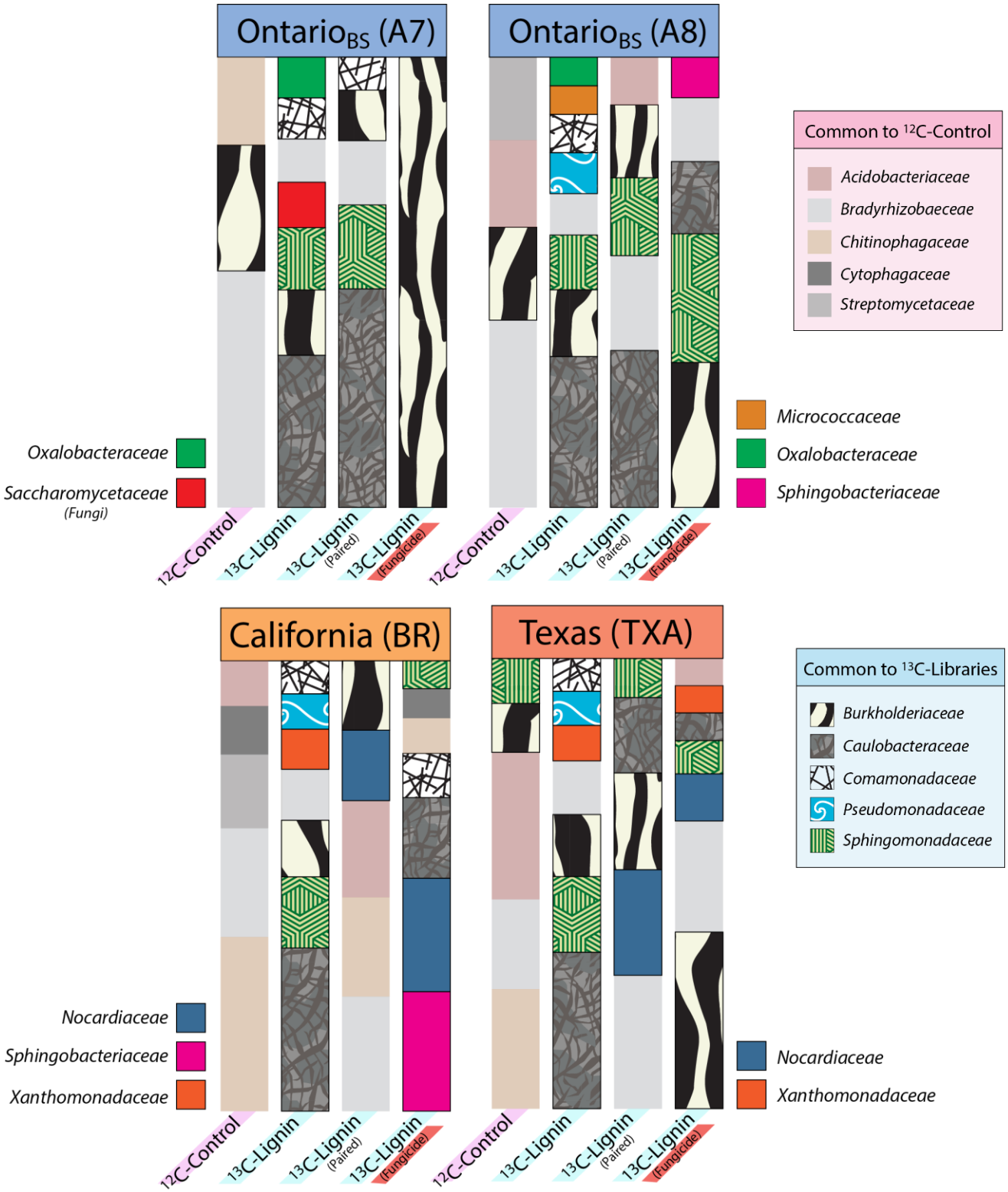
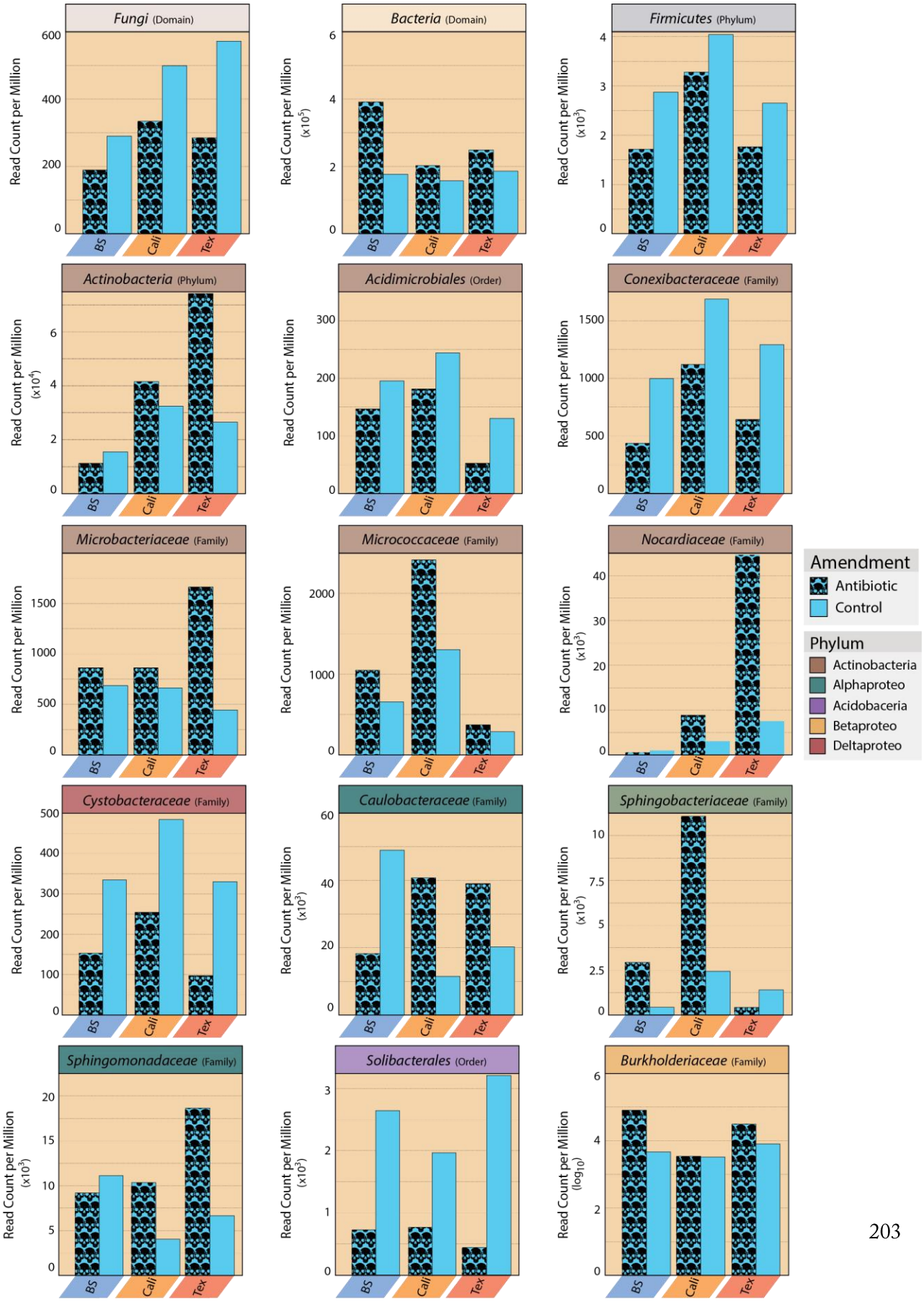


Figure 4.30. Abundances of select taxa previously identified as lignolytic in unassembled metagenomic libraries from soils amended ^{13}C-lignin with or without antibiotic. Each bar represents a single sample, except for in BS$_{ON}$ where n=2. Taxa were selected which exhibited notable trends of expansion or decline in antibiotic or control libraries.



4.2.5 Ecozone-Specific Lignin and Cellulose-degrading Populations

Of the total OTUs identified as differentially abundant between ^{12}C- and ^{13}C-pyrotag libraries (503), only 41 were abundant enough to be detected *in situ*, indicating a strong enrichment by SIP for low abundant OTUs. The majority of enrOTU found *in situ* were hemicellulose-degraders (90%), while only six lignolytic (*Solibacterales*, *iii1-15*, *Myxococcales* and *Gaiellales*) OTUs were found (no cellulolytic OTUs were found). This was consistent with the observation that hemicellulolytic and *in situ* libraries shared a majority of OTUs, in contrast to other libraries (Figure 4.6). Of the OTUs found *in situ*, their abundances in SIP-libraries was strongly correlated with *in situ* abundances (Figure 4.31). JP_{ON} and BS_{ON} sites had the greatest percentage of overlapping OTUs of any two ecozones (23%), while PP_{CA} and IDF_{BC} shared the second highest degree of overlap (17%). LP_{TX} had the lowest proportion of overlap with other ecozones (Figure 4.32).

Figure 4.31. Linear regression between *in situ* abundance of OTU deemed lignocellulolytic and their abundance in corresponding ^{13}C-pyrotag libraries.

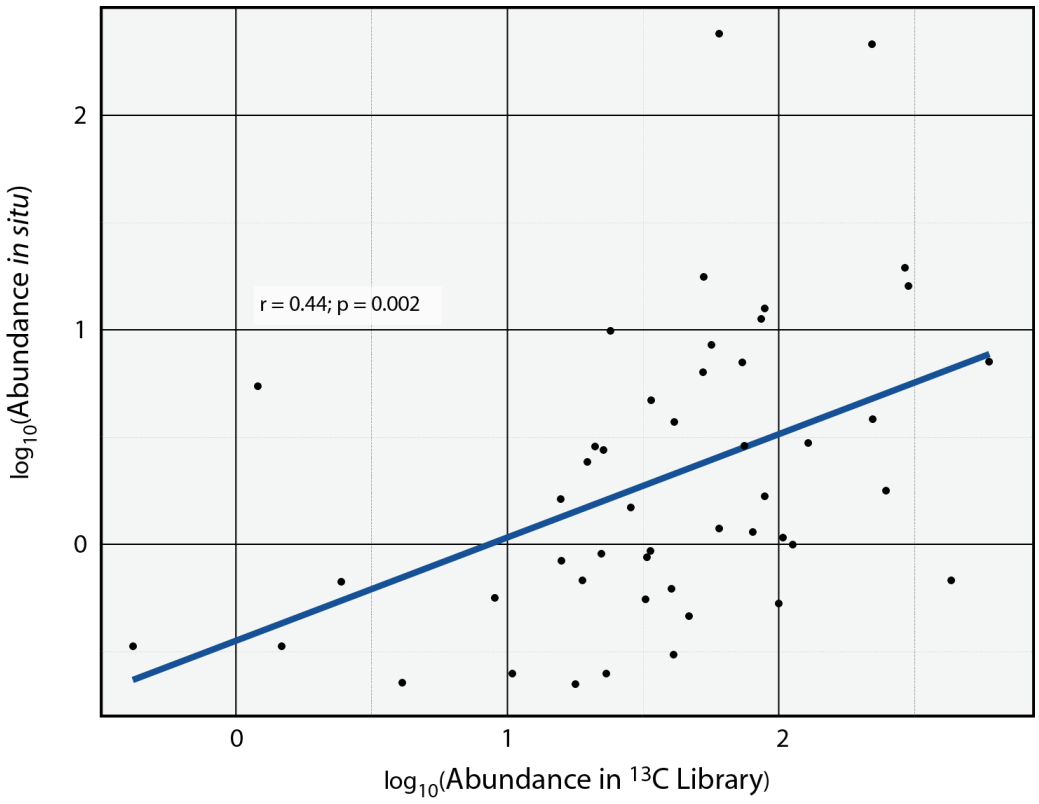
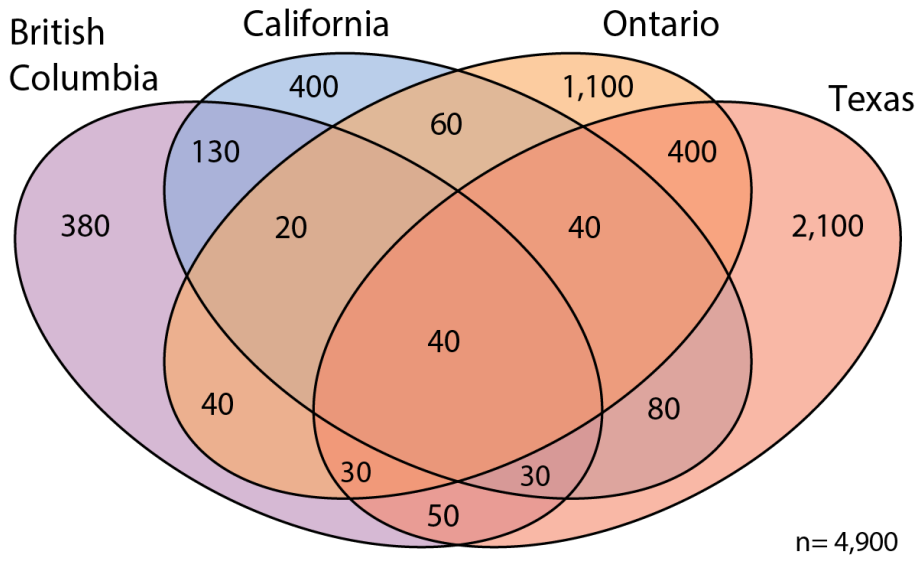


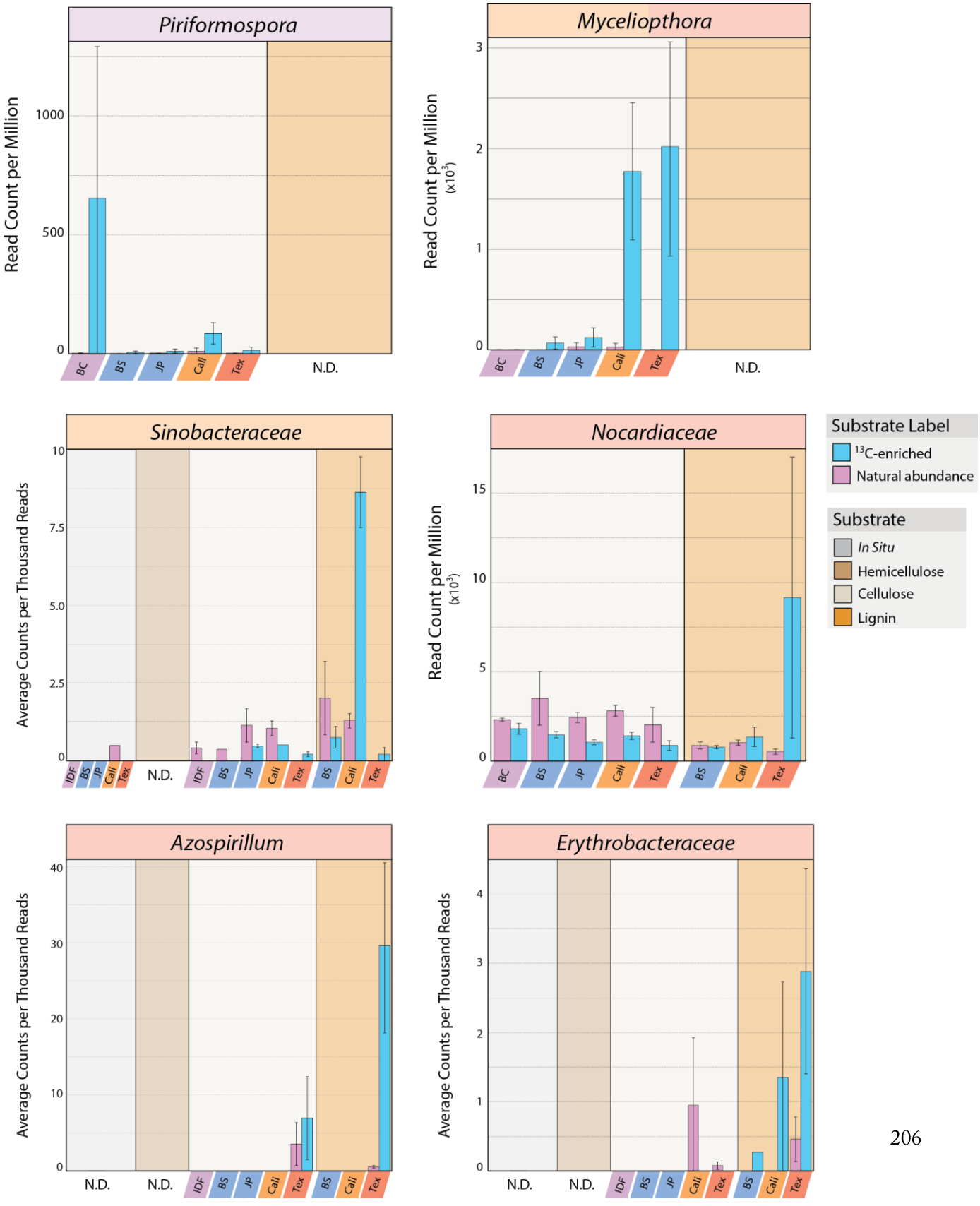
Figure 4.32. Venn diagram showing overlapping OTUs in ^{13}C-cellulose libraries among ecozones.



One of the most notable biogeographical features was the domination of bacterial cellulose-degraders in the IDF_{BC} ecozone and by a broadly different set of taxa than in others. Cellulolytic populations of *Deltaproteobacteria* (*MIZ46*), *Gammaproteobacteria* (*Cellvibrio*), *Planctomyces*, *Bacteroidetes* and *Verrucomicrobia* (*Opitutaceae*) were highly enriched in SIP-libraries from IDF_{BC}, but not elsewhere (Figure 4.9 and Appendix Figure C.2). Cellulolytic *Ascomycota*, prominent among all other ecozones, were also largely absent in IDF_{BC} (Figure 4.8; pg. 129). In contrast, cellulolytic *Piriformospora* (*Sebacinales; Basidiomycota*) were highly abundant, and to a lesser extent abundant in the other western ecozone PP_{CA} (Figure 4.33). The presence of cellulolytic *Sebacinales* in western ecozones was corroborated by the abundance pattern of LPMO family AA9 (Figure 4.23; pg. 158). Conversely, southern ecozones shared a predominance of cellulolytic *Ascomycota*, such as *Humicola*, *Arthrobotrys*, *Myceliopthora* and *Chaetomium* (Figure 4.33) also illustrated in LPMO family AA9 (Figure 4.23).

Biogeographical differences in lignolytic taxa were less pronounced than cellulolytic populations. A number of actinobacterial groups were specific to BS_{ON}, namely: *Gaiellaceae*,

Figure 4.33. Localized patterns of putatively cellulolytic or lignolytic taxa in either unassembled metagenomic (*Piriformospora*, *Myceliopthora* & *Nocardiaceae*) or pyrotag libraries (uncl. *Xanthomonadales*, *Azospirillum* & *Erythrobacteraceae*).



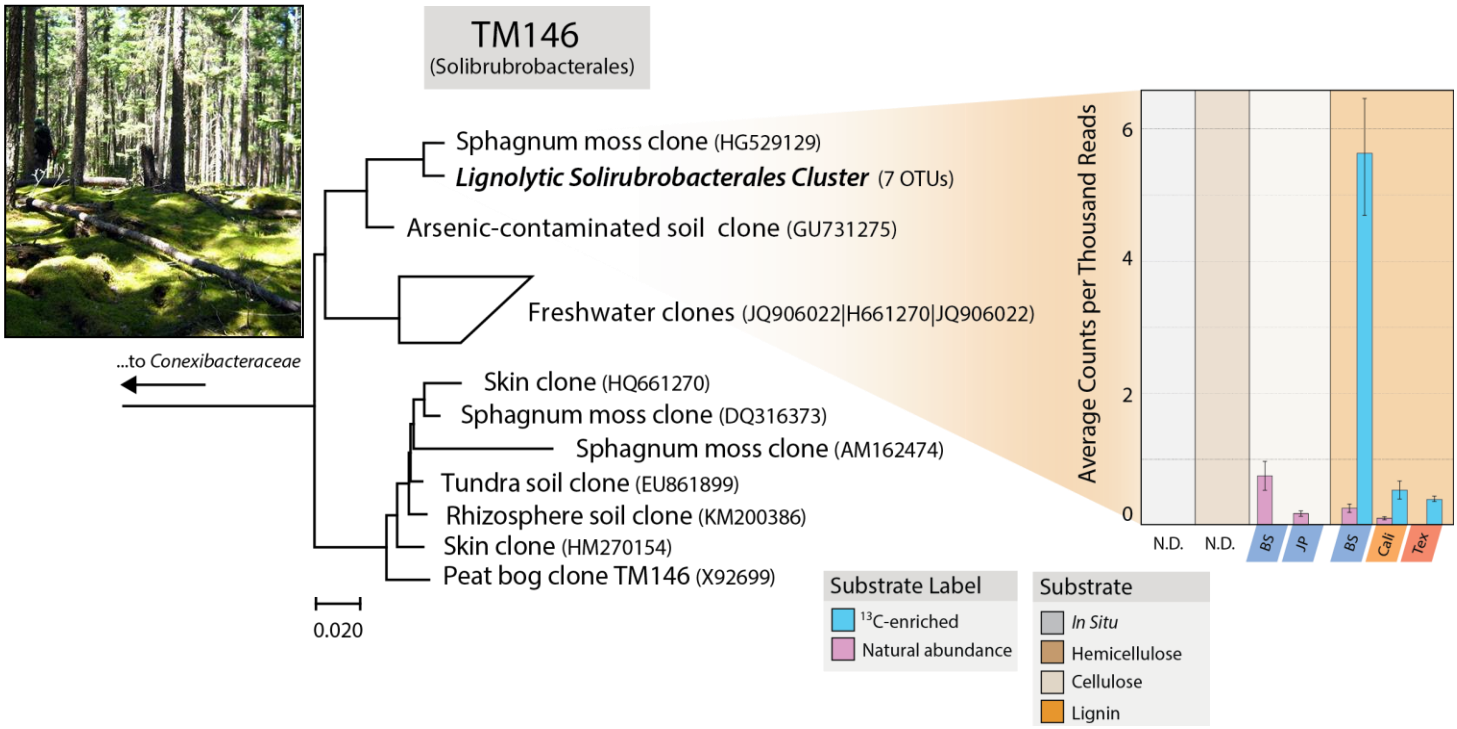
ACK-M1 and members of *Solirubrobacterales* clade 'TM146.' The prevalence of TM146 OTUs in BS_{ON} was notable given that soils there were blanketed with Sphagnum moss, not found at other ecozones. The clade *TM146* was named for a bacterial isolate from Sphagnum moss and contains a number of sequences from similar environments (Figure 4.34).

Lignolytic populations in LP_{TX} were distinct from other ecozones in both organic and mineral soils based on PLFA enrichment profiles (Figure 4.7; pg. 128). The majority of lignolytic OTUs exclusive to LP_{TX} (n=64) were members of genera common to all ecozones, illustrating fine-scale phylogenetic differences of localized populations. LP_{TX} also possessed a number of genera uncommon to other ecozones of which *Azospirillum* and *Nocardiaceae* were most abundant (Figure 4.33) and other exceptions included: *Ellin6513* (*Acidobacteria*), *Uliginosibacterium*, a number of *Actinobacteria* (*Salinispora*, *Nocardiopsaceae*, *Geodermatophilaceae*, *Pseudonocardiaceae* and *Gordoniaceae*), and *Holosporaceae*.

4.2.6 In situ Abundances of Lignocellulose-degrading Populations

Due to the poor overlap of OTUs between *in situ*, cellulose and lignin pyrotag libraries, the *in situ* abundances of lignocellulosic taxa could not be reliably assessed. During analyses performed in Chapter 3, one third of cellulolytic OTUs were detected *in situ* and, of those detected, their abundances were modest. Cellulolytic OTUs constituted between 0.08% and 1.4% of total reads with a median abundance of 0.42% among all samples. The most abundant single OTU (uncl. *Streptomycetaceae*) occupied, at most, 0.6% of a given sample with a mean abundance of 0.1%, followed by OTUs from *Janthinobacterium* (max: 0.5%), *Burkholderia* (0.4%), and uncl. *Microbacteriaceae* (0.3%). Of all enrOTU detected *in situ*, 90% were detected in the organic layer, suggesting SIP-incubations selected for low abundance taxa in mineral soils below the sequencing depth of *in situ* libraries.

Figure 4.34. Maximum parsimony phylogenetic tree for lignolytic OTUs classified to *Solirubrobacterales* (*Actinobacteria*) predominant in BS_{ON}. OTUs clustered in the 'TM146' clade (SILVA), named after a peat bog clone. *Inset* - Picture of sampling site in BS_{ON} with Sphagnum as predominant ground cover.

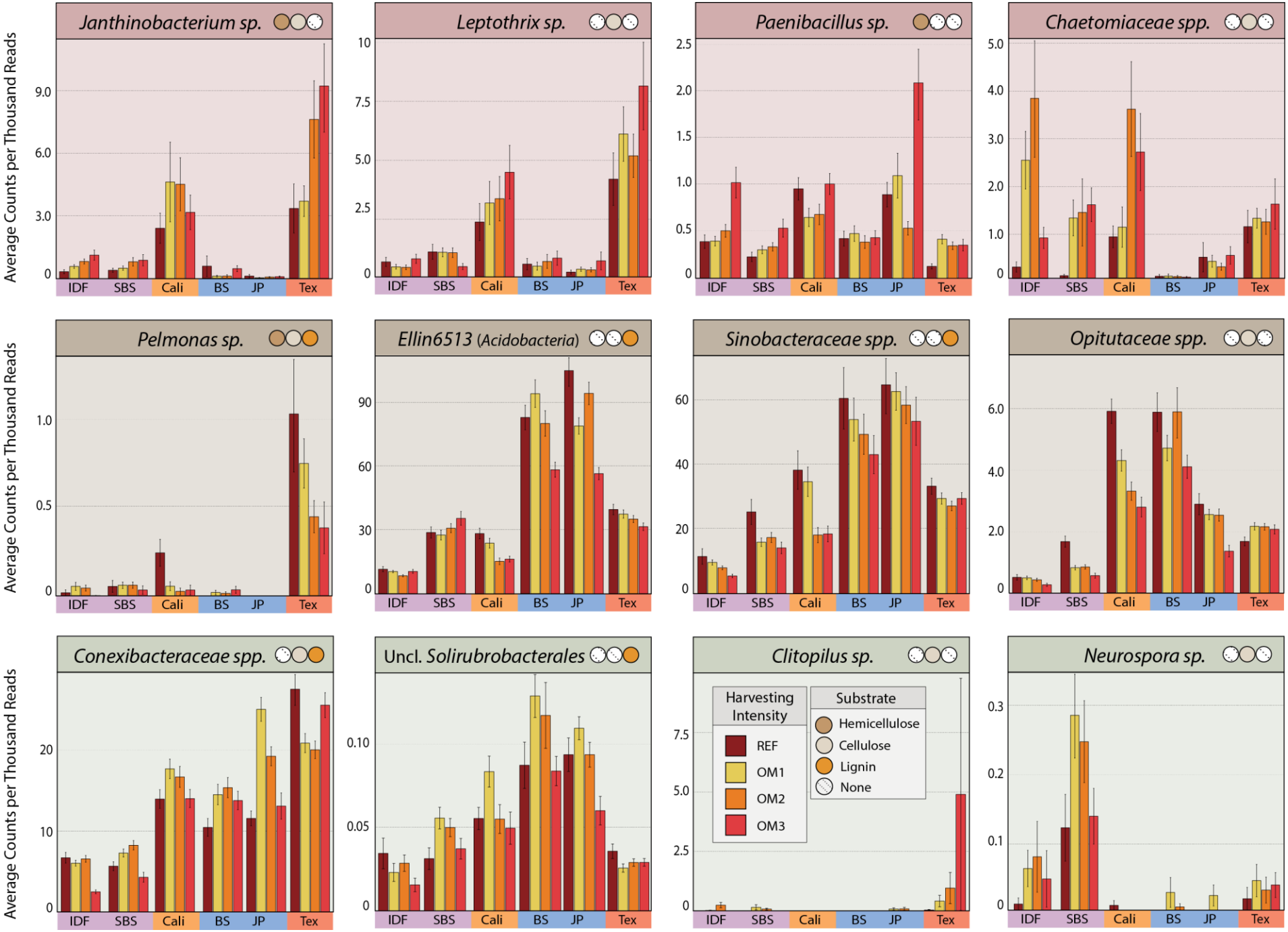


For fungi, six out of the sixteen enrOTU were detected *in situ* in low abundance (maximum 0.3% of any given sample), though, when binned by taxonomic classification, members of *Chaetomiaceae* occupied an average of ~2% of total *in situ* libraries. Consistent with observations in Chapter 3, taxa lacking reported lignocellulolytic activity were more common in mineral soils, including *Armatimonadetes* (hemicellulose | cellulose), *Candidatus Saccharibacter* (formerly *TM7*; hemicellulose), *Delta-* and *Gammaproteobacteria* (all three substrates) and *Acidobacteria* (lignin). In contrast, known cellulolytic and lignolytic taxa were more common in the organic layer, including an array of fungi, *Actinobacteria*, *Alpha-* and *Betaproteobacteria* (Table 4.1).

4.2.7 Impacts of Timber Harvesting on Lignocellulosic Taxa

Given the observations of fine-scale phylogenetic differences in response to harvesting reported in Chapter 3, the poor overlap between SIP and *in situ* OTUs make any characterization of harvesting impacts on lignocellulosic taxa highly speculative. In addition to the cellulolytic taxa affected by harvesting identified in section 3.3, namely, *Chaetomium*, *Opitutaceae*, *Streptomycetaceae* and *Caulobacteraceae*, the populations of a number of putatively lignocellulosic taxa may be affected. *In situ* populations of *Janthinobacterium, Paenibacillus, Cryptococcus* and *Leptothrix* all expanded in harvested plots (Figure 4.35; coloured red). Conversely, populations of putatively lignolytic taxa declined in harvested plots, such as *Pelomonas, Ellin6513* (*Acidobacteria*) and *Sinobacteraceae*, and cellulosic taxa, such as *Hypocrea* and *Sebacinaceae* (not shown) (Figure 4.35; coloured brown-grey). Notably, populations of *Pelomonas* and *Ellin6513* exhibited clearest declines in LP_{TX}, the only ecozone where they were identified as lignolytic. Three lignocellulosic groups had slight and consistent increased relative abundance at intermediate harvesting intensities, in particular in OM1: unclassified *Conexibacteraceae* and *Solirubrobacterales* (both *Actinobacteria*) and *Neurospora* (Fungi), (Figure 4.35; coloured green). *Clitopilus sp.*, previously identified as cellulolytic in PP_{CA} in Chapter 3, exhibited higher relative abundance at intermediate harvesting intensities, though appeared to also favour conditions in OM3 in LP_{TX} (Figure 4.35; coloured green).

Figure 4.35. Abundances of taxa designated putatively on one or more substrate among harvesting treatments in *in situ* pyrotag libraries coloured by whether populations expanded in harvested sites (shaded red), declined (shaded brown-grey) or peaked in OM1 or OM2 plots (shaded green).



4.3 Discussion

SIP surveying lignocellulolytic taxa in forest soils across North America expanded the diversity of putative degraders and provided evidence that, in many cases, contradicted or tempered conventional views of decomposer populations. Hemicellulolytic, cellulolytic and lignolytic microorganisms were identified in fourteen bacterial phyla and two fungal divisions, which were largely evident among all ecozones studied. Some of these groups exhibited exclusive activity on a single substrate, while others the capacity to degrade all three lignocellulosic polymers. The decomposition of DHP lignin in soil microcosms was largely bacteria-driven, predominated by Gram-negative groups, while fungi were substantial contributors to cellulose decomposition. Putatively lignocellulolytic taxa included a sizeable contingent of taxonomic groups with previously characterized *in vitro* activity, revealing their activity in soils and expanding knowledge of their phylogenetic diversity. This thesis reports, for the first time, the potential capacity to depolymerization lignin, and/or catabolize lignin bi-products, by members of *Acidobacteria*, *Chloroflexi*, *Elusimicrobia* and *Deltaproteobacteria* as well as novel groups within *Actinobacteria* and *Gammaproteobacteria*. The attribution of lignolytic activity was supported by the recovery of CAZy gene clusters encoding AA genes classified to the aforementioned groups. Similarly, for cellulose depolymerization, the substantial enrichment for *Planctomycetes*, *Armatimonadetes* and *Verrucomicrobia* revealed hereto unrecognized populations of decomposers in forest soils.

The ability of *Caulobacteraceae spp.* to degrade all three lignocellulosic substrates was unexpected, though, in retrospect, a number of lines of evidence suggest their involvement. The earliest evidence originates from work done by Henrici and Johnson (1935), who grew the first cultures of *Caulobacter* in cellulose-amended liquid culture. After their initial characterization, *Caulobacter* quickly became better known as model organisms for dimorphism and for

possessing strong adhesive 'holdfast' proteins (Poindexter, 1964). Recent attention has been given to their potential role in breakdown of lignocellulosic substrates after an array of CAZy genes were identified in the first sequenced genome (Nierman *et al.,* 2001). Subsequently, the capability of *C. crescentus* to degrade xylose was shown along with its expression of an unexpectedly high diversity of TonB-dependent sugar transporters (Hottes *et al.,* 2004; Blanvillain *et al.,* 2007). More recently, *C. crescentus* was shown to catabolize a broad range of carbohydrates, including cellobiose, both aerobically (Presley *et al.,* 2014) and anaerobically at temperatures up to 45°C (Song *et al.,* 2013). The capacity for lignin catabolism by *C. crescentus* is further supported by its growth on vanillic acid, benzoate and other aromatics as well the presence of a vanillate-inducible promoter in its genome (Chatterjee and Bourquin, 1987; Thanbichler *et al.,* 2007). Al-Thukair and Malik (2016) reported a *Caulobacter sp.* isolated from an oil-contaminated site that could degrade pyrene, while a laccase gene was previously identified in the *C. crescentus* genome based on homology, but has not been characterized (Li *et al.,* 2009). The potential role of *Caulobacter* species in decomposition in environmental samples has been shown in SIP-cellulose experiments in forest soil (Wang *et al.,* 2015) and a range of soils (Verastegui *et al.,* 2014), and in lignin-degrading cultures in tropical and temperate forest soils incubations (DeAngelis *et al.,* 2011; Pold *et al.,* 2015), though no study as conclusively or as explicitly as this research.

This thesis firmly solidifies the importance of genera within *Caulobacteraceae* as major lignocellulolytic groups. *Caulobacteraceae* were one of the few taxonomic groups significantly correlated with the degree of ^{13}C-enrichment from labeled lignin and their activity endured in fungicide treated soils. Lignin-degrading *Caulobacteraceae* were members of largely uncultured clades, which were more abundant in the mineral soil layer, supporting the existence of relatively unknown degraders of recalcitrant compounds in deeper, low nutrient soils. The differentiation

among cellulolytic/hemicellulolytic and lignolytic clades within *Caulobacter* and between its highly cellulolytic relative, *Asticcacaulis*, was supported by 16S rRNA gene libraries as well as CAZy content, suggesting the study of *Caulobacteraceae* may provide an interesting case study of niche partitioning. *Asticcacaulis* were originally differentiated from *Caulobacter* based on differences in stalk angle and localization of holdfast protein (Poindexter, 1964). So, while speculative, the differences in substrate utilization (lignin vs. cellulose) between these close relatives may relate to differences in cell structure and attachment. The observation of cellulolytic activity by *Asticcacaulis* was relatively novel with only one study reporting a cellulolytic *Asticcacaulis* isolate (Kim *et al.,* 2013) and another predicting activity based on genome content (Medie *et al.,* 2012). Notably, the closely related genus, *Phenylobacterium*, which possess large number of laccases and characterized phenol oxidase activity (Muller and Lingens, 1983; Lu *et al.,* 2015), were not enriched on any substrate in this study. Comparative studies of these genera, as well as species within uncultured clades of *Caulobacteraceae*, may yield insights into the evolutionary and natural history of what may become a new model group for the degradation of lignocellulose.

Caulobacteraceae were one of the taxonomic groups whose populations declined in the decades following timber harvesting (Chapter 3) and also in a study of soil warming on lignolytic populations (Pold *et al.,* 2015). Sensitivity to changes in moisture regime may be the most likely cause of population declines given *Caulobacter* are famously cultivable from oligotrophic fresh water habitats that include distilled water (Poindexter, 1964). Information to confirm such a sensitivity is limited, though *Caulobacter* populations were found to be one of the microbial populations to respond most rapidly to re-wetting of dry river sediments (Fazi *et al.,* 2008). Speculatively, moisture may play an important role in their dimorphic lifestyle in which colonization of plant biomass may occur during periods of wetness, followed by a strong

adherence to the substrate during drying. This may ensure proximity of the cell to the resource and confer greater benefit from secreted extracellular catabolic enzymes. Further research is necessary to uncover why *Caulobacteraceae* populations decline in warmer, drier soils and whether the ability to adhere to a substrate may be implicated in their efficacious decomposition of lignocellulosic polymers.

Many of the taxa designated here as lignocellulolytic have been observed in previous SIP studies, in enrichment cultures or have characterized lignocellulolytic isolates and enzymes. The predominance of *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes, Firmicutes* and *Actinobacteria* in decomposition in soil has been widely reported in culture-independent surveys (Lee *et al.,* 2011; Štursová *et al.,* 2012; DeAngelis *et al.,* 2015; Leung *et al.,* 2016). In the forest soils studied here, *Alpha-* and *Betaproteobacteria* exhibited the most prolific differential abundance in ^{13}C-libraries for all three lignocellulose polymers as well as in the recovery of CAZymes. Metatranscriptomic profiling of forest and grassland soil communities indicated that *Burkholderia* (*Betaproteobacteria*) and *Azospirillum* (*Alphaproteobacteria*) were far more abundant in forest soils and were correlated with increased abundances of aromatic and xenobiotic degradation genes (Nacke *et al.,* 2014). Members of both these genera were identified as lignolytic in the present research and have previously characterized lignolytic activity (Faure *et al.,* 1996; Woo *et al.,* 2014). Other prominent lignolytic *Alphaproteobacteria* identified were members of *Sphingomonadaceae*, from which novel lignin degradation genes (the *lig* genes of *S. paucimobilis* SYK-6) have been characterized (Masai *et al.,* 2007). *Sphingomonadaceae* were one of the most common groups from which genes encoding peroxidases and oxidases were recovered. *Sphingomonadaceae* were also one of the few taxonomic groups found to degrade hemicellulose and lignin, but not cellulose, others included *Micrococcaceae* (*Actinobacteria*) and *Sphingobacteriaceae* (*Bacteroidetes*).

Betaproteobacteria have been identified as the major degraders of cellulose (Štursová *et al.,* 2012) and hemicellulose (Leung *et al.,* 2016) in SIP-surveys of forest soils. All lignocellulolytic *Betaproteobacteria* identified in this thesis were members of *Burkholderiales* and represent relatively unknown taxa. Of the two major cellulolytic genera, *Leptothrix* and *Janthinobacterium*, both associated with mineral soils, only *Janthinobacterium* has previously reported evidence for cellulolytic activity (Avellaneda-Torres *et al.,* 2014). *Pelomonas* and *Variovorax*, found here to assimilate carbon from DHP-lignin, have never been implicated in lignin degradation, though *Pelmonas saccharophila* (formerly *Pseudomonas saccharophila*) can degrade polyaromatic hydrocarbons (Chen and Aitken 1999) and a species of *Variovorax* reportedly grows on homovanillate (Allison *et al.,* 1995), and close relatives able to degrade kraft lignin (Chen *et al.,* 2012). *Pelomonas* populations were also enriched in microcosms fed hemicellulose and cellulose, consistent with previous characterizations (Shil *et al.,* 2014). The predominance of *Alpha-* and *Betaproteobacteria* in degrading lignocellulose in forest soils was a major feature of this study and was broadly supported, though incomplete, in the literature.

Members of *Ascomycota* and *Basidiomycota* were the only fungi to exhibit cellulolytic or lignolytic activity. *Ascomycota* were the primary fungal degraders of cellulose, which has been reported in other SIP-cellulose experiments in forest soils (Štursová *et al.,* 2012) and longitudinal studies of decomposition (Voriskova and Baldrian, 2013). The minor proportion of reads classified to fungi in ^{13}C-lignin metagenomes was unexpected and was at odds with the levels of ^{13}C-enrichment observed in PLFA, comparable in some samples to bacterial enrichment. This discrepancy suggests that fungi assimilating ^{13}C into PLFAs were not replicating DNA at sufficient levels to be recovered during density gradient ultracentrifugation, resulting in their underrepresentation in sequencing data. This explanation is indirectly supported by the observation that fast-growing fungi and yeasts, with short doubling times, were the predominant

type of fungal taxa found in SIP-cellulose libraries, such as *Trichoderma*, *Neurospora* and *Chaetomium*. The sole putatively lignolytic fungi was also a yeast (*Saccharomycetaceae spp.*). Slow-growing *Agaricomycetes* would be the most likely fungal taxa to degrade lignin, and while reported to assimilate carbon from DHP-lignin (Haider and Trojanowki, 1975), may do so at a rate unamenable to SIP-DNA experiments.

Gram-negative taxa drove lignin decomposition in soil microcosms and were largely unaffected by fungicide, which dramatically reduced ^{13}C-enrichment of fungal and Gram-positive PLFAs. The relative importance of Gram-negative taxa in lignin decomposition (3-fold higher than fungi) was also reported in decomposers in semi-arid soils (Torres *et al.,* 2014). However, the same study reported 5- to 10-fold higher enrichment from lignin than cellulose, likely the result of using impure commercial ^{13}C-substrates. *Burkholderiaceae* exhibited the greatest growth in ^{13}C-assimilation following fungicide treatment, along with *Sphingobacteriaceae* (*Bacteroidetes*), *Sphingomonadaceae*, and *Xanthomonadaceae*. Lignolytic *Xanthomonas*, a common plant pathogen, have been characterized (Kern and Kirk, 1987). A *Sphingobacterium* isolate from deciduous woodland soil had notable lignolytic activity (Taylor *et al.,* 2012), leading to discovery of two novel superoxide dismutases potentially able to degrade lignin (Rashid *et al.,* 2015). This novel mechanism may help explain the surprising lack of lignin-modifying genes recovered from *Sphingobacteriaceae*, despite their clear enrichment in ^{13}C-lignin pyrotag libraries, most closely related to *Mucilaginibacter*.

The steep decline in ^{13}C-enrichment of Gram-positive PLFAs in fungicide treatments suggests some form of syntrophy between Gram-positive bacteria and fungi. Metagenomic data corroborated PLFA data, but showed a more modest decrease in fungal reads and expansion of bacteria. Overall, reads classified to *Actinobacteria* actually increased in antibiotic treatment, but two major groups of putatively lignolytic *Actinobacteria* decreased, namely *Acidimicrobiales*

and *Conexibacteraceae* (*Solirubrobacterales*). The increase in *Actinobacteria* was driven by populations of *Microbacteriaceae*, *Micrococcaceae* and *Nocardiaceae*, all in the order *Actinomycetales*, which expanded prodigiously in LP_{TX} soil following fungicide treatment. Members of the latter three families possess isolates with lignin-degrading capabilities and include the characterization of *dypB* genes in *Rhodococcus* (Taylor *et al.*, 2012; Ahmad *et al.*, 2011). The identification of *dyp* genes classified to *Nocardiaceae* from fungicide treated LP_{TX} soil underlines the potential relevance of DyP peroxidases in bacterial, soil-based lignin-degradation. The decline in ^{13}C-enrichment of Gram-positive PLFAs may have been driven by the substantial decline in *Firmicutes* population, yet no putatively lignolytic taxa were identified from this phylum. The possibility of non-targeted effects of the fungicides used is unlikely given that both cycloheximide (targets eukaryotal 60S ribosome) and amphotericin B (targets ergesterol) are produced by *Streptomyces*, and are routinely used in culturing *Actinobacteria* (Gontang *et al.*, 2007). There is also the possibility that Gram-positive PLFA markers are not exclusive and may be produced by fungi, though there is no evidence for this in the literature. PLFA enrichment profiles in the antibiotic experiments strongly suggest some degree of cross-feeding, yet the balance of evidence still supports a substantial capacity of bacteria to degrade lignin in forest soils, which was the central purpose for conducting the fungicidal treatments.

A number of lignin-modifying gene families were mainly comprised of bacterial reads, such as laccases, alcohol oxidases, benzoquinone reductases, quinone-dependent oxidoreductases and Dyp-type peroxidases, keeping with the near exclusivity of Dyp-type peroxidases in the bacterial domain (Colpa *et al.*, 2014). The sizeable increase of vanillyl alcohol oxidase genes in ^{13}C-lignin metagenomes was consistent with the chemical composition of DHP-lignin, comprised of oligomers of ring-labeled vanillyl alcohol. Laccases and benzoquinone reductases were also enriched in ^{13}C-libraries, but only after subsetting to taxa designated putatively

lignolytic from pyrotag analysis. Without subsetting, the abundance of AA genes in ^{13}C-lignin libraries was at parity or depleted relative to ^{12}C-libraries, revealing the ubiquity of oxido-reductase enzymes with homology to AA families and the challenge it presents to targeting specific lignolytic homologs.

The sole hemicellulolytic activity of *Streptomyces* was unexpected given their reputation as both cellulose and lignin-degraders. *Streptomyces* were one of the first characterized bacterial degraders of lignin (Crawford *et al.,* 1978), leading to the characterization of *Streptomyces viridosporus* T7A (Pasti *et al.,* 1990) and eventual discovery of small laccases (Majumdar *et al.,* 2014) and a Dyp-type peroxidase encoded in its genome (Davis *et al.,* 2013). This ground breaking work, partly inspired by the similarity in hyphal morphology to fungi, garnered *Streptomyces* attention. However, the lack of lignolytic *Streptomyces* in this study corroborates other reports of modest lignolytic activity. In one study, only a small subset of *Streptomyces* isolates exhibited relatively weak lignolytic activity on DHP lignin (Vetrovsky *et al.,* 2014). In another case, forest soil enrichments for lignolytic taxa were actually depleted in *Streptomyces* (Pold *et al.,* 2015). In this thesis, *Streptomyces* were only identified as hemicellulolytic, while *Kitasatospora* and other unclassified *Streptomycetaceae* were significantly active on both hemicellulose and cellulose. Yet, *Streptomycetaceae* had the highest proportion of CAZy clusters encoding putative Dyp genes and also the sole potential bacterial Class II peroxidase. This discrepancy may indicate an overrepresentation of *Streptomycetaceae* in databases used for annotating peroxidase genes, but also the possibility of more complex trophic requirements in *Streptomycetaceae*. *Streptomycetaceae* exhibited substantial activity in SIP experiments using ^{13}C-labeled whole plant tissue (Lee *et al.,* 2011), suggesting that lignin-degrading gene expression may depend on the presence of plant carbohydrates. Similarly, *Bacillus* possess characterized cellulolytic and lignolytic taxa (Tian *et al.,* 2014; Menendez *et al.,* 2015), yet were

only identified here as hemicellulolytic. It could be that *Bacillus* were identified as the earliest organisms to respond to xylose amendments in a similar SIP experiment, but were eventually overtaken by the slower-growing but more active *Bacteroidetes,* then *Micrococcales* (Pepe-Ranney *et al.,* 2016). Discrepancies between SIP studies and *in vitro* characterization are likely the result of both technical and ecological factors, stressing the necessity of complementary approaches.

The characterization of mineral soil provided evidence for distinct and novel lignocellulolytic populations in a largely overlooked niche for microbial decomposition. Cellulolytic populations were highly active in mineral soils, incorporating far more carbon relative to pre-existing biomass than populations in organic soils. Strong enrichment for long-established cellulolytic taxa, such as *Cellvibrio* (Berg *et al.,* 1972), *Cytophaga* (Stanier, 1942), *Sorangium* (Imsenecki and Solntzeva, 1936) and a long list of fungi, was evident in organic soils, while most mineral-associated cellulolytic taxa were implicated in cellulose-degradation for the first time, including the following bacterial groups: candidate division *FBP* (*Armatimonadetes*), *FW68* (*Armatimonadetes*), *Leptothrix* (*Betaproteobacteria*), *Janthinobacterium* (*Betaproteobacteria*) and *DH61* (*Planctomycetes*), and *MIZ46* (*Myxococcales*). The pronounced cellulolytic activity from members of *Armatimonadetes* was corroborated by a recent SIP-cellulose experiment (Wang *et al.,* 2015) and by the cellulolytic activity of one of the three cultured representatives of *Armatimonadetes* (Lee *et al.,* 2014). Lignolytic *Acidobacteria* were also more abundant in mineral soils, including members of poorly characterized subgroup 2 (*Ellin6531*) and 6 (*iii1-15*). All three Dyp2-type genes classified as acidobacterial were recovered from mineral soil metagenomes. This is the first assignment of lignolytic activity to members of these groups, though microarray profiling of lignin-bead amended tropical forest soil enrichments had suggested the involvement of *Acidobacteria* (DeAngelis *et al.,* 2011). SIP-

cellulose-based discovery of cellulolytic *Acidobacteria* (subgroup 1) by Eichorst and Kuske (2012) and Štursová *et al.,* (2012) was not observed in this study, resulting either from biogeographical differences or their use of impure ^{13}C-maize cellulose.

SIP shines light on the multitude of uncultured taxa, commonly described as 'microbial dark matter,' which proved effective in identifying a number of uncultured, putatively lignolytic bacteria. OTUs belonging to the myxobacterial family *Cystobacteraceae* showed a twenty-fold enrichment in ^{13}C-lignin libraries. Two CAZy clusters were recovered from *Cystobacteraceae* encoding a putative Dyp and laccase, respectively. OTUs clustered with *Cystobacter gracilis*, a species commonly isolated from decaying plant material that lacks the capacity to degrade cellulose (Reichenback, 2005), a phenotype consistent with our findings. A close relative of *Cystobacter*, *Stigmatella aurantiaca*, reportedly degrades phenolic compounds (dos Santos *et al.*, 2014). This suggests a putative role for *C. gracilis* in lignin degradation, recently proposed to represent its own genus within *Cystobacteraceae* (Garcia *et al.,* 2010).

Another newly designated phylum, *Elusimicrobia*, originally named 'Termite Group 1,' was roughly eight-fold enriched in ^{13}C-lignin pyrotag libraries. Sequences classified to *Elusimicrobia* occupied as much as 20% of bacterial 16S rRNA libraries in a study of lower termite guts (Boucias *et al.,* 2013) and have been found in a number of lignin-rich environments, such as leaf-cutter ant fungus gardens (Suen *et al.,* 2011) and peatlands (Urbanova and Barta 2014), as well other environments like ocean sediments (Oni *et al.*, 2015). Huang *et al.,* (2013) reported that termites fed woody diets were particularly populated by *Elusimicrobia*, with the highest populations occurring in termites fed pine, as opposed to poplar, maize or sorghum. Despite extensive circumstantial evidence for their role in lignin-degradation, there is no direct evidence of lignolytic activity. The sole cultured representative from the phylum, isolated from

the gut of a beetle larva, is strictly anaerobic and ferments sugars using typical anaerobic pathways and possessed no noteworthy AA or CAZyme gene content (Herlemann *et al.*, 2009).

Two novel groups of *Gammaproteobacteria* were designated as putatively lignolytic in addition to *Enterobacteriaceae,* which has two isolates with characterized lignin-degrading activity: *E. lignolyticus* (DeAngelis *et al.*, 2011) and *E. soli* (Manter *et al.*, 2011). The recovery of a CAZy cluster containing multiple laccase genes classified to *E. lignolyticus* is noteworthy. The putative lignolytic capability of members of purple sulfur bacteria (PSB), *Ecothiorhodospiraceae*, was evidenced in the differential abundance in ^{13}C-lignin metagenomes in all three ecozones assayed. While PSB are unlikely candidates as lignin degraders, the designation was weakly supported by the recovery of a CAZy cluster encoding an *Ecothiorhodospiraceae* CBM and laccase. The other novel group within *Gammaproteobacteria* was represented by a single OTU that clustered with uncultured '*Xanthomonadales incertae sedis Acidibacter*' in SILVA and was classified as *Piscirickettsiaceae*, a fish pathogen, with GreenGenes. *Piscirickettsiaceae* was among the top most differentially abundant families for AA4 (4.8-fold) and AA6 (2.9-fold) in unassembled ^{13}C-lignin metagenomes.

The capacity to degrade multiple lignocellulosic substrates strongly suggests an organism's involvement in the decomposition of plant matter. In this study, approximately 35% of putatively lignocellulolytic taxonomic groups were capable of degrading more than one substrate. This may be an underestimate given the incomplete coverage of ecozones for SIP-hemicellulose and lignin. However, few individual species exhibited multi-substrate use, with only five examples of OTUs degrading cellulose and lignin and also the minimal overlap between hemicellulose pyrotags and the other two SIP libraries. These findings suggest that few bacterial species have co-evolved metabolism of lignocellulosic polymers, implying that decomposition may be structured by a division of labour. This hypothesis was put forward by

Berlemont and Martiny (2013), whose comparative genomic study demonstrated that the genetic capacity to degrade crystalline cellulose was occurred in clusters of closely related species (~0.013% dissimilarity), while the capacity to degrade oligomeric forms of cellulose was more widely distributed within and among lineages. The findings reported here, that few OTUs based on a cutoff of 0.01% utilized more than one substrate, is in agreement with the degree of functional specialization suggested by Berlemont and Martiny. Similarly, Vetrovsky *et al.,* (2014), concluded that the co-metabolism of polysaccharides and DHP-lignin by *Streptomycetes* was negligible. On the other hand, the recovery of fungal draft genomes containing endoglucanases, lytic polysaccharide monooxygenases, oxidases and peroxidases was evidence for the multi-substrate degradative capacity of fungi, consistent with the long-established understanding of their life histories.

Similar to observations reported in Chapter 3, biogeographical differences in the composition of lignocellulolytic populations were evident among ecozones. These differences were related to differences in *in situ* abundances, but also commonly the presence or absence of a taxa. Lignocellulolytic populations from proximal ecozones, such as in Ontario, shared the greatest similarity, while LP_{TX} communities were most distinct. Cellulolytic populations exhibited the greatest differences according to the East-West divide, exemplified by the relative involvement of *Basidiomycota*, and also a North-South divide, based on increasing participation of *Chaetomiaceae* in southern sites. The western distribution of *Sebacinaceae* was a notable biogeographical feature as well as novel feature of the SIP-metagenomic data. *Sebacinaceae* are known for ectomycorrhizal associations and, while a few saprobic species have been described, cellulolytic activity has not previously been shown (Oberwinkler *et al.,* 2013; Weiß *et al.,* 2016). Differences between North American and European cellulose-degrading populations was apparent in the broad differences in cellulolytic taxa reported in Czech forest soils, namely

Collimonas, *Alkanindiges*, *Streptacidiphilus* and *Herminiimonas* (Štursová *et al.*, 2012). These genera were present in low abundances *in situ* in all ecozones studied here, but were not enriched in any ^{13}C-libraries. Similarly, *Myxococcales* were reportedly unique to forest soils in a SIP-cellulose survey of soils performed by Verastigui *et al.*, (2014) and, while *Myxococcales* were prominent cellulolytic taxa in some ecozones, they were not equally prominent in soil incubations among all ecozones. Biogeographical differences can be useful for the study of the physiology and ecology of the decomposer community. Such differences are likely to account for meaningful variation in the rate of decomposition among forests, demonstrated here by the correlation of the rate of enrichment with the abundances of a number of taxa.

Despite the overall success of SIP in addressing the goals of the research, there are notable limitations and caveats to the method. SIP microcosm experiments require conditions that encourage detectable levels of enrichment and favour certain taxa over others, such as faster growing *Ascomycota* over *Basidiomycota*. This might help explain why *Paenibacillus sp.* were not identified as putatively cellulolytic in SIP experiments despite being highly abundant in culture collections of cellulolytic taxa from the same soils (unpublished) and being detected *in situ* (~0.03% of total library). Yet, SIP may be the best approach to discriminate functional and non-functional populations relative to other environmental context-focused characterizations, as evidenced by the detailed phylogenetic analysis of *Caulobacteraceae,* and SIP offers a far less biased approximation to *in situ* conditions than conventional culturing-based surveys.

The most common criticism of SIP is the possibility of misattributing function due to cross-feeding that results from the assimilation of carbon by predators, parasites or opportunists. This is a particularly relevant concern given the extracellular nature of lignocellulose depolymerization. The clearest suggestion that cross-feeding had occurred in this study was the steep decrease in enrichment of Gram-positive PLFAs in soils incubated with fungicide. While

this is a relatively unambiguous indication that cross-feeding was occurring, the observation that the abundances of many Gram-positive taxa deemed putatively lignolytic actually increased in antibiotic treatments suggests the possibility of other, as of now, unknown explanations for this phenomenon. Cross-feeding may also have led to the putative designation of members of *Candidatus Saccharibacter* (formerly TM7) as cellulolytic in Chapter 3. This group contains obligate parasites, including the recent cultivation of a *TM7* parasite of *Actinobacteria* (He *et al.*, 2015). While this specific *TM7* parasite had only 85% 16S rRNA similarity to putatively cellulolytic OTUs, misattribution through tightly interdependent parasitism of *Actinobacteria* is a distinct possibility given the substantial cellulolytic activity of *Actinobacteria* observed. Similarly, in Chapter 4, a draft genome classified as *Parcubacterium*, suspected to be obligate symbionts (Nelson and Stegen, 2015), was recovered. In a previously published SIP-cellulose experiment, OTUs classified as *Xiphinematobacter* met the criteria for cellulolytic designation, yet are also obligate cytoplasmic symbionts of nematodes (El Zahar Haichar *et al.*, 2007). While the evidence for cross-feeding is noteworthy, time-course experiments showed that the same subset of PLFAs enriched on Day 4 remained the predominant enriched PLFAs and that the enrichment of other PLFAs was minimal. Štursová *et al.,* (2012) reported a similar lack of evidence for widespread cross-feeding, comparing incubations with cellulose at eight and fifteen days. Most importantly, confidence in SIP data should stem from the accuracy in which previously characterized lignocellulolytic taxa are found to occur.

Chapter 5: Conclusions

The research described in this thesis contributes new knowledge and perspective along with valuable data and methodology for understanding forest soil decomposers and post-harvesting selection pressures on soil microbial communities. This work is one of few studies to employ SIP to study broader ecological phenomena, rather than solely as a tool to target and identify novel functional taxa. SIP linked, for the first time, changes in cellulolytic populations with changes in overall activity. In that experiment, the increased relative abundance of *Chaetomiaceae*, and other bacteria associated with desert-environments, led to the discovery that desiccation, radiation and heat-tolerant taxa were thriving in soils. Not completely surprising, given the exposed nature of clear-cut forest soils, this phenomenon was documented in detail in this thesis, highlighting specific taxa relevant for monitoring and posing questions about the long-term implications of such a change. What might be the persistence and legacy of these stress-tolerant taxa across multiple harvesting cycles? Will their influence compound or dissipate over time? The commonalities identified between taxa thriving post-harvesting and following wildfire suggest that chronosequences from fire-affected forests may be reasonable analog systems for studying microbial succession in the decades following timber harvesting.

The potential long-term functional consequences of timber harvesting were apparent in a number of experimental results. The previously mentioned reductions in cellulolytic activity and net respiration in microcosms according to treatment (section 3.3) or, the decline of populations *Caulobacteraceae* and *Opitutaceae*, each correlated with total levels of enrichment from lignin and cellulose, respectively (section 4.2.7). The overall decline of methanotrophic populations was consistent with a growing body of research showing significant impacts on methane-uptake following harvest. The functional impact of the shifts in ectomycorrhizal fungi and decline of

rhizospheric bacteria are less clear, and these organisms represent populations of key interest for future research.

Varying the degree of organic matter removal (or retention) influenced the relative abundance of a number of populations in the decades following harvesting. OM retention had two major observable effects i) mitigating abiotic factors and the corresponding degree of population expansion or decline; and ii) fostering saprotrophic populations, including those specialized in wood decay. The potential of OM retention to mitigate abiotic changes and population shifts appeared to be the stronger of the two effects, given the modeling of abundance patterns. As such, this research found that OM retention may be a pragmatic tool in broadly managing the abiotic effects of disturbance on soil communities, of particular interest on methanotrophic populations. Yet, assessing the impact of intermediate levels of OM retention was more challenging given the lack of environmental data and the generalizability of patterns across ecozones. Therefore, the extent of differences between OM1 and OM2 treatments, most relevant to modern forestry practices, may not be fully clear and closer attention to differentiate the two should be made in future LTSP studies.

Comparisons of forest soils communities from across North America revealed the scope of variation in community structure. The degree of variation observed raises the possibility that soil management may require monitoring and assessment of soil ecology at the local level. Bacterial species were far more cosmopolitan than fungi and, thus, had a greater likelihood of demonstrating generalized responses to harvesting. The more discrete, localized distribution of fungi strongly supports the need to perform regional or local assessments, given their major role in forest soil ecology. Studying the sources of biogeographical variation, whether due to environmental conditions or ecotypic variation within closely related populations, can reveal aspects of the physiology, evolution and dispersal of forest soil microorganisms. Similarly, the

abundance patterns of species (OTUs) may inform understanding of physiology. For example, evidence for a saprobic, wood-degrading lifestyle of members of *Thelephora* may be inferred by their sizably expanded populations in OM1 and OM2. Despite broad biogeographical variation in community structure, a number of phyla were consistently associated with organic (*Alphaproteobacteria* and *Bacteroidetes*) and mineral layer soils (*Delta-* and *Gammaproteobacteria*, *TM7*, *Firmicutes*, *Armatimonadetes* and *Chloroflexi*). The lack of knowledge of taxa endemic to mineral soils should be a major focus in future studies of both harvesting impacts, given substantive expansion of *Chloroflexi* and *AD3,* as well as the unique cellulolytic (*Armatimonadetes, Planctomycetes* and *Verrucomicrobia*) and lignolytic taxa therein (*Acidobacteria* and *Sinobacteraceae*).

The potential of bacteria to degrade lignin has become more established in recent years through culture-based screening and enzyme characterization, but only a handful of studies have examined their activity from an environmental perspective. As such, this research offers the most extensive characterization of bacterial lignin-degraders from a wide range of forest soils to date. The work confirmed that a number of previously characterized genera were active in soil and implicated a number of novel taxa in an attempt to direct the search for novel lignin-modifying enzymes. This research marks an end to speculation on the involvement of *Caulobacter* and *Caulobacteraceae spp.* in the decomposition of all three major lignocellulose polymers. In doing so, it may open an interesting case-study in the evolutionary history and lignocellulolytic adaptation of a bacterial lineage with notably unique methods of dispersal and adhesion. The relatively diminutive role of fungi in lignin degradation in SIP microcosm experiments challenges the conventional perspective on the role of fungi as the most prominent members of the soil decomposer community. A similar break from convention was the finding that cellulolytic activity decreased when populations of fungi (*Chaetomiaceae*) expanded. These

results draw attention to the involvement of bacteria in degrading recalcitrant forms of carbon below the surface.

Given the aim of the LTSP network to study forest regeneration in the decades to come, this research provides comprehensive data on the state of microbial community structure prior to canopy closure. The data has been carefully curated, and made publicly available to facilitate the longitudinal study. The scope and scale of SIP work is unprecedented with replicated PLFA, pyrotag and whole shotgun metagenomic data from over one hundred soil samples. The recovery of ^{13}C-labeled DNA alone required over 70 ultracentrifugation runs, amounting 140 days of ultracentrifugation. To ensure the quality of research, a highly sensitive and accurate method for quantifying ^{13}C-content in DNA was developed along with a high-throughput method for recovering DNA from the CsCl gradient. Both were critical to optimize experiments and process the high volume of fractions recovered in processing so many samples. The improved assembly of metagenomic data illustrates the utility of SIP methods for culture-independent studies of highly diverse soil communities. Without the 3 to 20-fold increase in percentage of read assembled, considerable information about the CAZy content of lignocellulolytic taxa could not have been obtainable. And, given the quality and abundance of assembled data, this thesis may be just the beginning of a wealth of discovery by those prospecting for novel CAZymes.

Even with the efforts documented in this thesis to better understand the impacts of timber harvesting on soil communities, forest researchers are a far from a conceptual understanding of what microbial processes are occurring and what effects shifts in microbial communities might have. In this study, 30% of taxa impacted by harvesting were unclassifiable beyond the family level, and the total number of unclassified reads increased with increasing harvesting intensity. These stats reveal how far we are from a clear assessment of the ecology of forest soil communities and timber harvesting disturbance. For plant communities, timber harvesting

increased the richness of ground cover species, driven by the colonization of r-strategists even over the long-term (Roberts *et al.,* 2016). Microbial studies may borrow such broad ecological categorizations to begin inferring consequences of harvesting without knowledge of the ecology of all taxa. For example, metagenomics makes it possible to infer the relative abundance of microbial r-strategist versus K-strategists based on estimations of average genome size (Nayfach and Pollard, 2015). The concept of function-oriented phylogenetic databases, such as FUNGuild, may also prove useful in building towards a relevant functional characterization of microbial communities. Amidst the vast complexity and considerable unknowns of soil microbial ecology, long-term studies like the LTSP provide stable, comprehensive data collection, which leads to the discovery and formulation of valuable new perspectives.

Combining the study of forest soil microbial ecology with bioprospecting for commercially relevant biocatalysts is a shrewd strategy for advancing interests in the forest industry. Properly paired, they can improve forest management practices and contribute to basic research that can create new revenue streams for forest products. This thesis demonstrates that SIP research, too, can be used in combination to assess both ecological effects and, at the same time, recover sequence data of potential value for novel biocatalyst discovery. Given the importance of forestry to the Canadian economy, Canada is uniquely positioned to be a world leader in a genomics-based understanding of forest ecosystems, with the potential to develop substantive research expertise in forestry, microbial ecology and genomics.

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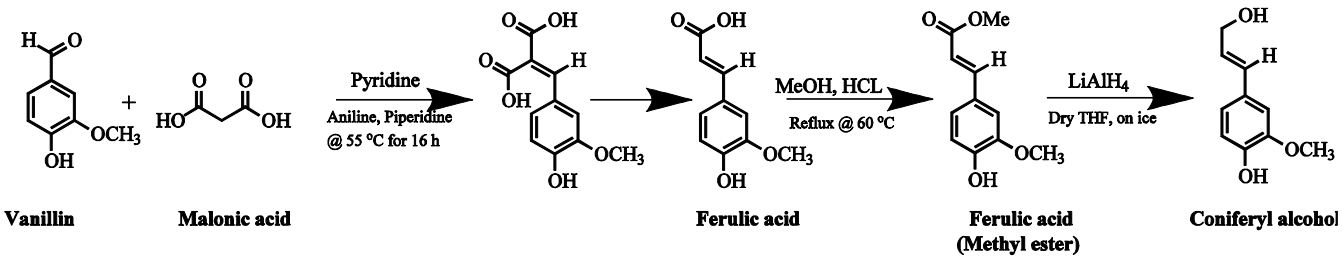
Appendix A

Synthesis of Coniferyl Alcohol from ^{13}C-ring labelled Vanillin

Written and performed by Dr. Rahul Singh



Aldol condensation of vanillin in the presence of malonic acid

Aldol condensation of 1:1 molar ratio of malonic acid and vanillin in the presence of catalytic amounts of piperidine and aniline (~6 drops) in pyridine. Thus, in a 3-neck flask (100 ml), 10 ml pyridine, 2.5 g vanillin and 2 g malonic acid were added and the mix was reflux at 55 °C for ~16 h.

Working up the reaction

Post reflux, the mix was removed from the flask and 60 ml chloroform was added (rinsed the flask with that as well). This mixture was extracted 6 times with acidic water (pH ~2). Essentially, ferulic acid precipitates in acidic water. Ferulic acid can be recovered as precipitate as well as extraction by ethyl acetate. After recovery, the yield was ~ 87%. Check on TLC (1:1 ethyl acetate/hexane) for the leftover vanillin (salvage in the case of labeled material).

Esterification of ferulic acid

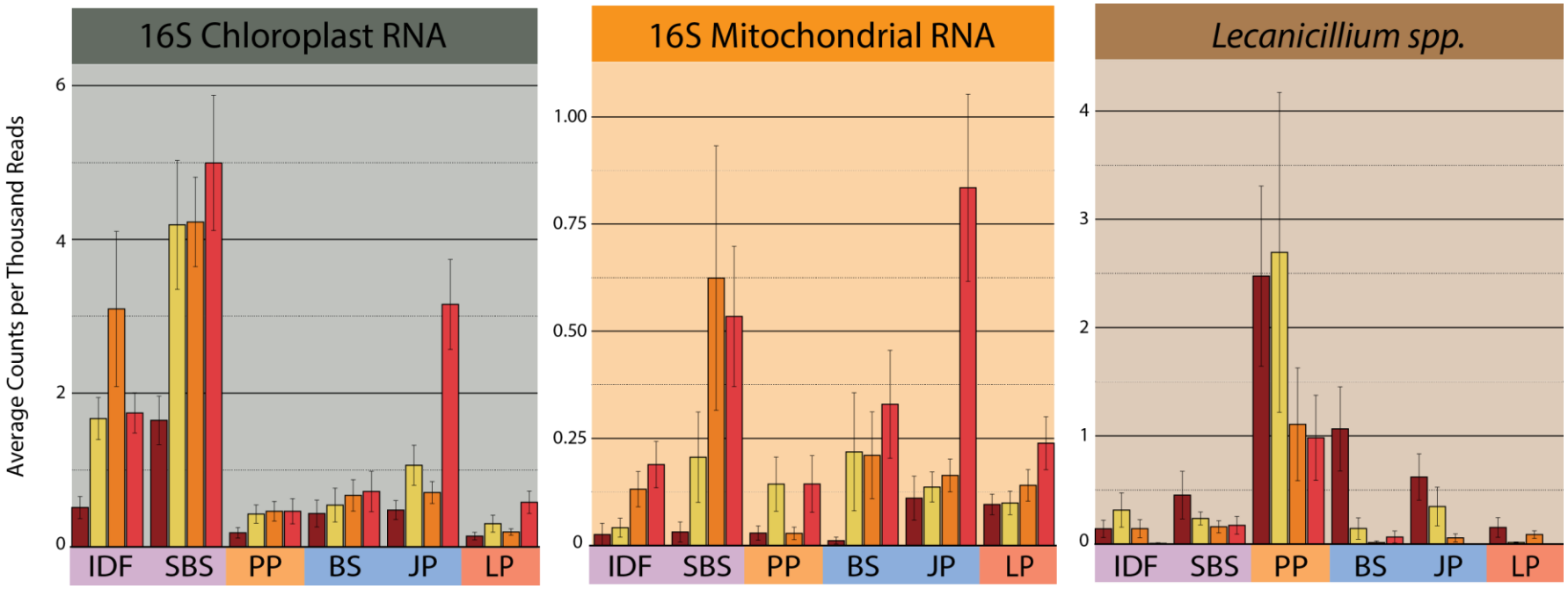
Esterification eases the reduction of ferulic acid to coniferyl alcohol. In a 3 neck flask, 2.5 g ferulic acid was mixed with 10 ml methanol and ~ 50 µl conc. HCL. This mixture was refluxed at 65-70 °C for 5-6 h. Test the progress of the reaction on TLC, if required add more HCL. Once finished, recover the sample using ethyl acetate and dry under vacuum. Expected yield is ~ 95%

Reduction with Lithium Aluminium Hydride (LiAlH)

A 2 neck flask was set up with an add funnel under positive nitrogen pressure. The flask was pre-weighed with 0.732 g of LiAlH, to which 60 ml of freshly distill THF was added slowly, while stirring. Methyl ester of ferulic acid (~ 2.5 g) was mixed with 14 ml of freshly distill dry THF and placed in the add funnel. The flask was kept on ice and ester was added drop wise really slowly. Towards the end, some additional THF was used to rinse the add funnel. The solution was stirred at room temperature for additional 2 h and then bought back at 0 °C. At this point, 12 ml ethyl acetate was added drop wise, followed by 70 ml of 2 N HCl. These were added slowly as the residual LiAlH was reactive initially. Also, note that stirring also gets difficult towards the end due to accumulation of salts. Finally, the product was extracted in ethyl acetate and dried under vacuum. Following drying, the product can be purified using silica chromatography.

Appendix B – Broad Ecological Impacts of Timber Harvesting

Figure B.1. Abundance pattern of taxa that illustrate the broad character of environmental change detectable in soil pyrotag libraries. Taxa from 16S rRNA libraries reveal the expansion of plant ground cover (Chloroplast and Mitochondrial rRNA genes). The decline of entomopathic fungi (*Lecanicillium spp.*) reveal potential changes in insect host populations. Error bars correspond to one standard error of the mean.



Appendix C – Detailed Descriptions of Lignocellulolytic Bacterial Populations

The most prominent bacterial hemicellulolytic genera were *Agrobacterium*, *Bacillus*, *Paenibacillus*, and *Streptomyces* (Figure C.1) and included taxa also active on cellulose, such as *Cellvibrio*, *Janthinobacterium*, *Cytophagaceae* and *Salinibacterium* (Figure C.2). The most prominent cellulose-degraders were members of the candidate division 'FBP' within *Armatimonadetes* (Figure C.2). Sequences annotated as FBP were, at best, 80% similar to any sequence in NCBI's curated rRNA database and clustered with sequences from uncultured organisms, distantly related to *Fimbriimonas ginsengisoli* in the SILVA tree. Other groups of the *Armatimonadetes* (*FW68*) were also designated cellulolytic (Figure C.2). Exclusively cellulolytic bacterial taxa included *Kitasatospora* and other unclassified *Streptomycetaceae; DH61* (*Planctomycetes*); *MIZ46* and *Polyangiaceae* (*Deltaproteobacteria*), and *Leptothrix* (*Burkholderiales*) (Figure C.2). Binning assembled metagenomic data from ^{13}C-cellulose libraries yielded one high-quality, and numerous lower quality, draft genomes classified as *Sorangium* (*Polyangiaceae*), as well as lower quality drafts classified by LCA as *Myxococcus, Cytophaga*, *Chthonomonas* (*Armatimonadetes*), and *Chthoniobacter* and *Pedosphaera* (*Verrucomicrobia*) (Figure 4.14). A number of high-quality draft genomes were recovered for cellulolytic fungi, including *Chaetomium*, *Neurospora* and *Pseudogymnoascus*.

Lignolytic bacterial taxa were typically from novel and uncultured clades or uncultured clades within better known families. Taxa originating from poorly characterized clades included *Acidobacteria* from Grp-2 (*Ellin6531*), Grp-3 (*Solibacterales*; SILVA clade 'Unknown Family_Candidatus Solibacter') and Grp-6 (*iii1-15*) (Figure C.3). These clades

Figure C.1. Abundance barplots of prominent hemicellulolytic taxa depicting differential abundance between ^{12}C- and ^{13}C-pyrotag libraries including *in situ* abundances across ecozones.

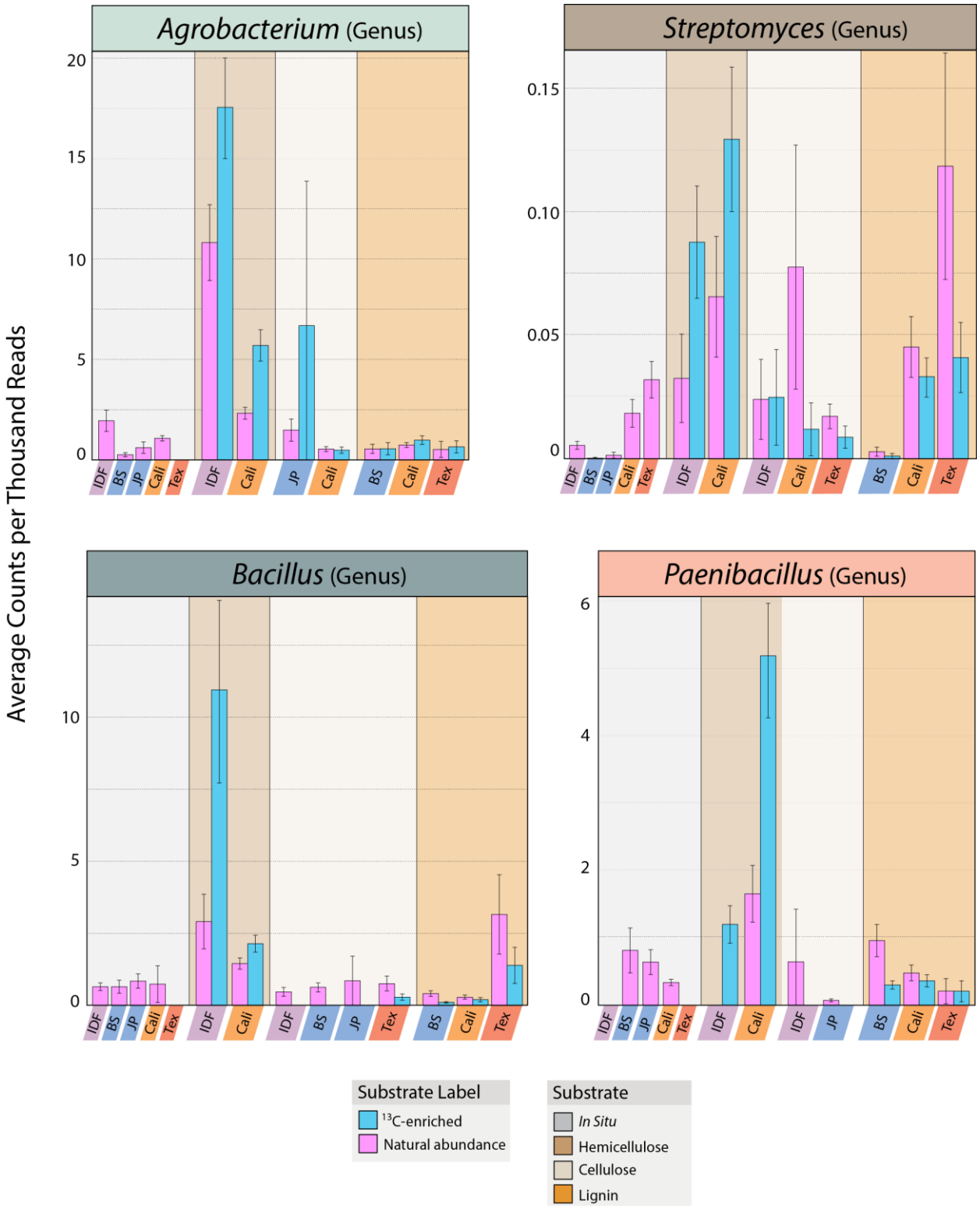


Figure C.2. Abundance barplots of prominent cellulolytic taxa depicting differential abundance between ^{12}C- and ^{13}C-pyrotag libraries including *in situ* abundances across ecozones.

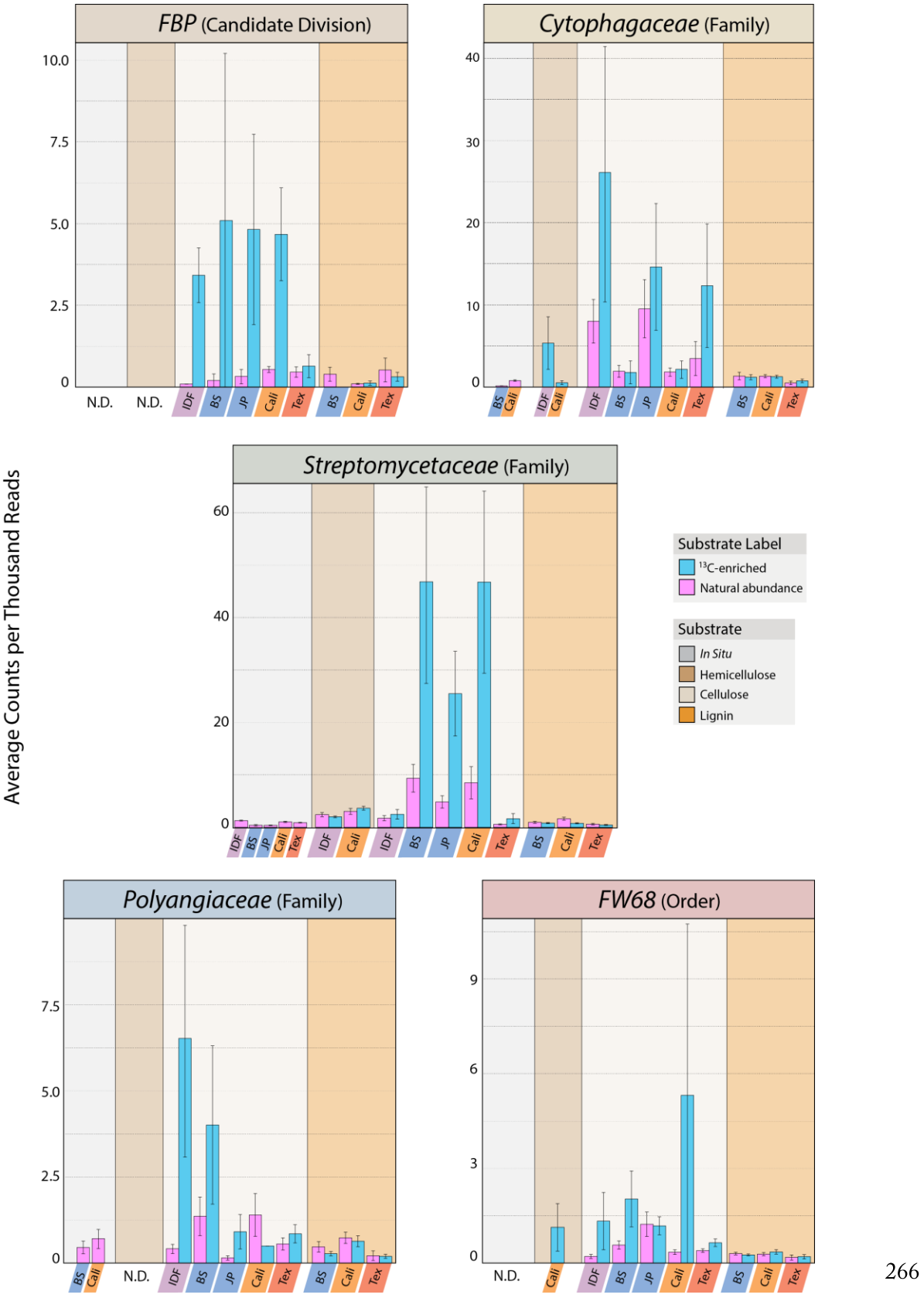
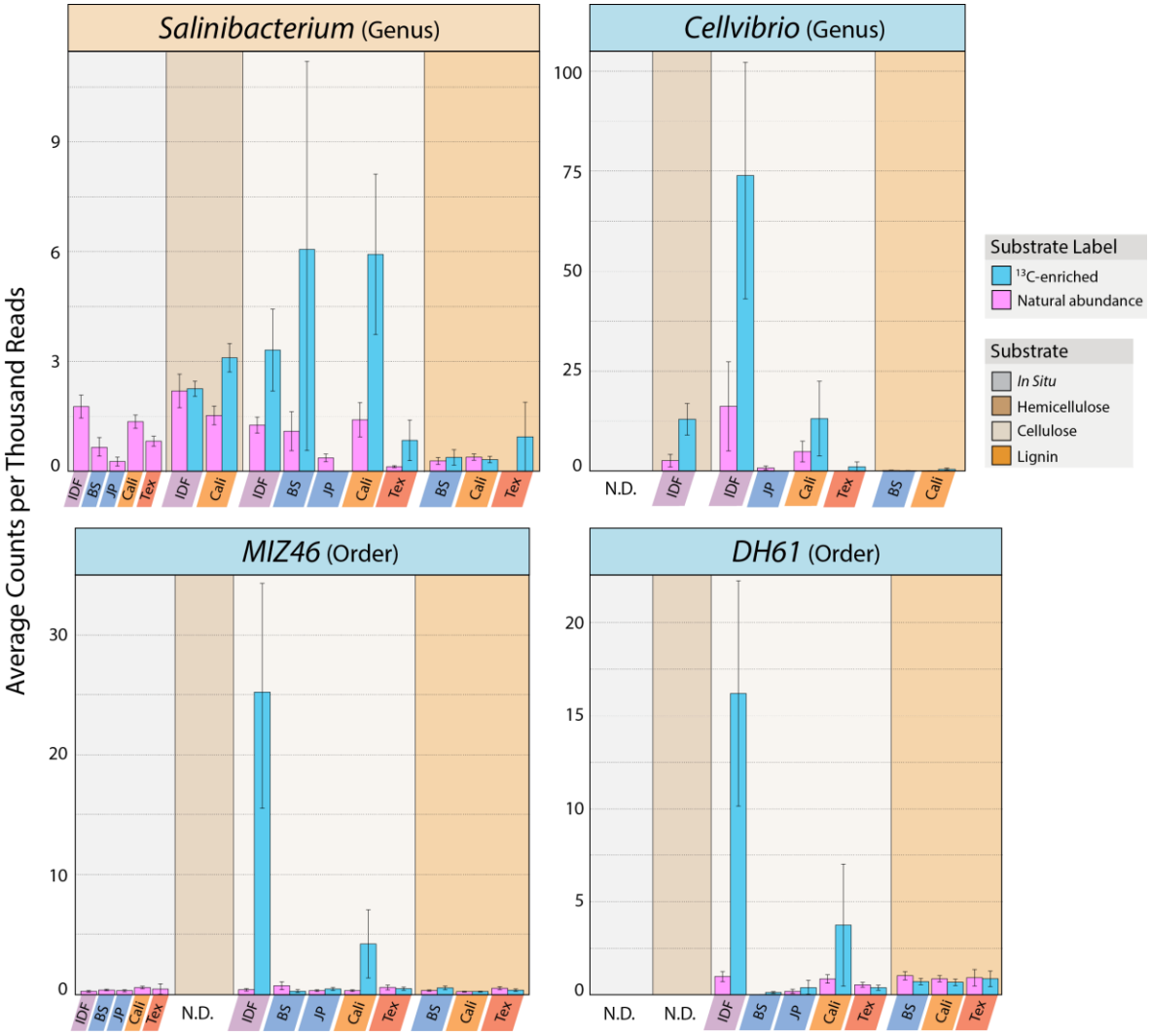
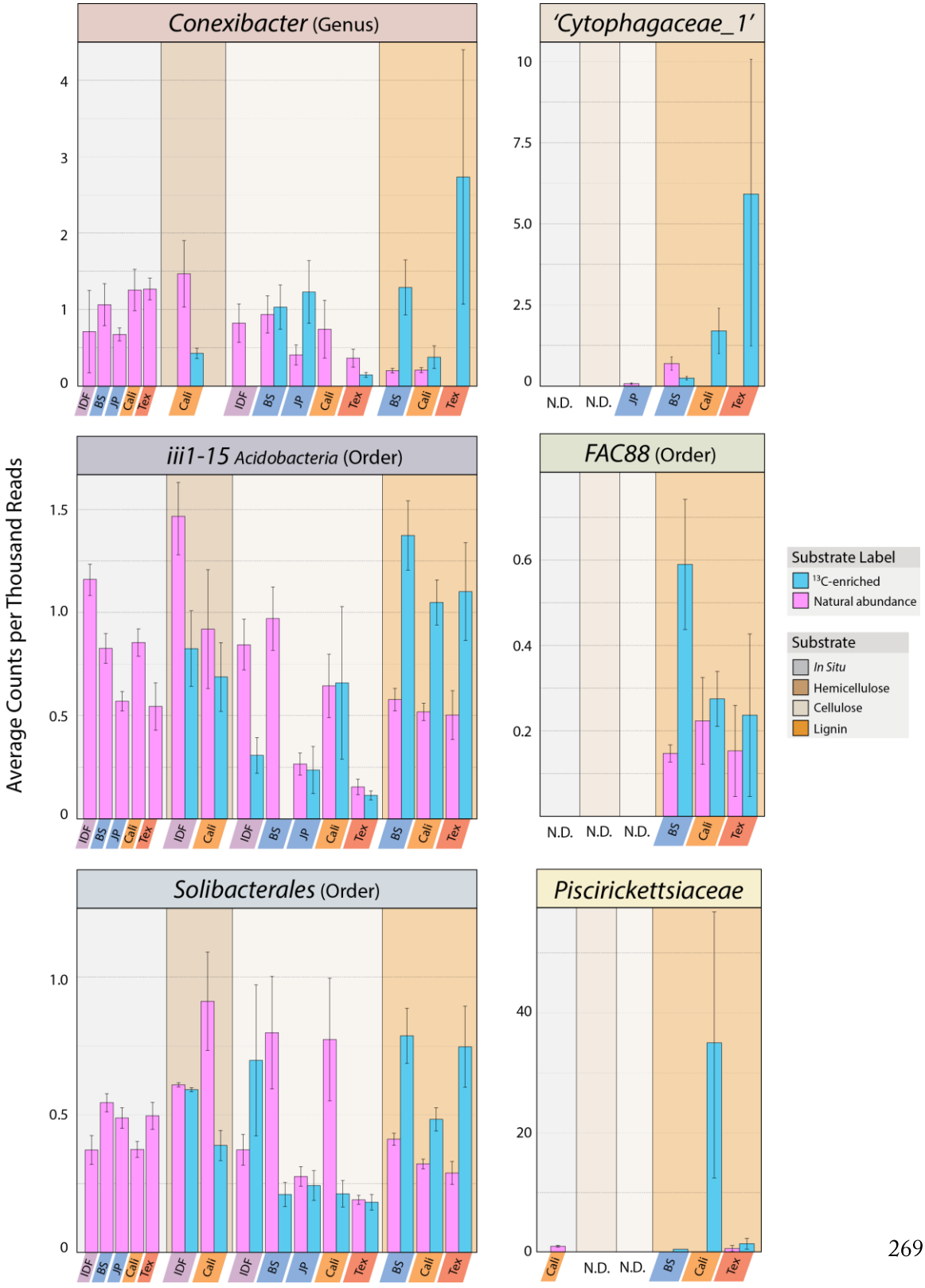


Figure C.2. *continued…*



possess a prodigious number of sequences in the SILVA repository (493, 1,466 and 3,817, respectively), but possess no cultured representatives. These groups were not related to the predominant groups of *Acidobacteria* common among in ^{12}C-libraries. Three low-quality draft genomes of *Acidobacteria* were recovered from ^{13}C-lignin libraries (Table E.13). Lignolytic *Actinobacteria* were identified from novel clades within *Conexibacteraceae* (SILVA clade 'YNPFFP1') and *Gaiellaceae*, as well as clades with cultured representatives such as *Conexibacter* (closest representative: *C. woesi*), *Lysinimonas* (*Microbacteriaceae*: *L. soli*), *Arthrobacter* (*Micrococcaceae*; *A. soli*), and *Jatrophihabitans* (*Frankinaceae*; *J. endophyticus*). Two low quality draft genomes classified to *Conexibacter* were recovered from ^{13}C-lignin libraries. Putatively lignolytic members of *Bacteroidetes* grouped with a clade of environmental sequences in the family *Cytophagaceae* (SILVA clade 'Cyotphagaceae_1) and with *Mucilaginibacter* (SILVA clade 'Mucilaginibacter_1'), which were closely related to cultured taxa *M. frigoritolerans* and *M. herbaticus*. Low-quality draft genomes classified as *Mucilaginibacter* and *Chitinophagaceae* were recovered from ^{13}C-lignin libraries. Other lignolytic OTUs from poorly characterized clades included *Elusimicrobia*, formerly Termite Group1, (*FAC88*; SILVA clade 'Lineage IIa'); *Sinobacteraceae* (SILVA clade 'Xanthomonadales Intercetae Sedis_Acidibacter'); *Cystobacteraceae* (SILVA clade 'uncultured' within *Myxococcales*) and *Piscirickettsiaceae* (SILVA clade 'uncultured' within *Xanthomonadales*). Lignolytic OTUs also clustered with a number of taxa available in culture collections, such *Bradyrhizobium canariense* ('*Bosea*' in Table 4.1), *Altererythrobacter dongtanensis* (*Erythrobacteraceae*), *Novosphingobium lentum* and *Sphingomonas echinoides* (*Sphingomonadaceae*), *Aquaspirillum polymorphum* (*Telmatospirillum*), *Azospirillum doebereinerae* (*Alphaproteobacteria*) and *Cystobacter gracilis* (*Cystobacteraceae*).

Figure C.3. Abundance barplots of prominent lignolytic taxa depicting differential abundance between ^{12}C- and ^{13}C-pyrotag libraries including *in situ* abundances across ecozones. 'Cytophagaceae_1' was classified as *Clostridium* (*Firmicutes*) by GreenGenes, but placed in the 'Cytophagaceae_1' clade in SILVA (*Bacteroidetes*).



Additional lignolytic taxa were identified from metagenomic libraries corresponding to *Cardiobacteriaceae*, *Enterobacteriaceae*, *Ectothiorhodospiraceae*, *Hyphomonadaceae* and *Pseudomonadaceae* (Figure C.4). Attempts to identify which genera within these families were differentially abundant were unsuccessful due to the lack of specificity of LCA classification, an example of which is provided for family *Ectothiorhodospiraceae* (Figure C.4). Another notable and problematic result of using LCA classification was illustrated by the classification of *Pantholops*, a Tibetan antelope from the ruminant family *Bovidae*, as differentially abundant in ^{13}C-lignin libraries (12-fold) among all ecozones. Given the nature of samples used, it is unlikely that the genome assembly frequently the top blast hit used in LCA (Accession: GCA_000400835) was the real source of these sequences. Instead, the Pantholops genome may contain contamination from lignolytic microorganisms or, though improbable, horizontally transferred genes from lignolytic organisms.

The genus *Burkholderia* and members of unclassified *Burkholderiaceae* were active on all three substrates. Lignolytic *Burkholderia* clustered with *B. nodosa* and *B. tropica* in SILVA. Members of *Variovorax* and unclassified *Comamonadaceae* were active on lignin, while *Leptothrix* and *Polaromonas*, both from family *Comamonadaceae*, were cellulolytic and hemicellulolytic. One unclassified *Comamonadaceae* OTU showed considerable cellulolytic activity across all ecozones and grouped with *Aquincola* in SILVA (Figure C.6). Metagenomic binning recovered one high quality draft genome classified as *Comamonadaceae* (Figure C.6) and a lower-quality draft from *Variovorax* (Table E.13). Cellulolytic *Oxalobacteraceae* related to *Janthinobacterium* and other unclassified groups within *Oxalobacteraceae*, while members of *Pelomonas* were active on all three substrates. Lignolytic OTUs from *Pelomonas* grouped with *Chlorochromatium* (*C. aggregatum*) in SILVA. A draft genome classified as *Collimonas sp.* (*Oxalobacteraceae*) was recovered from ^{13}C-lignin libraries.

Figure C.4. Abundances of prominent lignolytic taxa identified by differential abundance between ^{12}C- and ^{13}C-whole shotgun metagenomes for cellulose and lignin. These taxa were not identified in analyses of pyrotag libraries.

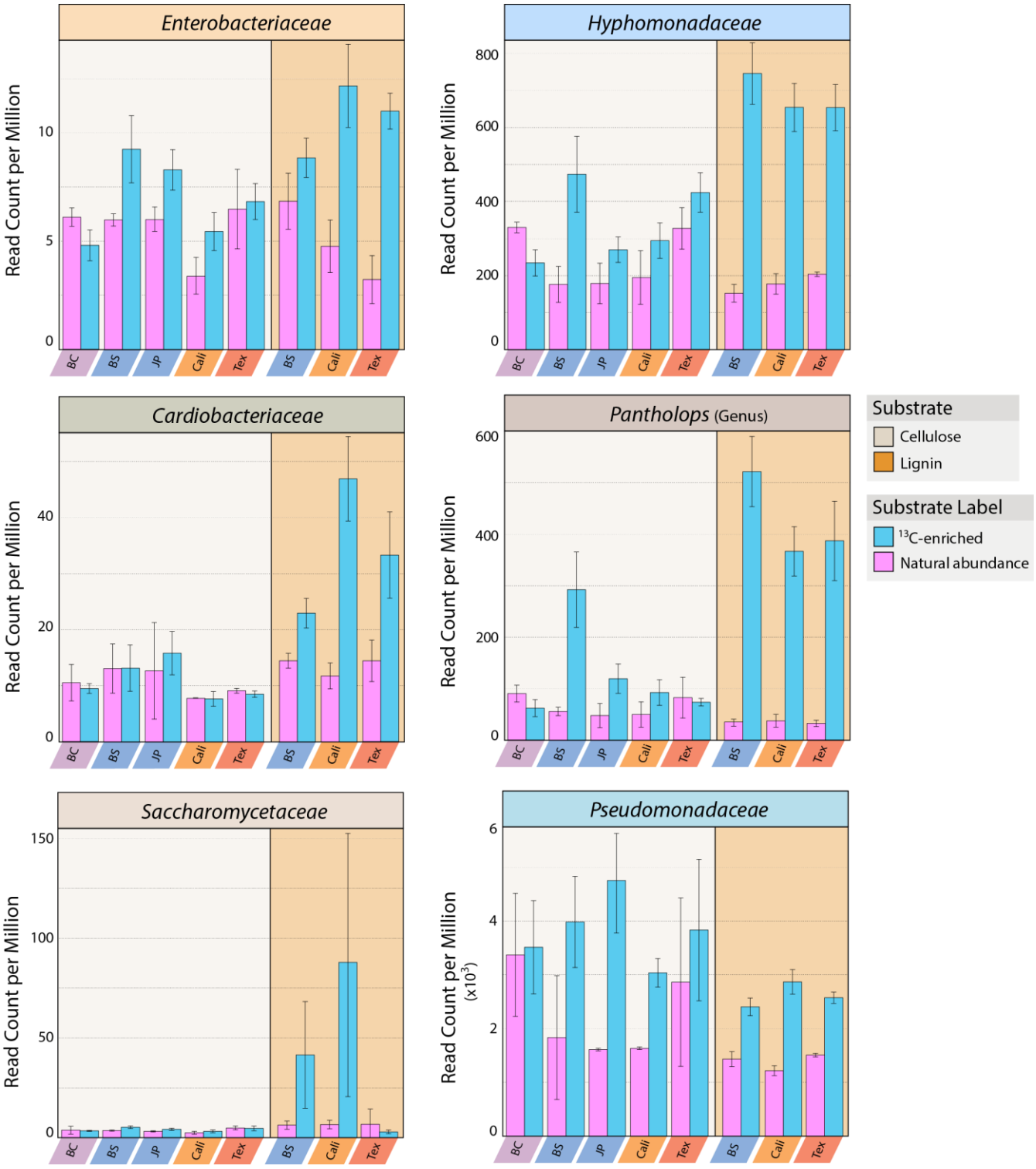


Figure C.5. Abundances of metagenomic reads classified to all genera within the family *Ectothiorhodospiraceae*. Each genus shows a nearly identical abundance pattern in which ^{13}C-lignin libraries are differentially more abundant than ^{12}C-lignin libraries. The consistency of this pattern is unlikely biological, but rather related to the use of LCA to classify metagenomic reads. These graphs demonstrate why genus-level classification with LCA is putative at best.

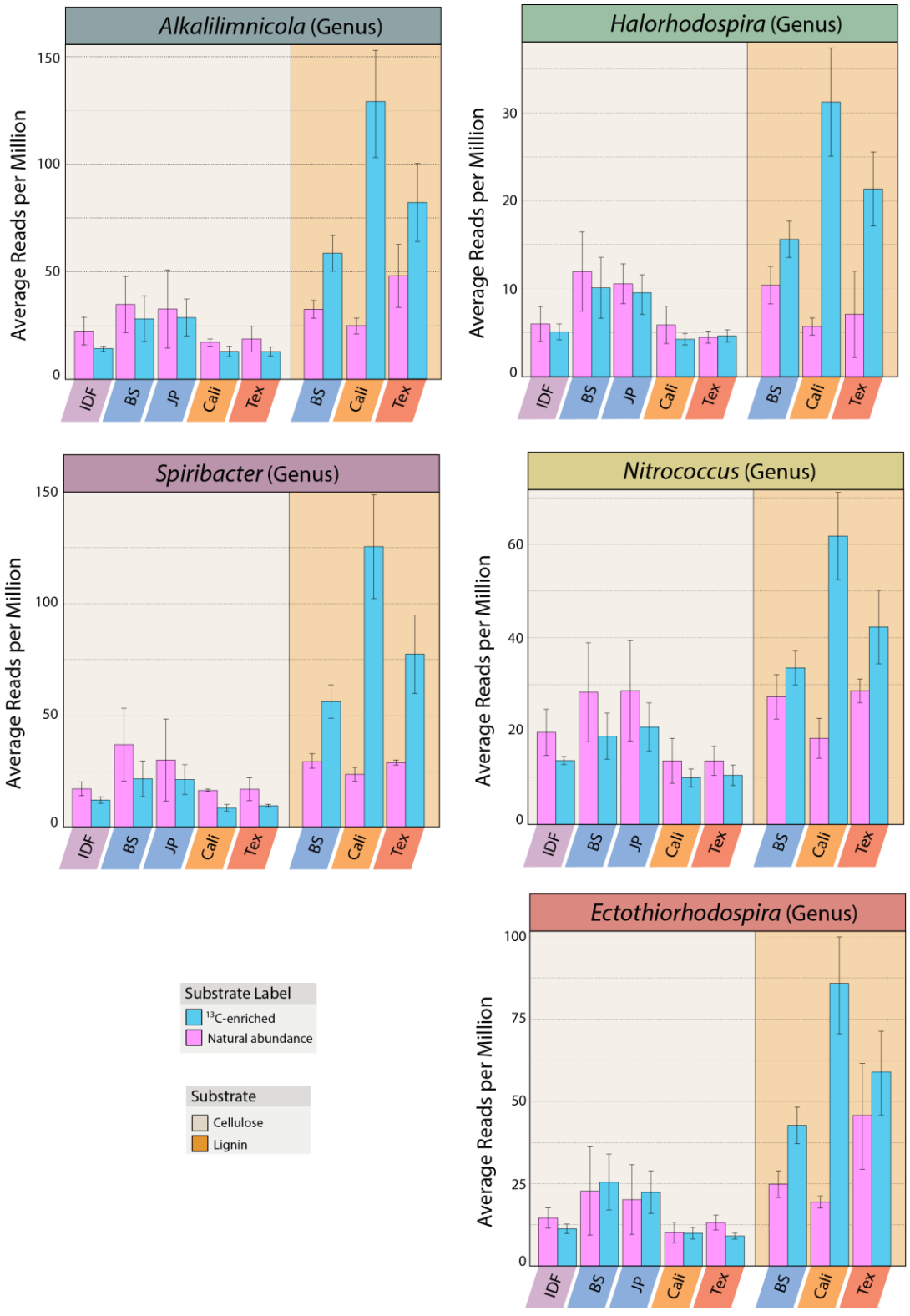


Figure C.6. Abundances of all prominently hemicellulolytic, cellulolytic and lignolytic genera of *Burkholderiales* based on differential abundance between ^{12}C- and ^{13}C-pyrotag libraries.

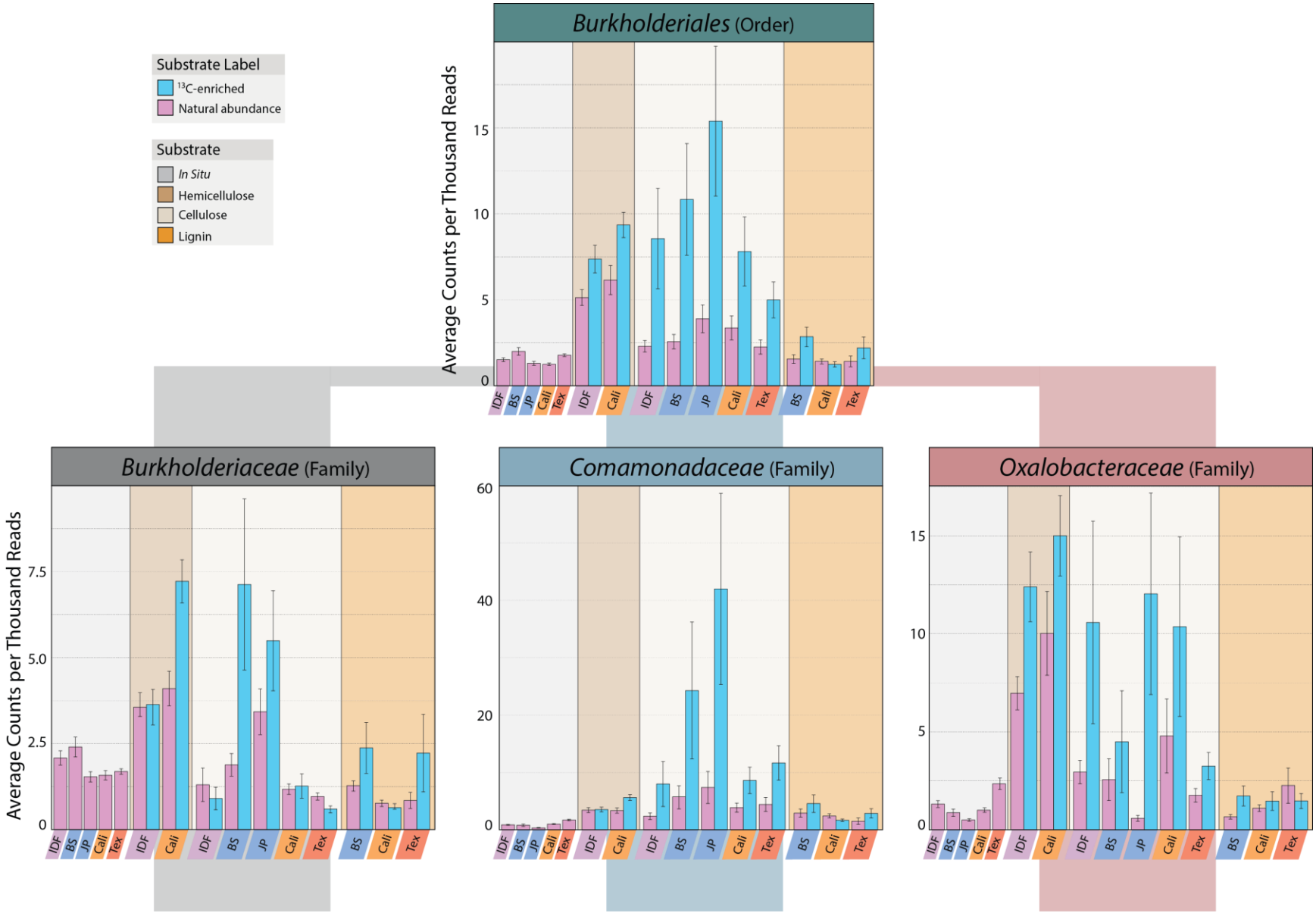


Figure C.6 … *continued*

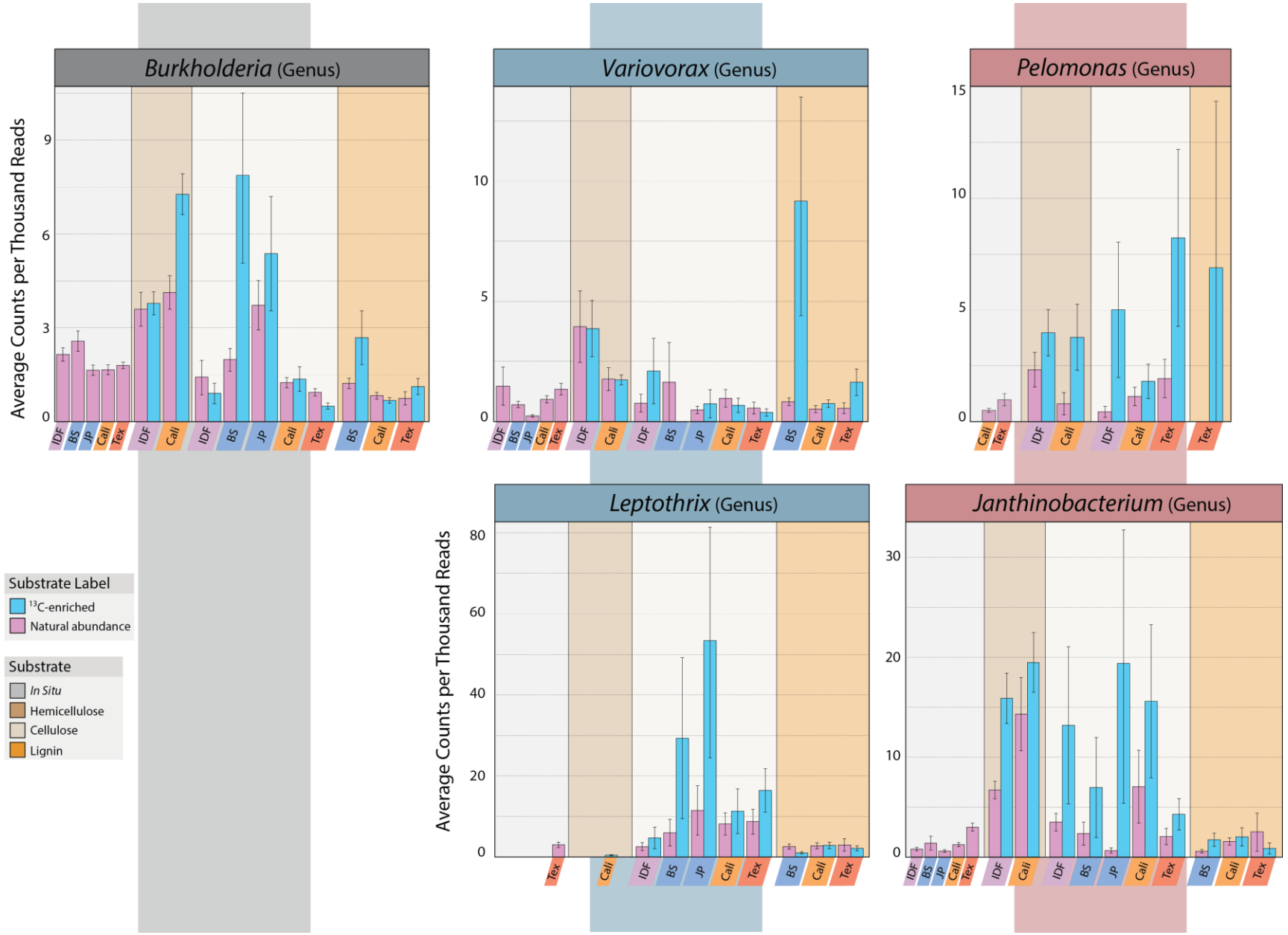
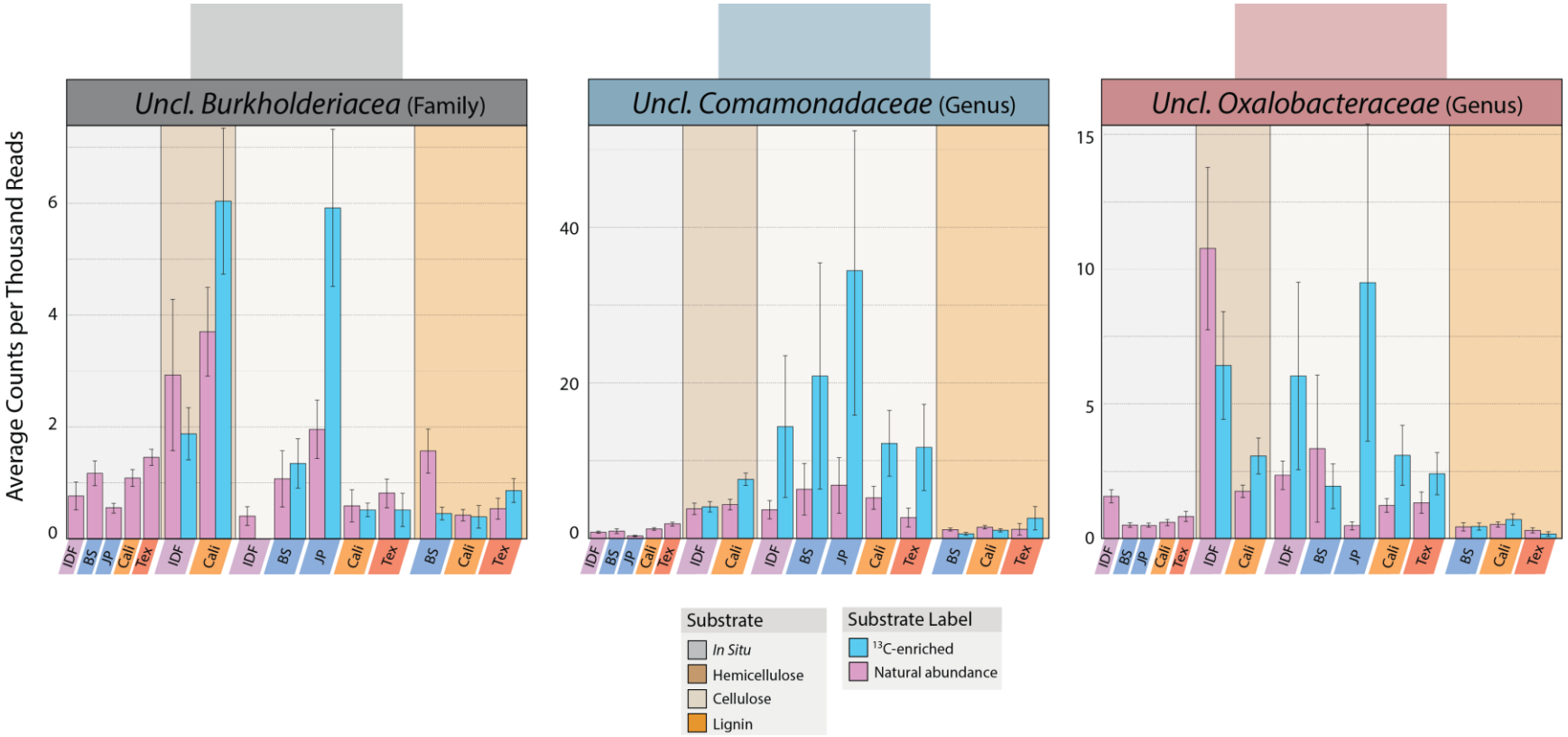


Figure C.6 … *continued*



Appendix D

Iso *Life*

Iso *Life* bv, P.O. Box 349 NL -6700 AH Wageningen
Visiting address: Droevendaalsesteeg 1, Bldg 107, NL -6708 PB Wageningen, The Netherlands
Info@isolife.nl Tel: +31 (0) 317 480512 www.isolife.eu

CERTIFICATE OF ANALYSIS

Article name:	U-^{13}C Cellulose high DP
Origin:	Maize stem *(Zea mays L.)*
Catalogue number:	U-10508
Lot number:	0901 –0273
Isotopic enrichment:	Uniform, 97 atom % ^{13}C
Chemical purity:	58 % glucose (w/w)
DP (average; by viscosity in cueen):	2,100
Average Mol. Wt.	350 kD
Crystallinity (by WAXS):	optional
Carbohydrate analysis:	optional
Residual lignin:	4.4 % (w/w)
Appearance:	fluffy, fibrous, off-white

April 15, 2010

Iso *Life*

Appendix E – Supplementary Tables

Table E.1. Summary of studies on the long-term impacts of timber harvesting on physicochemical properties of soil, forest productivity and soil biological.

Impact	Since Harvest	Major Conclusions	Geographic Location	Forest	Response Variable(s)	Focus	Reference
Affected	1 year	Forest floor removal affects nitrogen content.	Northern British Columbia, Canada	Coniferous	Stable isotope analysis	Chemical	Choi 2005
Affected	5 - 7 years	Differences in soil carbon pools with depth on account of organic matter removal.	Washington State, USA	Coniferous	Soil carbon	Chemical	Strahm 2009
Affected	4 years	Reduced methane uptake in clear-cut forest; no effect in selective cut.	Bavaria, Germany	Coniferous	Methane flux; soil properties	Chemical	Wu 2011
Affected	1 - 8 years	Decomposition rates slowed post-harvesting.	British Columbia, Canada	Coniferous	Mass loss	Chemical & Ecological	Prescott 2000
Affected	1 year	Arthrobacter populations increase with harvesting.	Northern British Columbia, Canada	Coniferous	PLFA; bacterial culturing	Ecological	Axelrood 2002
Affected	1 year	Reduction in Pseudomonas populations from harvesting.	Northern British Columbia, Canada	Coniferous	16S Library	Ecological	Axelrood 2002
Affected	13 years	Change in microbial community structure.	Northern British Columbia, Canada	Coniferous	Bacterial, archael and fungal phylogenetic genes	Ecological	Hartmann 2009
Affected	10 years	Change in microbial community structure.	Northern British Columbia, Canada	Coniferous	Bacterial and fungal phylogenetic genes	Ecological	Hartmann 2012
Affected	12 - 16 years	Modest changes in hemicellulolytic microbial populations.	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Stable isotope probing; bacterial and fungal phylogenetic genes	Ecological	Leung 2015
Affected	30 years	Reduced respiration and changes in microbial community.	Wyoming, USA	Coniferous	PLFA; CO2 flux; soil properties	Ecological	Chatterjee 2008
Affected	20 years	Changes in fungal abundance and microbial community structure.	Northern Alberta, Canada	Coniferous	PLFA; bacterial and fungal phylogenetic genes	Ecological	Hynes 2012
Affected	1 & 8 years	Diversity of omnivorous and predacious nematodes lowered in clearcuts.	British Columbia, Canada	Coniferous	Nematode populations	Ecological	Forge and Simard 2001
Affected	2 years	Soil microbial communities were altered by harvesting, but intensification did not cause further disturbance.	Northern Ontario	Coniferous	Bacterial and fungal phylogenetic genes; microbial biomass	Ecological	Smenderovac 2014
Affected	4 years	OM removal altered community structure.	Missouri, USA	Coniferous	PLFA	Ecological	Ponder 2002
Affected	5 - 11 years	No loss in species richness, but shift in community by harvesting.	Borneo	Palm	Bacterial phylogenetic genes	Ecological	Lee-Cruz (2013)
Affected	1 - 42 years	Partial cutting reduced biomass; significant variation unexplained by cutting intensity.	Global	Mixed	Tree biomass	Productivity	Zhou 2013

Impact	Since Harvest	Major Conclusions	Geographic Location	Forest	Response Variable(s)	Focus	Reference
Affected | Negative	2 - 4 years	CO2 flux reduced by organic matter removal.	Northern Ontario	Coniferous	Soil CO2 flux; soil properties	Chemical	Fleming 2006a
Affected | Negative	40 years	Soil respiration reduced from organic matter removal.	Northern Ontario	Coniferous	Soil CO2 flux; soil properties	Chemical	Webster 2016
Affected | Negative	6 - 10 years	Decreaes in microbial biomass and protease, phosphatase and dehydrogenase activities.	Northern British Columbia, Canada	Coniferous	Microbial C & N; enzyme activity	Ecological	Tan 2008
Affected | Negative	12 years	Lignocellulose degrading gene abundance reduced.	Northern British Columbia, Canada	Coniferous	Metagenomic DNA libraries	Ecological	Cardenas 2015
Affected | Negative	1 - 20 years	Net decrease in microbial biomass and activity.	Global	Mixed	Biomass and respiration	Ecological	Holden 2013
Affected | Positive	2 years	Minor increase in decomposition with harvesting, likely due to physical rather than biological changes	Northern British Columbia, Canada	Coniferous	Litter mass; mesofauna; soil properties	Chemical & Ecological	Kranabetter 1999
Neutral	3 - 7 years	No negative impact of forest floor removal on N transformation rates	Northern British Columbia, Canada	Coniferous	Microbial C & N	Chemical	Tan 2005
Neutral	10 years	CO2 flux unaffected, slight soil C increase with harvesting	North Carolina, USA	Coniferous	Soil CO2 flux; soil properties	Chemical	Butnor 2006
Neutral	5 years	Organic removal had negatively affected by harvesting	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Total C & N	Chemical	Sanchez 2005
Neutral	3 - 7 years	No net changes microbial CN content or respiration	Northern British Columbia, Canada	Coniferous	Microbial C & N; respiration; soil properties	Chemical	Mariani 2006
Neutral	1 - 10+	Harvesting had little to no effect on soil C and N in meta-analysis	Global	Mixed	Total C & N	Chemical	Johnson and Curtis 2001
Neutral	10 years	No net differences in tree biomass	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Tree biomass; soil properties	Productivity	Sanchez 2006
Neutral	10 years	Few consistent effects on planted tree biomass	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Tree biomass; foliar nutrients	Productivity	Ponder 2012
Neutral	10 years	Tree biomass unaffected by organic matter removal	Washington State, USA	Coniferous	Tree biomass	Productivity	Holub 2013
Tempoeral | Variable	3 years	Increased rate of tree growth after 1 year, followed by reductions	British Columbia, Canada	Coniferous	Tree biomass	Productivity	Kneeshaw 2002
Temporal | Affected	1, 2, 4, 5 & 7 years	Long-term shift in ratio of bacterial and fungal abundance	Wisconsin, USA	Deciduous	PLFA	Ecological	Lewandowski 2015
Temporal | Affected	20, 40 & 40+ years	Diversity of fungi reduced in harvested sites	Northern Alberta, Canada	Mixed	Culturable fungi	Ecological	Lumley 2001
Temporal | Affected	2 years	Lignocellulose mineralization initially increased, then decreated as well as reductions in other soil elments like S, and K	Missouri, USA	Coniferous	14-C lignocellulose mineralization; soil properties	Ecological & Chemical	Spratt 2000

Impact	Since Harvest	Major Conclusions	Geographic Location	Forest	Response Variable(s)	Focus	Reference
Temporal | Affected | Negative	3 & 10 years	Nutrient depletion over time	Vancouver Islanc, Canada	Coniferous	Foliar and microbial C & N; soil properties	Chemical & Ecological	Chang et al 1995
Temporal | Neutral	10 years	Soil CO2 flux w as impact follow ing harvesting, but returned to near pre-harvested state after 10 years	Central Ontario, Canada	Deciduous	Soil CO2 flux; soil properties	Chemical	Peng and Thomas 2006
Temporal | Neutral	3 - 6 years	N mineralization unaffected by organic matter removal	North Carolina, USA	Deciduous	Nitrogen contents	Chemical	Lee 2003
Temporal | Neutral	10-11 years	Soil chemistry unaffected by harvesting	Northern Alberta, Canada	Coniferous	Foliar nutrients; soil properties	Chemical & Productivity	Kishchuk et al. 2015
Temporal | Variable	1 - 20 years	Impacts on soil properties variable by time post-harvesting, soil depth and geography	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Soil properties	Chemical	Thiffault 2011
Variable	40 + years	Soil N replenished under stem-only harvesting, uncertain under w hole-tree	Norw ay	Coniferous	N contents	Chemical	Merila 2014
Variable	4 - 8 years	Elemental concentrations w ere variably impacted post-harvestin	Southern Sw eden	Coniferous	Soil properties	Chemical	Gronflaten 2008
Variable	5 years	Impacts on tree survival and biomass variable by geography & tree species	LTSP - Broadly Distributed Across USA and Canada	Coniferous	Tree survival, biomass	Chemical & Productivity	Fleming 2006b
Variable	2 years	Organic removal had variable influence on tree biomass	Northern British Columbia, Canada	Coniferous	Tree biomass,;foliar N	Chemical |& Productivity	Kamaluddin 2005
Variable	1 year	Nitrifying communities unaffected; reduction in soil respiration; no impact on tree grow th	Ottaw a, Canada	Mixed	Culturing; CO2 flux; nitrogen contents	Ecological & Productivity	Hendrickson 1985

Table E.2. Extensive compilation of all known lignolytic and cellulolytic bacteria, including both predicted function (based on genomic content) and validated function. Fungal degraders have been included in this list, but without a comprehensive effort. Table ordered by substrate, activity, then according to descending taxonomic rank.

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium smegmatis
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium tuberculosis
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus erythropolis TA421
Taylor 2012	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus erythropolis A5.1
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus fascians
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus equi
Taylor 2012	Aromatics	Validated	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Microbacterium phyllosphaerae A1.1
Taylor 2012	Aromatics	Validated	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Microbacterium marinilacus A1.2
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces cyaneus CECT 3335
Tian 2014	Aromatics	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Thermobifida	Thermobifida fusca
Tian 2014	Aromatics	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus halodurans
Tian 2014	Aromatics	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus subtilis
Tian 2014	Aromatics	Validated	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia cepacia
Tian 2014	Aromatics	Validated	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia sp. VE22
Tian 2014	Aromatics	Validated	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	Escherichia coli JM109
Tian 2014	Aromatics	Validated	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae
Ghodake 2009	Aromatics	Validated	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter calcoaceticus NCIM 2890
Tian 2014	Aromatics	Validated	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas fluorescens GcM5-1A
Tian 2014	Aromatics	Validated	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas aeruginosa BCH
Medie 2012	Cellulose	Predicted	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Acidobacterium	Acidobacterium capsulatum ATCC 51196
Medie 2012	Cellulose	Predicted	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Acidobacterium	Acidobacterium sp. MP5ACTX9
Medie 2012	Cellulose	Predicted	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Koribacter	Candidatus Koribacter versatilis Ellin345
Medie 2012	Cellulose	Predicted	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Terriglobus	Terriglobus saanensis SP1PR4
Medie 2012	Cellulose	Predicted	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Solibacter	Solibacter usitatus Ellin6076
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Acidothermales	Acidothermaceae	Acidothermus	Acidothermus cellulolyticus 11B ATCC 43068
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium adolescentis ATCC 15703
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium animalis subsp. lactis AD011
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium animalis subsp. lactis Bb12
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium animalis subsp. lactis DSM 10140
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium animalis subsp. lactis V9
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium dentium Bd1
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. BBMN68
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. JDM301
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. JCM 1217
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum NCC2705
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. infantis 157F
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. longum F8
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum DJO10A
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium longum subsp. infantis JCM 1222
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium pseudocatenulatum D2CA
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Catenulisporales	Catenulisporaceae	Catenulispora	Catenulispora acidiphila DSM 44928
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium sp. JLS
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium sp. KMS
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium sp. MCS

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	Mycobacterium vanbaalenii PYR-1
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus equi 103S
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Corynebacteriales	Tsukamurellaceae	Tsukamurella	Tsukamurella paurometabola DSM 20162
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	Frankia	Frankia alni ACN14a
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	Frankia	Frankia sp. EAN1pec
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Geodermatophilales	Geodermatophilaceae	Geodermatophilus	Geodermatophilus obscurus DSM 43160
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Glycomycetales	Glycomycetaceae	Stackebrandtia	Stackebrandtia nassauensis DSM 44728
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineococcus	Kineococcus radiotolerans SRS30216
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Beutenbergiaceae	Beutenbergia	Beutenbergia cavernae DSM 12333
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas fimi ATCC 484
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas flavigena DSM 20109
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium	Brachybacterium faecium DSM 4810
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Jonesiaceae	Jonesia	Jonesia denitrificans DSM 20603
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Clavibacter	Clavibacter michiganensis subsp. michiganensis NCPPB 382
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Clavibacter	Clavibacter michiganensis subsp. sepedonicus ATCC33113
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	Leifsonia xyli subsp. xyli str. CTCB07
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	Microbacterium testaceum StLB037
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	Arthrobacter phenanthrenivorans Sphe3
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Xylanimonas	Xylanimonas cellulosilytica DSM 15894
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micrococcales	Sanguibacteraceae	Sanguibacter	Sanguibacter keddieii DSM 10542
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora aurantiaca ATCC 27029
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora sp. L5
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Salinispora	Salinispora arenicola CNS-205
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Salinispora	Salinispora tropica CNB-440
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Verrucosispora	Verrucosispora maris AB-18-032
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	None	None	Thermobispora	Thermobispora bispora DSM 43833
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Kribbella	Kribbella flavida DSM 17836
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	Nocardioides sp. JS614
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinosynnema	Actinosynnema mirum DSM 43827
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Amycolatopsis	Amycolatopsis mediterranei U32
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Saccharopolyspora	Saccharopolyspora erythraea NRRL2338
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces avermitilis MA-4680
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces bingchenggensis BCW-1
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces coelicolor A3(2)
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces flavogriseus ATCC 33331
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces griseus subsp. griseus NBRC 13350
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces scabiei 87.22
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Nocardiopsis	Nocardiopsis dassonvillei subsp. dassonvillei DSM 43111
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Thermobifida	Thermobifida fusca YX
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Streptosporangium	Streptosporangium roseum DSM 43021
Medie 2012	Cellulose	Predicted	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Thermomonospora	Thermomonospora curvata DSM 43183
Medie 2012	Cellulose	Predicted	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Coriobacterium	Coriobacterium glomerans PW2
Medie 2012	Cellulose	Predicted	Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	Conexibacter	Conexibacter woesei DSM 14684
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidetes	incertae sedis	Rhodothermaceae	Rhodothermus	Rhodothermus marinus DSM 4252
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteriodes	Bacteriodes xylanisolvens XB1A
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides fragilis 638R
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides fragilis YCH46
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides helcogenes P 36-108 ATCC 35417
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides salanitronis DSM 18170
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Paludibacter	Paludibacter propionicigenes WB4

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Prevotella denticola F0289
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Prevotella ruminicola 23
Medie 2012	Cellulose	Predicted	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	Alistipes shahii WAL8301
Medie 2012	Cellulose	Predicted	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga hutchinsonii ATCC 33406
Medie 2012	Cellulose	Predicted	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Dyadobacter	Dyadobacter fermentans DSM 18053
Medie 2012	Cellulose	Predicted	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Leadbetterella	Leadbetterella byssophila DSM 17132
Medie 2012	Cellulose	Predicted	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma	Spirosoma linguale DSM 74
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	Capnocytophaga ochracea DSM 7271
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Cellulophaga	Cellulophaga algicola DSM 14237
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Cellulophaga	Cellulophaga lytica DSM 7489
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	Flavobacterium johnsoniae UW101
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Zunongwangia	Zunongwangia profunda SM-A87 SMA-87
Medie 2012	Cellulose	Predicted	Bacteroidetes	Flavobacteriia	Flavobacteriales	None	Flavobacteriales	Flavobacteriales bacterium HTCC2170
Medie 2012	Cellulose	Predicted	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Chitinophaga	Chitinophaga pinensis DSM 2588
Medie 2012	Cellulose	Predicted	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Pedobacter heparinus DSM 2366
Medie 2012	Cellulose	Predicted	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Pedobacter saltans DSM 12145
Medie 2012	Cellulose	Predicted	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp. 21
Medie 2012	Cellulose	Predicted	Chloroflexi	Chloroflexia	Chloroflexales	Roseiflexaceae	Roseiflexus	Roseiflexus castenholzii DSM 13941
Medie 2012	Cellulose	Predicted	Chloroflexi	Chloroflexia	Herpetosiphonales	Herpetosiphonaceae	Herpetosiphon	Herpetosiphon aurantiacus ATCC 23779
Medie 2012	Cellulose	Predicted	Deinococcus-the	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	Deinococcus geothermalis DSM 11300
Medie 2012	Cellulose	Predicted	Deinococcus-the	Deinococci	Deinococcales	Trueperaceae	Truepera	Truepera radiovictrix DSM 17093
Medie 2012	Cellulose	Predicted	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	Fibrobacter succinogenes subsp. succinogenes S85
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Alicyclobacillus	Alicyclobacillus acidocaldarius subsp. DSM 446
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus amyloliquefaciens FZB42
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus cellulosilyticus DSM 2522
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus clausii KSM-K16
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus halodurans C-125
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus licheniformis ATCC 14580 / DSM13
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus pumilus SAFR-032
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus subtilis BSn5
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus subtilis subsp. subtilis str. 168
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus subtilis subsp. spizizenii str. W23
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus subtilis subsp. natto BEST195
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus polymyxa SC2
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus polymyxa E681
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus sp. JDR-2
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus sp. Y412MC10
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus brevis ATCC 367
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus crispatus ST1
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	Lactococcus lactis subsp. cremoris NZ9000
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	Lactococcus lactis subsp. cremoris MG1363
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	Lactococcus lactis subsp. lactis KF147
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	Lactococcus lactis subsp. cremoris SK11
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus gallolyticus subsp. ATCC BAA-2069
Medie 2012	Cellulose	Predicted	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus gallolyticus UCN34
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium acetobutylicum ATCC 824
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium acetobutylicum EA 2018
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium cellulolyticum H10
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium cellulovorans 743B
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium lentocellum DSM 5427

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium phytofermentans ISDg95
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium saccharolyticum WM1
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium thermocellum ATCC 27405
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium thermocellum DSM 1313
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium eligens ATCC 27750
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium rectale ATCC 33656
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium rectale DSM 17629
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium rectale M104/1
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium siraeum 70/3
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium siraeum V10Sc8a
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio	Butyrivibrio fibrisolvens 16/4
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio	Butyrivibrio proteoclasticus B316
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	Coprococcus sp. ART55/1
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	Roseburia intestinalis XB6B4
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	Roseburia intestinalis M50/1
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	None	Anaerocellum	Anaerocellum thermophilum DSM 6725
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Ruminococcus albus DSM 20455
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Ruminococcus sp. 18P13
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor hydrothermalis 108
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor kristjanssonii 177R1B
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor kronotskyensis 2002
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor obsidiansis OB47
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor owensensis OL
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor saccharolyticus DSM 8903
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Thermoanaerobacter	Thermoanaerobacterium thermosaccharolyticum DSM 571
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	Thermoanaerobacteraceae	Thermoanaerobacter	Thermoanaerobacter italicus Ab9
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	Thermoanaerobacteraceae	Thermoanaerobacter	Thermoanaerobacter mathranii A3 DSM11426
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	Thermoanaerobacteraceae	Thermoanaerobacter	Thermoanaerobacter mathranii subsp. mathranii str. A3
Medie 2012	Cellulose	Predicted	Firmicutes	Clostridia	Thermoanaerobactera	Thermoanaerobacteraceae	Thermoanaerobacter	Thermoanaerobacter tengcongensis MB4
Medie 2012	Cellulose	Predicted	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	Planctomyces brasiliensis DSM 5305
Medie 2012	Cellulose	Predicted	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Rhodopirellula	Rhodopirellula baltica SH1
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Caulobacterales	Caulobacteraceae	Asticcacaulis	Asticcacaulis excentricus CB 48
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Caulobacterales	Caulobacteraceae	Caulobacter	Caulobacter crescentus CB15
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Caulobacterales	Caulobacteraceae	Caulobacter	Caulobacter crescentus NA1000
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Caulobacterales	Caulobacteraceae	Caulobacter	Caulobacter segnis ATCC 21756
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Caulobacterales	Caulobacteraceae	Caulobacter	Caulobacter sp. K31
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	Bradyrhizobium japonicum USDA 110
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Methylobacteriaceae	Methylobacterium	Methylobacterium radiotolerans JCM 2831
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Methylobacteriaceae	Methylobacterium	Methylobacterium sp. 4-46
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium radiobacter K84
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium sp. H13-3
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium tumefaciens str. C58
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium vitis S4
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium etli CFN 42
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium etli CIAT 652
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium leguminosarum bv. trifolii WSM1325
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium leguminosarum bv. trifolii WSM2304
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium leguminosarum bv. viciae 3841
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Rhizobium	Rhizobium sp. NGR234
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Sinorhizobium	Sinorhizobium medicae WSM419

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhodobacterales	Hyphomonadaceae	Hirschia	Hirschia baltica ATCC 49814
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Rhodospirillales	Rhodospirillaceae	Azospirillum	Azospirillum sp. B510
Medie 2012	Cellulose	Predicted	Proteobacteria	Alphaproteobact	Sphingomonadales	Sphingomonadaceae	Sphingobium	Sphingobium japonicum UT26S UT26S (=NBRC 101211)
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia ambifaria MC40-6
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia ambifaria AMMD
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia cenocepacia HI2424
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia cenocepacia J2315
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia cenocepacia AU 1054
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia gladioli BSR3
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia glumae BGR1
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia multivorans ATCC 17616
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia phymatum STM815
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia sp. CCGE1002
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum CFBP2957
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum GMI1000
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum IPO1609
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum PSI07
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum MolK2
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia solanacearum CMR15
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Comamonadaceae	Acidovorax	Acidovorax avenae subsp. citrulli AAC00-1
Medie 2012	Cellulose	Predicted	Proteobacteria	Betaproteobacte	Burkholderiales	Comamonadaceae	Ramlibacter	Ramlibacter tataouinensis TTB310
Medie 2012	Cellulose	Predicted	Proteobacteria	Deltaproteobacte	Myxococcales	Cystobacteraceae	Stigmatella	Stigmatella aurantiaca DW4/3-1
Medie 2012	Cellulose	Predicted	Proteobacteria	Deltaproteobacte	Myxococcales	Myxococcaceae	Myxococcus	Myxococcus xanthus DK1622
Medie 2012	Cellulose	Predicted	Proteobacteria	Deltaproteobacte	Myxococcales	Polyangiaceae	Sorangium	Sorangium cellulosum 'So ce 56'
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Aeromonadales	Aeromonadaceae	Aeromonas	Aeromonas salmonicida subsp. salmonicida A449
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Alteromonadales	Colwelliaceae	Colwellia	Colwellia psychrerythraea 34H
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	Pseudoalteromonas atlantica T6c
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Alteromonadales	Shewanellaceae	Shewanella	Shewanella violacea DSS12
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Cellvibrio	Cellvibrio japonicus Ueda107
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Saccharophagus	Saccharophagus degradans 2-40
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Teredinibacter	Teredinibacter turnerae T7901
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Citrobacter	Citrobacter rodentium ICC168
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Cronobacter	Cronobacter turicensis
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Dickeya	Dickeya dadantii 3937
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Dickeya	Dickeya dadantii Ech586
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Dickeya	Dickeya dadantii Ech703
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Dickeya	Dickeya zeae Ech1591
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae subsp. cloacae ATCC 13047
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae NCTC 9394
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter sakazakii ATCC BAA-894
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter sp. 638
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia amylovora ATCC 49946
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia amylovora CFBP1430
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia amylovora IL-5
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia pyrifoliae DSM 12163
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia pyrifoliae Ep1/96
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia sp. Ejp617
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia tasmaniensis Et1/99
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae NTUH-K2044
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae 342

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae subsp. pneumoniae MGH 78578
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Klebsiella	Klebsiella variicola At-22
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pantoea	Pantoea ananatis AJ13355
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pantoea	Pantoea ananatis LMG 20103
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pantoea	Pantoea sp. At-9b
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pantoea	Pantoea vagans C9-1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pectobacterium	Pectobacterium atrosepticum SCRI1043
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pectobacterium	Pectobacterium carotovorum subsp. carotovorum PC1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Pectobacterium	Pectobacterium wasabiae WPP163
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Rahnella	Rahnella sp. Y9602
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia enterocolitica subsp. enterocolitica 8081
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia enterocolitica subsp. palearctica 105.5R(r)
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia enterocolitica subsp. palearctica Y11
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis Antiqua
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis biovar Medie 2012valis str. Harbin 35
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis D106004
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis D182038
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis KIM
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis Nepal516
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis biovar Microtus str. 91001
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis Pestoides F
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pestis Z176003
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pseudotuberculosis IP 31758
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pseudotuberculosis IP 32953
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pseudotuberculosis PB1/+
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Yersinia	Yersinia pseudotuberculosis YPIII
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Legionellales	Legionellaceae	Legionella	Legionella longbeachae NSW150
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Oceanospirillales	Hahellaceae	Hahella	Hahella chejuensis KCTC 2396
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas fluorescens SBW25
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas putida BIRD-1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas putida F1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas syringae pv. phaseolicola 1448A
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas syringae pv. tomato str. DC3000
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas syringae pv. syringae B728a
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Vibrionales	Vibrionaceae	Photobacterium	Photobacterium profundum SS9
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Vibrionales	Vibrionaceae	Vibrio	Vibrio furnissii NCTC 11218
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	Pseudoxanthomonas suwonensis 11-1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas albilineans
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas axonopodis pv. citri str. 306
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas campestris pv. Campestris B100
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas campestris pv. campestris str. 8004
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas campestris pv. campestris str. ATCC 33913
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas campestris pv. vesicatoria str. 85-10
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas oryzae pv. oryzae MAFF 311018
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas oryzae pv. oryzae KACC10331
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas oryzae pv. oryzae PXO99A
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xylella	Xylella fastidiosa M23
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xylella	Xylella fastidiosa Temecula1
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xylella	Xylella fastidiosa M12
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xylella	Xylella fastidiosa subsp. fastidiosa GB514

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Medie 2012	Cellulose	Predicted	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xylella	Xylella fastidiosa 9a5c
Medie 2012	Cellulose	Predicted	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta	Spirochaeta thermophila DSM 6192
Medie 2012	Cellulose	Predicted	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema	Treponema succinifaciens DSM 2489
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga lettingae TMO
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga maritima MSB8
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga naphthophila RKU-10
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga neapolitana DSM 4359
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga petrophila RKU-1
Medie 2012	Cellulose	Predicted	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga sp. RQ2
Medie 2012	Cellulose	Predicted	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	Opitutus terrae PB90-1
Medie 2012	Cellulose	Predicted	Verrucomicrobia	Opitutae	Puniceicoccales	Puniceicoccaceae	Coraliomargarita	Coraliomargarita akajimensis DSM 45221
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Acidothermales	Acidothermaceae	Acidothermus	Acidothermus cellulolyticus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas biazotea
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas cellasea
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas fimi
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas flavigena
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas gelida
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas iranensis
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas persica
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas terrae
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas	Cellulomonas uda
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Curtobacterium	Curtobacterium flaccumfaciens
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Cellulosimicrobium	Cellulosimicrobium cellulans
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Cellulosimicrobium	Cellulosimicrobium cellulans
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Xylanimonas	Xylanimonas cellulosilytica
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora aurantiaca
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora chalcea
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora melanospora
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora propionici
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora	Micromonospora ruminantium
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	None	None	Thermobispora	Thermobispora bispora
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinosynnema	Actinosynnema mirum
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces albogriseolus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces aureofaciens
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces cellulolyticus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces flavogriseus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces lividans
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces nitrosporeus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces olivochromogenes
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces reticuli
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces rochei
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces thermovulgaris
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces viridosporus
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Thermobifida	Thermobifida alba
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Thermobifida	Thermobifida cellulolytica
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Nocardiopsaceae	Thermobifida	Thermobifida fusca
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Streptosporangium	Streptosporangium subroseum
Koeck 2014	Crystalline	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Thermomonospora	Thermomonospora curvata
Koeck 2014	Crystalline	Validated	Bacteroidetes	Bacteroidetes	incertae sedis	Rhodothermaceae	Rhodothermus	Rhodothermus marinus
Koeck 2014	Crystalline	Validated	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides cellulosilyticus

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Koeck 2014	Crystalline	Validated	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides sp. P-1
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga aurantiaca
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga haloflava
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga hutchinsonii
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga krzemieniewska
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	Cytophaga rosea
Koeck 2014	Crystalline	Validated	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Sporocytophaga	Sporocytophaga myxococcoides
Koeck 2014	Crystalline	Validated	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	Flavobacterium johnsoniae
Koeck 2014	Crystalline	Validated	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	Fibrobacter	Fibrobacter succinogenes
Koeck 2014	Crystalline	Validated	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Caldibacillus	Caldibacillus cellulovorans
Koeck 2014	Crystalline	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus circulans
Koeck 2014	Crystalline	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus pumilis
Koeck 2014	Crystalline	Validated	Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Thermoactinomyces	Thermoactinomyces sp. YX
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium cellulofermentans
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium cellulovorans
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium chartatabidum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium longisporum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium sp. C7
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium thermopapyrolyticum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Lachnoclostridium	Lachnoclostridium phytofermentans
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium alkalicellulosi
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium cellulolyticum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium cellulosi
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium clariflavum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium josui
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium papyrosolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium stercorarium
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium straminisolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium termitidis
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Ruminiclostridium	Ruminiclostridium thermocellum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium	Eubacterium cellulosolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio	Butyrivibrio fibrisolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Cellulosilyticum	Cellulosilyticum lentocellum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Cellulosilyticum	Cellulosilyticum ruminicola
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium	Lachnoclostridium celerecrescens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium	Lachnoclostridium herbivorans
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium	Lachnoclostridium populeti
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	None	Thermoanaerobacter	Thermoanaerobacter cellulolyticus
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Acetivibrio	Acetivibrio cellulolyticus
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Acetivibrio	Acetivibrio cellulosolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Pseudobacteroides	Pseudobacteroides cellulosolvens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	Ruminiclostridium aldrichii
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	Ruminiclostridium caenicola
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	Ruminiclostridium cellobioparum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	Ruminiclostridium hungatei
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminiclostridium	Ruminiclostridium sufflavum
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Ruminococcus albus
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Ruminococcus flavefaciens
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Halanaerobiales	Halanaerobiaceae	Halocella	Halocella cellulosilytica
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor bescii

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor hydrothermalis
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor kristjanssonii
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor kronotskyensis
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor lactoaceticus
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor obsidiansis
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	incertae sedis	Caldicellulosiruptor	Caldicellulosiruptor saccharolyticus
Koeck 2014	Crystalline	Validated	Firmicutes	Clostridia	Thermoanaerobactera	Thermoanaerobacteraceae	Thermoanaerobacter	Thermoanaerobacter thermocopriae
Koeck 2014	Crystalline	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Alcaligenaceae	Achromobacter	Achromobacter piechaudii
Koeck 2014	Crystalline	Validated	Proteobacteria	Deltaproteobacte	Myxococcales	None	Myxobacter	Myxobacter sp. AL-1
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Cellulomonas	Cellulomonas gilvus
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Cellvibrio	Cellvibrio japonicus
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Cellvibrio	Cellvibrio mixtus
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Cellvibrionales	Cellvibrionaceae	Cellvibrio	Cellvibrio vulgaris
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Enterobacteriales	Enterobacteriaceae	Dickeya	Dickeya dadantii
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Xanthomonadales	Xanthomonadaceae	Rudaea	Rudaea cellulosilytica
Koeck 2014	Crystalline	Validated	Proteobacteria	Gammaproteobac	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas sp.
Koeck 2014	Crystalline	Validated	Thermotogae	Thermotogae	Thermotogales	Fervidobacteriaceae	Fervidobacterium	Fervidobacterium islandicum
Koeck 2014	Crystalline	Validated	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga maritima
Koeck 2014	Crystalline	Validated	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	Thermotoga neapolitana
Vicuna 1988	Lignin	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	Corynebacterium sp.
Zimmermann	Lignin	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	Corynebacterium sp.
Woo 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Gordoniaceae	Gordonia	Gordonia sp.
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia	Nocardia sp. DSM 1069
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Rhodococcus jostii RHA1
Bugg 2011	Lignin	Validated	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	Arthrobacter globiformis
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus	Micrococcus luteus
Taylor 2012	Lignin	Validated	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus	Micrococcus luteus E1.1
Taylor 2012	Lignin	Validated	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus	Micrococcus luteus B5.3
Zimmermann	Lignin	Validated	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Arthrobacter	Arthrobacter simplex
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Amycolatopsis	Amycolatopsis sp. 75iv2
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Nocardia	Nocardia autotrophica
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces viridosporus T7A
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces flavovirens
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces badius ATCC 39117
Tian 2014	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces psammoticus
Zimmermann	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces rimosus
Bugg 2011	Lignin	Validated	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces coelicolor
Zimmermann	Lignin	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Thermomonospora	Thermomonospora mesophila
Zimmermann	Lignin	Validated	Actinobacteria	Actinobacteria	Streptosporangiales	Thermomonosporaceae	Actinomadura	Actinomadura sp.
Zimmermann	Lignin	Validated	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	Flavobacterium sp.
Tian 2014	Lignin	Validated	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp. CKTN2
Goran 2015	Lignin	Validated	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp. T2
Duan 2014	Lignin	Validated	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp. HY-H
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus megaterium
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus pumilus
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus atrophaeus
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus licheniformis
Bugg 2011	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Aneurinibacillus	Aneurinibacillus aneurinilyticus
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus sp. ITRC-S6
Tian 2014	Lignin	Validated	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	Paenibacillus sp. CBMAI 868

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
Zimmermann :	Lignin	Validated	Proteobacteria	Alphaproteobact	Rhizobiales	Beijerinckiaceae	Beijerinckia	Beijerinckia sp.
Taylor 2012	Lignin	Validated	Proteobacteria	Alphaproteobact	Rhizobiales	Brucellaceae	Ochrobactrum	Ochrobactrum pseudogrignonense A4.3
Taylor 2012	Lignin	Validated	Proteobacteria	Alphaproteobact	Rhizobiales	Brucellaceae	Ochrobactrum	Ochrobactrum rhizosphaerae C4.1
Si 2015	Lignin	Validated	Proteobacteria	Alphaproteobact	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium sp. S5-1
Gonzalez 199	Lignin	Validated	Proteobacteria	Alphaproteobact	Rhodobacterales	Rhodobacteraceae	Sagittula	Sagittula stellata E-37
Zimmermann :	Lignin	Validated	Proteobacteria	Alphaproteobact	Sphingomonadales	Sphingomonadaceae	Pseudomonas	Pseudomonas paucimobilis
Tian 2014	Lignin	Validated	Proteobacteria	Alphaproteobact	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas paucimobilis SYK-6
Zimmermann :	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Alcaligenaceae	Achromobacter	Achromobacter sp.
Woo 2014	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia sp. LIG30
Tian 2014	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Pandoraea	Pandoraea sp. B-6
Zimmermann :	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Pseudomonas	Pseudomonas cepacia
Zimmermann :	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Burkholderiaceae	Pseudomonas	Pseudomonas multivorans
Chen 2012	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Comamonadaceae	Comamonas	Comamonas sp. B-9
Zimmermann :	Lignin	Validated	Proteobacteria	Betaproteobacte	Burkholderiales	Comamonadaceae	Pseudomonas	Pseudomonas acidovorans
Woo 2014	Lignin	Validated	Proteobacteria	Betaproteobacte	Neisseriales	Chromobacteriaceae	Aquitalea	Aquitalea sp.
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Aeromonadales	Aeromonadaceae	Aeromonas	Aeromonas sp.
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Citrobacter	Citrobacter freundii IITRL1
Harazono 20(	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Citrobacter	Citrobacter sp. VA53
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Citrobacter	Citrobacter sp. IITRSU7
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter aerogenes
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter lignolyticus SCF1
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter soli
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae KBH3
Zimmermann :	Lignin	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Erwinia	Erwinia sp.
Zimmermann :	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter sp.
Zimmermann :	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Moraxellaceae	Pseudomonas	Pseudomonas cruciviae
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas putida mt-2
Tian 2014	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas putida GB-1
Zimmermann :	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas ovalis
Zimmermann :	Lignin	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas pseudoalcaligenes
Kern 1987	Lignin	Validated	Proteobacteria	Gammaproteoba	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas sp.
Tamboli 2011	Phenols	Validated	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	Sphingobacterium sp. ATM
Tian 2014	Phenols	Validated	Proteobacteria	Alphaproteobact	Rhizobiales	Brucellaceae	Brucella	Brucella sp. GXY-1
Tian 2014	Phenols	Validated	Proteobacteria	Alphaproteobact	Sphingomonadales	Sphingomonadaceae	Sphingobium	Sphingobium chlorophenolicum
Tian 2014	Phenols	Validated	Proteobacteria	Gammaproteoba	Enterobacteriales	Enterobacteriaceae	Enterobacter	Enterobacter cloacae DG-6
Tian 2014	Phenols	Validated	Proteobacteria	Gammaproteoba	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas sp. SUK1
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Gymnopilus	Gymnopilus chrysopellus PR-1187
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Agaricales	Fistulinaceae	Fistulina	Fistulina hepatica
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	Schizophyllum	Schizophyllum commune H4-8
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	Schizophyllum	Schizophyllum commune Loenen D
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	Schizophyllum	Schizophyllum commune Tattone D
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Boletales	Coniophoraceae	Coniophora	Coniophora puteana
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	Hydnomerulius	Hydnomerulius pinastri
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	Serpula	Serpula lacrymans S7.3
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	Serpula	Serpula lacrymans S7.9
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	Serpula	Serpula lacrymans var shastensis SHA21-2
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	Schizopora	Schizopora paradoxa KUC8140
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Geastrales	Sphaerobolaceae	Sphaerobolus	Sphaerobolus stellatus
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Gloeophyllales	Gloeophyllaceae	Gloeophyllum	Gloeophyllum trabeum
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Gloeophyllales	Gloeophyllaceae	Neolentinus	Neolentinus lepideus

Source	Substrate	Activity	Phylum	Class	Order	Family	Genus	Full Name
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Gomphales	Gomphaceae	Ramaria	Ramaria rubella (R. acris) UT-36052-T
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Jaapiales	Jaapiaceae	Jaapia	Jaapia argillacea
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Daedalea	Daedalea quercina
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Laetiporus	Laetiporus sulphureus var. sulphureus
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Postia	Postia placenta MAD 698-R
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Postia	Postia placenta MAD-698-R-SB12
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Wolfiporia	Wolfiporia cocos MD-104 SS10
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	Antrodia	Antrodia sinuosa
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	Fomitopsis	Fomitopsis pinicola FP-58527 SS1
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Fibroporia	Fibroporia radiculosa
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Polyporus	Polyporus arcularius
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Sistotremastrum	Sistotremastrum niveocremeum HHB9708 ss-11.0
JGI	Cellulose	Validated	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Sistotremastrum	Sistotremastrum suecicum
JGI	Cellulose	Validated	Basidiomycota	Dacrymycetes	NA	Dacrymycetaceae	Calocera	Calocera cornea
Bucher 2004	Lignin	Validated	Ascomycota	Dothideomycetes	Pleosporales	Aigialaceae	Ascocratera	Ascocratera manglicola
Bucher 2004	Lignin	Validated	Ascomycota	Dothideomycetes	Pleosporales	Pleosporales incertae sedis	Astrosphaeriella	Astrosphaeriella striatispora
Bucher 2004	Lignin	Validated	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Cryptovalsa	Cryptovalsa halosarceicola
Bucher 2004	Lignin	Validated	Ascomycota	Sordariomycetes	Xylariales	Xylariales incertae sedis	Linocarpon	Linocarpon bipolaris
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Agaricales incertae sedis	Plicaturopsis	Plicaturopsis crispa
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	Gymnopus	Gymnopus androsaceus JB14
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurotus	Pleurotus ostreatus PC15
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurotus	Pleurotus ostreatus PC9
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	Galerina	Galerina marginata
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Panellus	Panellus stipticus KUC8834
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	Auricularia	Auricularia subglabra
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	Exidia	Exidia glandulosa
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	Phlebia	Phlebia brevispora HHB-7030 SS6
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	Fomitiporia	Fomitiporia mediterranea
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Trametes	Trametes ljubarskyi CIRM1659
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	Trametes	Trametes versicolor
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	Ganoderma	Ganoderma sp. 10597 SS1
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Lentinaceae	Lentinus	Lentinus tigrinus ALCF2SS1-6
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Lentinaceae	Lentinus	Lentinus tigrinus ALCF2SS1-7
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Lentinaceae	Lentinus	Lentinus tigrinus
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Bjerkandera	Bjerkandera adusta
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Ceriporiopsis	Ceriporiopsis (Gelatoporia) subvermispora B
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Obba	Obba rivulosa 3A-2
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	Phanerochaete	Phanerochaete carnosa HHB-10118-Sp
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	Phanerochaete	Phanerochaete chrysosporium RP-78 v2.2
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	Phlebiopsis	Phlebiopsis gigantea
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Artolenzites	Artolenzites elegans CIRM1663
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Cerrena	Cerrena unicolor
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Dichomitus	Dichomitus squalens
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Pycnoporus	Pycnoporus cinnabarinus BRFM 137
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Pycnoporus	Pycnoporus coccineus BRFM 310
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Pycnoporus	Pycnoporus coccineus CIRM1662
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Pycnoporus	Pycnoporus sanguineus BRFM 1264
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Trichaptum	Trichaptum abietinum
JGI	Lignin	Validated	Basidiomycota	Agaricomycetes	Russulales	Stereaceae	Stereum	Stereum hirsutum FP-91666 SS1
JGI	Lignin	Validated	Basidiomycota	Dacrymycetes	NA	Dacrymycetaceae	Calocera	Calocera viscosa
JGI	Lignin	Validated	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	Tremella mesenterica
Bucher 2004	Lignin	Validated	NA	NA	NA	NA	NA	Rhizophila marina

Table E.3. Overview of all sampling sites within the ecozones utilized in this study, including sampling information, climatic information and the date harvesting took place. Data was sourced from collaborators (Dr. Matt Busse, Dr. Andy Scott, Dr. Paul Hazlett and Dr. David Morris) and Ponder et al. 2012 and Hartmann et al. 2012

Name	Site	Sample Collection	Lat.	Long.	Elevation (m)	Soil Classification	Tree Cover	Climatic Zone	Annual Temp (°C)	Precipitation (mm)	Year Est.	Country
Fensom	A7	7/3/2011	49.07	-89.41	445	Orthic Dystric Brunisol	Black Spruce	Dfb, Humid Continental w arm summer	2.4	266	1995	CANADA
Fensom	A8	7/4/2011	49.08	-89.38	450	Orthic Dystric Brunisol	Black Spruce	Dfb, Humid Continental w arm summer	1.8	266	1995	CANADA
Fensom	A9	7/5/2011	49.07	-89.39	442	Gleyed Dystric Brunisol	Black Spruce	Dfb, Humid Continental w arm summer	1.5	266	1995	CANADA
Brandy City	BR	6/22/2011	39.55	-121.04	1135	Mesic Ultic Haploxeralfs	Ponderosa pine, sugar pine, w hite fir, giant sequoia	Csa, Mediterranean hot summer	11.2	55	1995	USA
Blodgett	BL	9/16/2011	38.88	-120.64	1350	Mesic Ultic Haploxeralfs	Ponderosa pine, sugar pine, w hite fir, giant sequoia	Csa, Mediterranean hot summer	11.2	55	1994	USA
Low ell Hill	LH	9/16/2011	39.26	-120.78	1268	Mesic Ultic Haploxeralfs	Ponderosa pine, sugar pine, w hite fir, giant sequoia	Csa, Mediterranean hot summer	11.2	55	1995	USA
Wells	JW	7/7/2011	46.42	-83.37	228	Orthic Humo-Ferric Podzol	Jack Pine, Black Spruce, Red Pine	Dfb, Humid Continental cool summer	4.4	248	1993-1994	CANADA
Superior	JS	8/4/2011	47.57	-82.85	426	Orthic Dystric Brunisol	Jack Pine, Black Spruce	Dfb, Humid Continental cool summer	1.7	250	1993-1994	CANADA
Eddy	JE	8/3/2011	46.75	-82.25	490	NA	Jack Pine, Balsam fir, White birch	Dfb, Humid Continental cool summer	2.8	242	1993-1994	CANADA
Kurth	TXA	3/12/2012	31.11	-95.15	88	Aquic Glossudalfs	Loblolly Pine, Beautyberry, Y aupon, Sw eetgum, Oaks	Cfa, Humid subtropical	19.0	253	1997	USA
Kurth	TXB	3/12/2012	31.11	-95.15	88	Aquic Glossudalfs	Loblolly Pine, Beautyberry, Y aupon, Sw eetgum, Oaks	Cfa, Humid subtropical	19.0	253	1997	USA
Kurth	TXC	3/12/2012	31.11	-95.15	88	Aquic Glossudalfs	Loblolly Pine, Beautyberry, Y aupon, Sw eetgum, Oaks	Cfa, Humid subtropical	19.0	253	1997	USA
O'Connor Lake	OC	6/26/2010	50.88	-120.35	1075	Brunisolic Gray Luvisol	Douglas fir	Dfb, Humid Continental w arm summer	2.5	300	1999	CANADA
Black Pines	BP	6/22/2010	50.93	-120.28	1180	Brunisolic Gray Luvisol	Douglas fir, Lodgepole pine	Dfb, Humid Continental w arm summer	2.5	300	1999	CANADA
Dairy Creek	DC	6/25/2010	50.85	-120.42	1150	Brunisolic Gray Luvisol	Douglas fir, Subalpine fir, Lodgepole pine	Dfb, Humid Continental w arm summer	2.5	300	1999	CANADA
Log Lake	LL	7/9/2008	54.35	-122.61	780	Orthic Humo-Ferric Podzol	Subalpine fir, Douglas fir, Interior Spruce	Dfc, Boreal cool summer	2.2	415	1994	CANADA
Topley	TO	7/11/2008	52.32	-126.31	1100	Orthic Gray Luvisol, Gleyed Gray Luvisol	Lodgepole pine, Subalpine fir, Interior spruce	Dfc, Boreal cool summer	2.2	415	1994	CANADA
Skulow Lake	SL	8/14/2009	52.32	-121.92	1050	Orthic Gray Luvisol	Lodgepole pine, Interior spruce	Dfc, Boreal cool summer	2.2	415	1994	CANADA

Table E.4. Overview of all PLFA, pyrotag and metagenomic libraries collected from ecozones, substrates and soil layers.

Experiment	Ecozone	Horizon	Treatment	16S rRNA Libraries	ITS Libraries
'in situ' Harvesting Effects (Chapter 3)	British Columbia (IDF)	Org	REF	9	9
			OM1	27	27
			OM2	27	27
			OM3	0	0
		Min	REF	9	9
			OM1	27	27
			OM2	27	27
			OM3	27	27
	British Columbia (SBS)	Org	REF	12	12
			OM1	30	30
			OM2	30	30
			OM3	0	0
		Min	REF	12	12
			OM1	30	30
			OM2	31	33
			OM3	32	33
	Ontario (BS)	Org	REF	9	7
			OM1	8	8
			OM2	9	9
			OM3	9	9
		Min	REF	9	9
			OM1	9	9
			OM2	9	9
			OM3	9	9
	Ontario (JP)	Org	REF	7	9
			OM1	18	17
			OM2	16	17
			OM3	1	1
		Min	REF	9	9
			OM1	18	18
			OM2	17	15
			OM3	18	18
	California	Org	REF	9	9
			OM1	9	9
			OM2	9	9
			OM3	9	8
		Min	REF	9	9
			OM1	9	9
			OM2	9	9
			OM3	9	9
	Texas	Org	REF	8	7
			OM1	18	19
			OM2	15	20
			OM3	12	15
		Min	REF	8	7
			OM1	15	21
			OM2	19	17
			OM3	13	12

Table E.4... *continued*

Experiment	Library	Horizon	Treatment	PLFA	16S rRNA Libraries	ITS Libraries
SIP-Cellulose Harvesting Effects (Chapter 3)	^{13}C-Library	Org	REF	9	3	3
			OM1	9	3	2
			OM2	9	3	3
			OM3	9	3	3
		Min	REF	9	3	3
			OM1	9	3	3
			OM2	9	3	3
			OM3	9	3	3
	^{12}C-Library	Org	REF	1	3	3
			OM1	-	3	2
			OM2	-	3	2
			OM3	-	3	2
		Min	REF	1	3	3
			OM1	-	3	3
			OM2	-	3	3
			OM3	-	3	3

Table E.4... *continued*

	Ecozone	Horizon	Substrate	PLFA	^{13}C-Pyro	^{12}C-Pyro	^{13}C-Meta	^{13}C-Anti	^{12}C-Meta
SIP-Biodiversity (Chapter 4)	British Columbia (IDF)	Org	*'in situ'*	-	-	12	-	-	-
			Hemicellulose	-	3	4	-	-	-
			Cellulose	3	6	3	3	-	1
			Lignin	-	-	-	-	-	-
		Min	'in situ'	-	-	12	-	-	-
			Hemicellulose	-	6	4	-	-	-
			Cellulose	3	3	3	3	-	1
			Lignin	-	-	-	-	-	-
	Ontario (BS)	Org	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	3	3	3	3	-	1
			Lignin	10	4	8	4	-	3
		Min	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	0	2	3	3	-	1
			Lignin	10	8	7	8	4	4
	Ontario (JP)	Org	'in situ'	-	-	7	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	1	3	3	3	-	1
			Lignin	0	-	-	-	-	-
		Min	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	2	3	3	3	-	1
			Lignin	0	-	-	-	-	-
	California	Org	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	8	4	-	-	-
			Cellulose	9	3	3	3	-	1
			Lignin	10	6	9	5	-	3
		Min	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	15	3	-	-	-
			Cellulose	9	3	3	3	-	1
			Lignin	10	9	6	9	2	4
	Texas	Org	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	3	3	3	3	-	1
			Lignin	3	2	-	1	-	0
		Min	'in situ'	-	-	9	-	-	-
			Hemicellulose	-	-	-	-	-	-
			Cellulose	0	3	3	1	-	1
			Lignin	5	3	2	3	2	2

Table E.5. Overview of soil properties among ecozones, soil layers and harvesting treatments. Within groups of treatments, significantly different values ($p < 0.05$) have been denoted by lettering based on Tukey's Honest Significant Difference.

Organic Layer																
		REF				OM1				OM2				OM3			
	Ecozone	mean	std. error	n	sig	mean	std. error	n	sig	mean	std. error	n	sig	mean	std. error	n	sig
Moisture Content (w/w)	SBS	63.81	13.91	12	**a**	57.21	8.11	30	**b**	56.84	9.63	30	**b**	-	-	-	-
	IDF	68.13	11.08	9	ns	67.12	12.64	27	ns	68.13	13.30	27	ns	-	-	-	-
	BS	65.28	8.01	9	**a**	52.50	11.12	9	**b**	51.36	12.10	9	**b**	50.24	12.43	9	**b**
	JP	48.08	14.74	9	**a**	37.47	8.11	18	**b**	40.75	11.62	16	**ab**	-	-	-	-
	CALIFORNIA	33.17	18.20	9	ns	29.73	19.61	9	ns	29.28	16.81	9	ns	27.96	15.07	9	ns
	TEXAS	29.71	7.44	9	**b**	28.16	5.37	25	**b**	19.36	9.15	21	**a**	26.25	6.97	17	**b**
pH	SBS	4.72	0.49	9	ns	4.93	0.26	27	ns	4.89	0.33	27	ns	-	-	-	-
	IDF	5.25	0.19	9	**b**	5.48	0.23	27	**a**	5.56	0.25	27	**a**	-	-	-	-
	BS	4.45	0.29	9	**b**	4.87	0.33	9	**ab**	4.84	0.41	9	**ab**	5.16	0.39	9	**a**
	JP	3.83	0.23	9	ns	3.97	0.23	18	ns	3.92	0.23	16	ns	-	-	-	-
	CALIFORNIA	5.30	0.55	9	**ab**	4.40	0.61	9	**b**	5.11	0.60	9	**ab**	5.28	0.37	9	**a**
	TEXAS	4.46	0.27	9	**b**	4.83	0.37	24	**a**	4.69	0.47	21	**ab**	4.45	0.19	15	**b**
Bulk Density (g/cm^3)	SBS	-	-	-	-	1.58	0.14	27	ns	1.63	0.10	27	ns	-	-	-	-
	IDF	-	-	-	-	1.29	0.15	27	**a**	1.37	0.13	27	**b**	-	-	-	-
	BS	0.13	0.02	9	**b**	0.19	0.03	9	**a**	0.22	0.03	9	**a**	0.19	0.03	9	**a**
	JP	7.43	1.47	9	ns	6.77	0.98	18	ns	7.48	1.64	16	ns	-	-	-	-
	CALIFORNIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TEXAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total Carbon (%)	SBS	33.83	11.64	9	**a**	27.78	6.32	27	**b**	26.07	6.28	27	**b**	-	-	-	-
	IDF	44.29	0.09	9	**a**	36.18	2.32	27	**b**	31.22	11.03	27	**c**	-	-	-	-
	BS	44.42	1.54	9	**a**	38.72	4.81	9	**a**	42.00	4.23	9	**a**	31.53	8.22	9	**b**
	JP	40.53	4.06	9	ns	40.18	4.31	18	ns	39.48	2.75	16	ns	-	-	-	-
	CALIFORNIA	38.23	4.87	6	**a**	41.28	2.43	6	**a**	29.66	3.89	6	**b**	27.72	10.20	5	**b**
	TEXAS	17.98	4.81	9	**a**	11.32	1.77	9	**b**	10.97	5.44	8	**b**	12.21	2.40	8	**b**
Total Nitrogen (%)	SBS	0.89	0.25	9	ns	0.78	0.18	27	ns	0.77	0.21	27	ns	-	-	-	-
	IDF	1.24	0.14	9	ns	1.13	0.18	27	ns	1.03	0.36	27	ns	-	-	-	-
	BS	1.08	0.08	9	**a**	1.00	0.16	9	**ab**	1.13	0.12	9	**a**	0.84	0.27	9	**b**
	JP	1.24	0.10	9	ns	1.16	0.08	18	ns	1.17	0.13	16	ns	-	-	-	-
	CALIFORNIA	1.26	0.19	6	**a**	1.24	0.26	6	**ab**	1.02	0.19	6	**ab**	0.88	0.21	5	**b**
	TEXAS	0.63	0.16	9	**a**	0.44	0.08	9	**b**	0.43	0.19	8	**b**	0.43	0.06	8	**b**
C:N Ratio	SBS	37.44	3.82	9	**a**	35.72	3.86	27	**ab**	34.13	2.78	27	**b**	-	-	-	-
	IDF	36.23	4.20	9	**a**	32.81	6.01	27	**ab**	30.45	8.38	27	**b**	-	-	-	-
	BS	41.44	2.72	9	**a**	39.29	5.93	9	**ab**	37.24	2.94	9	**b**	38.43	3.50	9	**ab**
	JP	32.89	2.97	9	ns	35.33	3.69	18	ns	34.59	4.55	16	ns	-	-	-	-
	CALIFORNIA	30.67	3.78	6	ns	34.84	8.87	6	ns	29.73	5.20	6	ns	31.05	7.10	5	ns
	TEXAS	28.60	1.95	9	**a**	25.69	1.90	9	**b**	25.42	1.95	8	**b**	28.02	2.14	8	**a**

Table E.5... *continued*

Mineral Layer																
		REF				OM1				OM2				OM3			
	Ecozone	mean	std. error	n	sig	mean	std. error	n	sig	mean	std. error	n	sig	mean	std. error	n	sig
Moisture Content (w/w)	SBS	21.73	7.50	11	ns	23.86	5.87	31	ns	24.11	6.02	29	ns	23.30	6.20	30	ns
	IDF	31.63	10.81	9	ns	33.66	10.17	27	ns	31.91	10.37	27	ns	30.02	12.91	27	ns
	BS	25.96	4.12	9	**a**	22.79	3.17	9	**a**	21.61	5.28	9	**ab**	17.98	2.95	9	**b**
	JP	15.42	3.09	9	**a**	9.97	4.51	18	**b**	12.89	3.40	17	**ab**	11.73	4.07	18	**b**
	CALIFORNIA	22.49	7.07	9	ns	26.54	6.99	9	ns	24.99	8.43	9	ns	24.19	7.60	9	ns
	TEXAS	14.70	3.19	9	ns	14.60	5.14	24	ns	17.16	6.87	21	ns	15.87	5.19	16	ns
pH	SBS	5.15	0.41	8	ns	5.18	0.38	28	ns	5.15	0.28	26	ns	5.31	0.33	27	ns
	IDF	5.48	0.23	9	**b**	5.59	0.26	27	**b**	5.60	0.13	27	**b**	5.75	0.12	27	**a**
	BS	5.33	0.27	9	**b**	5.39	0.30	9	**b**	5.48	0.24	9	**ab**	5.74	0.28	9	**a**
	JP	4.97	0.41	9	**b**	5.18	0.14	18	**ab**	5.20	0.14	17	**ab**	5.27	0.16	18	**a**
	CALIFORNIA	6.11	0.44	9	**a**	5.54	0.31	9	**b**	5.86	0.28	9	**ab**	5.62	0.39	9	**b**
	TEXAS	-	-	-	**-**	4.94	0.25	24	**a**	4.97	0.43	21	**a**	4.60	0.27	16	**b**
Bulk Density (g/cm^3)	SBS	-	-	-	-	1.57	0.14	28	ns	1.64	0.10	26	ns	1.60	0.14	27	ns
	IDF	-	-	-	-	1.29	0.15	27	**b**	1.37	0.13	27	**b**	1.50	0.14	27	**a**
	BS	1.08	0.25	9	**b**	1.16	0.20	9	**ab**	1.27	0.20	9	**ab**	1.32	0.26	9	**a**
	JP	7.43	1.47	9	ns	6.77	0.98	18	ns	7.51	1.59	17	ns	7.30	1.31	18	ns
	CALIFORNIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TEXAS	-	-	-	-	1.30	0.02	24	**a**	1.27	0.05	21	b	1.32	0.01	16	**a**
Total Carbon (%)	SBS	1.22	0.30	8	**b**	1.50	0.55	28	**b**	1.76	0.84	26	**a**	1.44	0.42	27	**b**
	IDF	2.19	0.29	9	ns	2.42	0.59	27	ns	6.69	12.66	27	ns	1.99	0.32	27	ns
	BS	2.00	0.68	9	**ab**	2.42	1.02	9	**a**	1.47	0.74	9	**b**	1.39	0.54	9	**b**
	JP	1.92	0.82	9	ns	2.13	0.95	18	ns	2.14	0.87	17	ns	2.80	3.68	18	ns
	CALIFORNIA	6.12	1.20	9	**a**	6.11	1.30	9	**a**	4.90	0.96	9	**b**	5.14	1.05	9	**ab**
	TEXAS	-	-	-	**-**	0.92	0.11	24	**a**	0.95	0.20	21	**ab**	0.80	0.11	16	**b**
Total Nitrogen (%)	SBS	0.06	0.01	8	**a**	0.07	0.02	28	**ab**	0.09	0.04	26	**b**	0.07	0.02	27	**ab**
	IDF	0.10	0.00	9	ns	0.12	0.02	27	ns	0.27	0.45	27	ns	0.10	0.01	27	ns
	BS	0.09	0.02	9	ns	0.10	0.04	9	ns	0.07	0.03	9	ns	0.07	0.03	9	ns
	JP	0.13	0.06	9	ns	0.14	0.09	18	ns	0.19	0.15	17	ns	0.17	0.16	18	ns
	CALIFORNIA	0.29	0.06	9	ns	0.25	0.06	9	ns	0.23	0.06	9	ns	0.26	0.06	9	ns
	TEXAS	-	-	-	**-**	0.05	0.00	24	**a**	0.05	0.00	21	**a**	0.04	0.00	16	**b**
C:N Ratio	SBS	21.03	2.24	8	ns	20.16	2.20	28	ns	19.77	1.22	26	ns	20.53	2.15	27	ns
	IDF	21.90	2.87	9	ns	20.82	3.35	27	ns	21.28	4.10	27	ns	20.42	2.55	27	ns
	BS	21.77	2.82	9	ns	23.65	2.73	9	ns	20.76	2.59	9	ns	20.60	1.76	9	ns
	JP	17.84	3.29	9	ns	18.19	4.94	18	ns	17.86	5.65	17	ns	18.79	6.82	18	ns
	CALIFORNIA	21.10	1.42	9	**b**	24.69	4.50	9	**a**	21.28	0.77	9	**b**	19.62	0.99	9	**b**
	TEXAS	-	-	-	**-**	16.90	1.17	24	**b**	18.95	3.20	21	a	17.83	1.48	16	**ab**

Table E.6 Full list of bacterial and fungal taxa showing clear expansion or decline in response to harvesting.

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Response	Mean Abundance	Max. Abundance	Response ratio
Acidobacteria	Acidobacteriales	Acidobacteriaceae	Edaphobacter	BC, JP, CA, TX	Org	Variable	0.58	13.22	0.7
Acidobacteria	Acidobacteriales	Acidobacteriaceae	Acidopila	ON, BC, CA	-	Expanded	0.26	1.49	10.1
Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	BC, ON	Org	Variable	0.82	40.33	0.6
Acidobacteria	Acidobacteriales	Acidobacteriaceae	Telmatobacter	TX	Org	Expanded	0.20	1.27	15.7
Acidobacteria	Acidobacteriales	Koribacteraceae	Candidatus Koribacter	ON, BC, TX	-	Variable	0.52	18.02	1.5
Acidobacteria	Holophagales	Holophagaceae	Geothrix	All	Org	Expanded	0.35	2.25	14.2
Actinobacteria	Actinomycetales	Geodermatophilaceae	Blastococcus	BC, CA	-	Expanded	0.28	10.62	31.8
Actinobacteria	Actinomycetales	Geodermatophilaceae	Modestobacter	BC, CA	Org	Expanded	0.69	23.06	25.3
Actinobacteria	Actinomycetales	Geodermatophilaceae	Geodermatophilus	SBS, CA , TX	Org	Expanded	0.24	1.37	3.0
Actinobacteria	Actinomycetales	Kineosporiaceae	Kineosporia	BC, CA	Org	Declined	0.28	4.27	0.7
Actinobacteria	Actinomycetales	Microbacteriaceae	Subtercola	CA	Org	Expanded	0.33	1.15	11.7
Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	ON, BC, CA	-	Expanded	0.35	11.12	1.7
Actinobacteria	Actinomycetales	Micromonosporaceae	Dactylosporangium	BC, CA, TX	Org	Declined	0.25	2.14	0.7
Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	BC, BS, CA, TX	Org	Variable	1.03	92.86	0.8
Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	BC, ON, TX	-	Expanded	0.34	3.03	3.7
Actinobacteria	Actinomycetales	Nocardioidaceae	Kribbella	BC, TX	-	Variable	0.29	6.42	0.6
Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	BC, CA, TX	Min	Expanded	0.56	13.31	0.7
Actinobacteria	Actinomycetales	Pseudonocardiaceae	Actinomycetospora	CA, TX	Org	Expanded	0.46	17.85	3.2
Actinobacteria	Actinomycetales	Streptomycetaceae	Streptacidiphilus	BC, CA, TX	-	Declined	0.49	18.48	0.3
Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	BC, CA, TX	Org	Declined	0.31	13.90	0.8
Actinobacteria	Rubrobacterales	Rubrobacteraceae	Rubrobacter	CA	Min	Expanded	0.16	0.33	7.3
Actinobacteria	Gaiellales	Gaiellaceae	Gaiellaceae	All	Min	Expanded	0.39	20.48	2.0
Actinobacteria	Solirubrobacterales	unclassified	Solirubrobacter	BC, JP, CA, TX	Org	Declined	0.25	5.97	0.3
AD3	NA	NA	ABS-6	All	Min	Expanded	0.86	26.41	1.6
Armatimonadetes	NA	NA	0319-6E2	ON, BC, CA	Min	Expanded	0.20	1.76	2.2
Bacteroidetes	Cytophagales	Cytophagaceae	Spirosoma	CA	Org	Expanded	0.16	0.90	4.1
Bacteroidetes	Saprospirales	Chitinophagaceae	Flavisolibacter	IDF, ON, CA, TX	-	Expanded	0.25	1.69	2.4
Bacteroidetes	Saprospirales	Chitinophagaceae	Segetibacter	ON, CA	Min	Expanded	0.23	0.90	2.4
Chloroflexi	H39	NA	H39	ON, BC, CA	Min	Expanded	0.24	8.84	2.4
Chloroflexi	SBR1031	oc28	oc28	ON, BC, TX	-	Expanded	0.21	1.86	2.6
Chloroflexi	AKIW781	NA	AKIW781	BC, CA	Org	Expanded	0.21	1.38	6.8
Chloroflexi	NA	NA	Gitt-GS-136	BC, BS, CA	Min	Expanded	0.46	7.70	2.6

Table E.6... *continued*

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Response	Mean Abundance	Max. Abundance	Response ratio
Chloroflexi	B12-WMSP1	NA	B12-WMSP1	SBS, ON, CA	Min	Expanded	0.20	1.87	6.1
Chloroflexi	Thermogemmatisporales	Thermogemmatisporaceae	Thermogemmatisporaceae	SBS, JP, CA, TX	Min	Expanded	0.32	7.88	1.7
Chloroflexi	NA	NA	P2-11E	All	Min	Expanded	0.77	22.54	2.5
Chloroflexi	Ellin6537	NA	Ellin6537	All	-	Expanded	0.17	0.85	4.5
Cyanobacteria	Nostocales	Nostocaceae	Nostoc	BC	Org	Expanded	0.33	5.22	7.6
Firmicutes	Bacillales	Alicyclobacillaceae	Alicyclobacillus	BC, CA, TX	Min	Expanded	0.59	9.84	2.0
Firmicutes	Bacillales	Bacillaceae	Bacillus	BC, TX	Min	Expanded	0.28	5.83	1.4
Firmicutes	Bacillales	Bacillaceae	Geobacillus	BC	Min	Expanded	0.37	3.86	3.9
Firmicutes	Bacillales	Thermoactinomycetaceae	Thermoactinomycetaceae	BC	Min	Expanded	0.33	3.61	5.3
Firmicutes	Clostridiales	Clostridiaceae	Clostridium	BC, JP, CA, TX	Min	Expanded	0.25	3.52	2.8
GAL15	NA	NA	GAL15	ON, TX	Min	Expanded	0.20	2.50	4.5
Proteobacteria	Xanthomonadales	Rhodanobacteraceae	Luteibacter	BC, ON, TX	Org	Declined	0.62	18.97	0.5
Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC, ON	Org	Declined	0.44	5.38	0.4
Proteobacteria	Rhizobiales	Beijerinckiaceae	Methylocella	All	Org	Declined	0.25	4.08	0.7
Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodomicrobium	IDF, JP, CA, TX	Min	Declined	0.97	9.23	0.8
Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	JP, CA, TX	Min	Variable	1.01	86.45	1.0
Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	ON, CA, TX	Org	Expanded	0.38	10.75	6.2
Proteobacteria	Rhizobiales	Rhizobiaceae	Ensifer	IDF, CA, TX	-	Expanded	0.14	0.22	Harv.Only
Proteobacteria	Rhizobiales	Xanthobacteraceae	Ancylobacter	BC, CA, TX	Org	Declined	0.24	1.71	0.2
Proteobacteria	Rhizobiales	Xanthobacteraceae	Labrys	BC, CA, TX	Org	Declined	0.34	5.59	0.7
Proteobacteria	Rhodospirillales	Acetobacteraceae	Acidocella	BC, JP, CA, TX	Org	Variable	0.78	31.58	0.6
Proteobacteria	Rhodospirillales	Acetobacteraceae	Acidiphilium	BC, JP, TX	Org	Variable	0.48	10.42	0.9
Proteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	IDF	Org	Expanded	0.51	1.39	Harv.Only
Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	BC, CA, TX	Org	Declined	0.23	6.41	0.2
Proteobacteria	Burkholderiales	Comamonadaceae	Ramlibacter	BC, BS, CA, TX	Min	Expanded	0.48	8.26	2.7
Proteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	BC, CA	Org	Declined	0.25	0.90	0.0
Proteobacteria	Burkholderiales	Comamonadaceae	Pelomonas	CA, TX	Org	Declined	0.30	3.59	0.4
Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	BC, BS, CA	-	Expanded	0.34	4.00	5.6
Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	BC, CA, TX	-	Expanded	1.07	34.07	2.3
Proteobacteria	Burkholderiales	Oxalobacteraceae	Cupriavidus	JP, TX	Min	Expanded	0.28	4.27	2.5
Proteobacteria	Ellin6067	NA	Ellin6067	ON, BC, TX	Min	Expanded	0.51	13.28	1.7
Proteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	IDF, BS, CA, TX	Min	Expanded	0.34	15.16	2.1

Table E.6... *continued*

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Response	Mean Abundance	Max. Abundance	Response ratio
Proteobacteria	Myxococcales	Cystobacteraceae	Cystobacter	JP, CA, TX	-	Expanded	0.24	1.92	2.5
Proteobacteria	Nevskiales	Sinobacteraceae	Sinobacteraceae	All	-	Declined	1.16	66.63	0.7
Proteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio	TX	Min	Expanded	0.30	1.82	8.8
Proteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	BC, BS, CA, TX	Org	Declined	0.29	3.27	0.2
Proteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter	IDF	Min	Expanded	0.47	0.82	Harv.Only
Verrucomicrobia	Opitutales	Opitutaceae	Opitutus	ON, BC, CA	Org	Declined	0.23	11.56	0.5
Chloroplast	Trebouxiophyceae	Coccomyxa	Coccomyxa	SBS, ON, CA, TX	Org	Expanded	0.24	1.25	7.9
Chloroplast	NA	NA	Chloroplast	All	-	Expanded	0.55	49.81	3.4
Chloroplast	NA	NA	Stramenopiles	BC	Org	Expanded	0.23	1.27	11.2
Proteobacteria	Rickettsiales	mitochondria	mitochondria	All	-	Expanded	0.36	15.83	7.9
Ascomycota	Capnodiales	Incertae sedis	Phaeotheca fissurella	BC, CA	Org	Expansion	0.29	1.71	8.8
Ascomycota	Dothideales	Dothioraceae	Hormonema	CA	Org	Expansion	0.60	52.23	10.5
Ascomycota	Microthyriales	Microthyriaceae	Tothia	BC, CA	Org	Decline	0.91	16.25	0.0
Ascomycota	Pleosporales	NA	Pleosporales sp 2 MU 2012	BC	Org	Expansion	1.80	52.00	13.9
Ascomycota	Pleosporales	Sporormiaceae	Preussia	CA	Org	Expansion	0.54	23.89	0.7
Ascomycota	Pleosporales	Venturiaceae	Venturia	CA | BC	Org	Decline	1.47	210.98	1.1
Ascomycota	Pleosporales	Venturiaceae	Rhizosphaera	CA	Org	Expansion	1.20	31.32	5.0
Ascomycota	Pleosporomycetidae	Gloniaceae	Cenococcum	BC, ON, CA	-	Decline	4.69	331.43	0.3
Ascomycota	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	BC, BS	Org	Variable	0.78	56.67	0.5
Ascomycota	Chaetothyriales	Herpotrichiellaceae	Capronia	BC	Org	Variable	2.18	159.86	0.2
Ascomycota	Chaetothyriales	Herpotrichiellaceae	Rhinocladiella	BC	Org	Decline	0.77	3.19	0.0
Ascomycota	Chaetothyriales	NA	Chaetothyriales sp EXP0559F	BC, CA	Org	Decline	1.95	162.09	0.0
Ascomycota	Eurotiales	Elaphomycetaceae	Elaphomyces	ON, CA, TX	-	Decline	14.44	334.23	0.0
Ascomycota	Eurotiales	Trichocomaceae	Sagenomella	BC, ON	Min	Expansion	3.07	102.85	2.1
Ascomycota	Eurotiales	Trichocomaceae	Penicillium arenicola	BC, ON	Min	Expansion	1.92	44.97	1.4
Ascomycota	Eurotiales	Trichocomaceae	Talaromyces	BC, ON	Org	Expansion	2.02	60.00	1.4
Ascomycota	Lecanorales	Cladoniaceae	Cladoniaceae	ON, BC	Org	Expansion	0.81	26.42	65.3
Ascomycota	Lecanorales	NA	Lecanorales	BC, ON, CA	-	Expansion	0.74	26.42	37.5
Ascomycota	Helotiales	Dermateaceae	Cryptosporiopsis	BC	-	Expansion	0.48	19.14	2.4
Ascomycota	Helotiales	Dermateaceae	Mollisia	CA	Org	Expansion	0.78	20.81	14.8
Ascomycota	Helotiales	Helotiaceae	Crocicreas	BC,ON, CA	Org	Decline	1.10	150.27	0.6
Ascomycota	Helotiales	Helotiaceae	Rhizoscyphus ericae	BC, ON	-	Expansion	2.56	263.51	5.3

Table E.6... *continued*

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Response	Mean Abundance	Max. Abundance	Response ratio
Ascomycota	Helotiales	Hyaloscyphaceae	Hyaloscyphaceae sp I GK 2010	BC, ON	Org	Decline	0.78	6.76	0.0
Ascomycota	Helotiales	Hyaloscyphaceae	Mycoarthris	SBS, CA	Org	Decline	0.94	21.91	0.1
Ascomycota	Helotiales	Incertae sedis	Cadophora finlandica	BC, ON, CA	Min	Expansion	3.05	322.32	2.7
Ascomycota	Helotiales	Incertae sedis	Rhexocercosporidium	BC, BS, CA	Min	Expansion	1.03	50.12	4.5
Ascomycota	Helotiales	Incertae sedis	Tetracladium	BC, ON	Org	Expansion	0.52	14.22	3.5
Ascomycota	Incertae sedis	Incertae sedis	Leohumicola incrustata	BC, ON,	Min	Expansion	4.14	73.63	7.8
Ascomycota	Leotiales	Leotiaceae	Leotiaceae	SBS, BS	-	Expansion	6.20	190.66	159.7
Ascomycota	NA	NA	Ascomycota sp F45	BC, ON, CA	-	Decline	1.32	20.23	0.0
Ascomycota	Rhytismatales	Rhytismataceae	Lophodermium molitoris	ON	-	Expansion	0.49	4.88	15.7
Ascomycota	Thelebolales	Thelebolaceae	Thelebolus	IDF	Org	Decline	2.38	432.70	0.1
Ascomycota	NA	NA	fungal sp TRN36	BC, ON, CA	-	Expansion	0.80	16.15	49.4
Ascomycota	NA	NA	ascomycete sp olrim916	BC, BS	Min	Expansion	1.14	59.16	13.0
Ascomycota	NA	NA	Ascomycete CR 2004	BC, CA	Org	Decline	0.49	4.90	0.0
Ascomycota	NA	NA	Ascomycota sp X33	BC	-	Expansion	1.93	118.72	11.8
Ascomycota	NA	NA	ascomycete sp CH Co24	ON	Org	Expansion	0.60	121.94	10.3
Ascomycota	NA	NA	Ascomycota sp PIMO 418	CA	Org	Expansion	1.14	74.68	46.9
Ascomycota	Pezizales	Pyronemataceae	Otidea	BC, CA	-	Decline	8.16	85.16	0.0
Ascomycota	Hypocreales	Bionectriaceae	Clonostachys rosea catenulata	TX	Min	Decline	1.89	500.00	0.0
Ascomycota	Hypocreales	Cordycipitaceae	Lecanicillium	All	Org	Decline	0.82	14.22	0.2
Ascomycota	Hypocreales	Cordycipitaceae	Lecanicillium sp. BESC 246b	ON, BC	-	Expansion	0.35	3.15	24.7
Ascomycota	Hypocreales	Hypocreaceae	Hypocrea	SBS, ON, CA, TX	Org	Decline	1.13	37.29	0.4
Ascomycota	Hypocreales	Nectriaceae	Gibberella	BC, CA, TX	Org	Expansion	0.74	21.22	16.7
Ascomycota	Hypocreales	Nectriaceae	Cylindrocarpon sp JAT1366	BC, BS	-	Expansion	0.84	32.31	4.8
Ascomycota	Sordariales	Chaetomiaceae	Trichocladium opacum	BC, ON	Min	Expansion	0.66	14.59	6.0
Basidiomycota	Agaricales	Amanitaceae	Amanitaceae	TX	-	Decline	10.07	290.76	0.4
Basidiomycota	Agaricales	Cortinariaceae	Cortinarius	BC, ON, CA	-	Decline	5.27	427.35	0.3
Basidiomycota	Agaricales	Hydnangiaceae	Laccaria	ON, CA	-	Expansion	5.13	185.14	1.6
Basidiomycota	Agaricales	Hygrophoraceae	Hygrophorus	BC, ON, CA	-	Decline	17.59	316.98	0.1
Basidiomycota	Agaricales	Inocybaceae	Inocybe impexa	SBS, ON	Min	Expansion	5.34	183.45	24.3
Basidiomycota	Agaricales	Lyophyllaceae	Lyophyllum	BC	Org	Decline	1.27	36.95	0.0
Basidiomycota	Agaricales	Mycenaceae	Mycena corynephora	TX	-	Expansion	2.14	62.74	7.5
Basidiomycota	Agaricales	Tricholomataceae	Tricholoma triste	IDF	-	Decline	1.06	12.71	0.0

Table E.6... *continued*

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Response	Mean Abundance	Max. Abundance	Response ratio
Basidiomycota	Atheliales	Atheliaceae	Piloderma	All	-	Decline	18.70	907.86	0.2
Basidiomycota	Boletales	Gomphidiaceae	Gomphidiaceae	BC, CA	Min	Expansion	0.98	6.85	38.5
Basidiomycota	Boletales	Gomphidiaceae	Chroogomphus	BC	-	Expansion	0.72	6.85	45.7
Basidiomycota	Boletales	Rhizopogonaceae	Rhizopogon fuscorubens	TX	-	Decline	0.83	25.78	0.5
Basidiomycota	Boletales	Suillaceae	Suillus pseudobrevipes	BC, ON	Min	Expansion	60.18	1000.00	111.0
Basidiomycota	Boletales	Suillaceae	Suillus tomentosus	BC	-	Expansion	4.62	403.77	36.2
Basidiomycota	Gomphales	Gomphaceae	Ramaria	IDF, TX	-	Decline	1.73	14.94	0.0
Basidiomycota	Russulales	Russulaceae	Russula	BC, ON, CA	Min	Decline	17.15	514.38	0.4
Basidiomycota	Sebacinales	Sebacinaceae	Sebacina	BC	Org	Decline	1.65	78.74	0.5
Basidiomycota	Thelephorales	Thelephoraceae	Pseudotomentella	BC, ON	-	Decline	4.24	238.57	0.0
Basidiomycota	Trechisporales	NA	uncultured Trechisporales	BC, BS, TX	-	Expansion	3.56	116.62	3.5
Basidiomycota	NA	NA	Agaricomycotina sp	BC, ON	-	Expansion	4.34	84.19	106.4
Basidiomycota	Filobasidiales	Filobasidiaceae	Cryptococcus	SBS, ON	-	Expansion	2.48	99.67	1.5
Chytridiomycota	Monoblepharidales	Oedogoniomycetaceae	Oedogoniomyces	CA	-	Expansion	0.46	3.22	17.3
Chytridiomycota	NA	NA	Monoblepharidomycetes	CA, ON	Min	Expansion	0.65	4.60	4.3
Uncl. Fungi	NA	NA	fungal sp aurim625	ON, BC	Min	Expansion	1.62	67.77	6.7
Zygomycota	Mortierellales	Mortierellaceae	Mortierella sp WD35C	BC, ON	Min	Expansion	0.75	8.85	2.5
Zygomycota	Mucorales	Umbelopsidaceae	Umbelopsis vinacea	BC, BS, CA	Org	Decline	0.39	2.51	0.0

Table E.7. Full list of bacterial and fungal taxa showing population expansion in intermediate intensities of OM-removal.

Phylum	Order	Family	Taxa	Ecozones	Soil Layer	Max Abundance OM1|OM2	Mean Abundance	Max Abundance REF|OM3	Response ratio
Proteobacteria	Rhizobiales	Phyllobacteriaceae	Phyllobacterium	IDF	None	20.96	0.52	8.50	2.4
Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	BC	Org	10.75	0.38	9.90	1.1
Proteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	IDF	Org	2.54	0.33	0.70	4.7
Basidiomycota	Agaricales	Clavariaceae	Ramariopsis	BC	None	16.32	5.25	67.99	2.0
Basidiomycota	Agaricales	Entolomataceae	Clitopilus sp. 1_T_778	TX	Org	9.22	0.72	0.53	8.7
Basidiomycota	Agaricales	Hygrophoraceae	Hygrocybe sp	SBS, JP	None	145.08	4.30	16.88	13.3
Basidiomycota	Agaricales	NA	Agaricales sp.	IDF	Org	53.88	1.50	10.88	3.8
Basidiomycota	Atheliales	Atheliaceae	Piloderma sphaerosporum	SBS, BS	Org	907.86	23.70	344.53	3.4
Basidiomycota	Atheliales	Atheliaceae	Piloderma olivaceum	SBS, JP	Org	614.16	21.51	363.71	3.1
Basidiomycota	Boletales	Gomphidiaceae	Chroogomphus vinicolor	SBS	None	2.32	0.36	1.24	5.1
Basidiomycota	Boletales	Suillaceae	Suillus cothurnatus	JP	None	19.67	0.98	10.66	1.8
Basidiomycota	Hymenochaetales	Schizoporaceae	Hyphodontia aspera	BC	None	547.24	8.52	35.76	20.7
Basidiomycota	Thelephorales	Thelephoraceae	Thelephora sp. ECM1	BC, ON, CA	Org	212.67	2.46	22.03	11.7
Ascomycota	Hypocreales	Incertae sedis	Myrothecium	TX	None	13.96	0.96	1.88	3.8
Ascomycota	Microascales	Microascaceae	Doratomyces stemonitis	BC	None	35.20	1.11	0.20	81.2
Ascomycota	Sordariales	Lasiosphaeriaceae	Podospora	BC, TX	Org	53.91	2.01	10.47	3.8
Ascomycota	Xylariales	Amphisphaeriaceae	Discosia sp.1_KT_2010	TX	None	5.01	0.21	1.65	4.6
Ascomycota	Pezizales	Tuberaceae	Tuber sp. 17_KA_2010	CA	Min	106.17	20.44	0.00	OM 1|OM 2 only
Ascomycota	Geoglossales	Geoglossaceae	Geoglossum	BC	None	117.42	4.47	25.92	4.1
Ascomycota	Helotiales	Incertae sedis	Tetracladium	IDF	Org	14.22	0.52	5.68	3.3
Ascomycota	Rhytismatales	Rhytismataceae	Colpoma sp. PDD_91607	ON	Org	6.72	1.91	8.83	1.1
Ascomycota	Eurotiales	Trichocomaceae	Penicillium adametzii	JP	Org	123.47	2.09	72.22	1.5
Ascomycota	Verrucariales	NA	Verrucariales	BC, ON	None	234.90	1.92	58.45	1.7
Ascomycota	Capnodiales	Incertae sedis	Elasticomyces elasticus	BC	Org	10.44	0.92	3.35	5.1
Ascomycota	Capnodiales	Mycosphaerellaceae	Mycosphaerella sp. AM_2006c	BC	Org	38.64	0.82	2.98	8.0
Unclassified	NA	NA	Fungal sp. AB22	IDF, CA	Org	8.72	0.52	2.51	5.0
Unclassified	NA	NA	Fungal sp. OTU_084	TX	None	0.39	0.12	0.13	6.8

Table E.8. Overview of site characteristics for environmental and soil parameters and microbial activity. Values denoted by letters are statistically significant ($p < 0.05$) by lettering group based on Tukey's Honest Significant Difference.

		Blodgett	Brandy City	Lowell Hill
Site Data	Year Established	1994	1995	1995
	Lat / Long	38.88N / 120.64W	39.55N / 121.04W	39.26N / 120.78W
	Elevation	1350 m	1135 m	1268 m
	Soil type	Mesic Ultic Haploxeralfs (Loamy)		
Soil composition (n=9)	Average Percent Carbon	$8^{A} \pm 0.36$	$7^{B} \pm 0.15$	$4.7^{C} \pm 0.14$
	Average Percent Nitrogen	$0.37^{A} \pm 0.019$	$0.32^{B} \pm 0.007$	$0.2^{C} \pm 0.004$
	Average C:N Ratio	22.1 ± 0.45	22.2 ± 0.42	23.5 ± 0.77
	Average pH	5.69 ± 0.04	5.77 ± 0.08	5.81 ± 0.09
Respiration (n=36)	Average mg CO2 per g soil	$1.13^{A} \pm 0.05$	1.02 ± 0.04	$0.97^{B} \pm 0.05$
PLFA Biomass (n = 12)	Average delta-^{13}C	$1400^{A} \pm 100$	$3300^{B} \pm 400$	$2500^{B} \pm 300$
	Total ^{13}C Biomass (umols C per g soil)	0.33^{A}	0.63^{B}	0.53
	Total ^{12}C Biomass (umols C per g soil)	46.5^{A}	25.8^{B}	21.3^{B}

Technical error (S.D.) for delta-^{13}C is 80, which is equivalent to an average of ± 0.0003 umols C

Table E.9. Overview of soil properties and cellulolytic activity (i.e. ^{13}C-enrichment of PLFA or DNA) among soil layers and harvesting treatments. All values correspond to the mean ± standard error. Within groups of treatments, significantly different values ($p < 0.05$) have been denoted by lettering based on Tukey's Honest Significant Difference.

		Organic Layer				**Mineral Layer**			
		REF	OM1	OM2	OM3	REF	OM1	OM2	OM3
Soil composition (n=9)	Average Percent Carbon	37.0 ± 2.2	41.5^{A} ± 1.0	33.0 ± 2.4	31.5^{B} ± 3.6	5.9 ± 0.5	7.6 ± 0.7	-	6.4 ± 0.8
	Average Percent Nitrogen	1.21 ± 0.09	1.34 ± 0.11	1.21 ± 0.13	1.10 ± 0.13	0.26 ± 0.02	0.31 ± 0.04	-	0.32 ± 0.04
	Average C:N Ratio	31.1 ± 1.5	32.5 ± 3.3	28.2 ± 1.7	29.5 ± 2.2	22.3 ± 0.9	25.4^{A} ± 1.3	-	20.0^{B} ± .03
	Average pH	5.47 ± 0.2	4.35^{A} ± 0.2	5.1 ± 0.2	5.2^{B} ± 0.1	6.1 ± 0.2	5.5 ± 0.1	-	5.6 ± 0.1
Repiration (n=36)	Average mg CO_2 per g soil	-	-	-	-	1.20^{A} ± 0.04	1.01^{B} ± 0.04	-	0.90^{C} ± 0.05
PLFA Biomass Measures (n= 9)	Average Delta ^{13}C	1,600 ± 110	1,100 ± 130	2,300 ± 170	1,400 ± 70	$6,400^{A}$ ± 870	4,800 ± 650	4,800 ± 700	$3,200^{B}$ ± 540
	Total ^{13}C carbon (umols ^{13}C per g soil)	0.96^{A}	0.45^{B}	0.62	0.68	0.42^{A}	0.43^{A}	0.41^{A}	0.24^{B}
	Total ^{12}C carbon (umols ^{13}C per g soil)	32.2^{A}	19.0^{B}	16.6^{B}	25.8^{A}	5.0^{A}	6.6^{B}	6.2	5.0
	Median number of enriched FA	33^{A} ± 0.9	27^{C} ± 1.2	27^{B} ± 0.7	25^{C} ± 0.5	29 ± 1.2	29 ± 0.7	29 ± 0.6	27 ± 0.7
	Fungal:Bacteria Ratio (^{13}C)	0.78^{A} ± 0.07	0.95^{A} ± 0.05	1.68^{B} ± 0.21	1.95^{B} ± 0.48	0.64 ± 0.13	0.71 ± 0.15	0.92 ± 0.13	1.03 ± 0.23
DNA enrichment (n=3)	Atom % ^{13}C above natural abundance	4.8 ± 1.0	4.5 ± 1.5	5.6 ± 1.1	3.3 ± 0.5	14.6^{A} ± 2.2	9.9 ± 1.1	10.3 ± 0.6	9.1^{B} ± 1.1

Technical error (S.D.) for Delta-values is 80, which is equivalent to an average of ± 0.0003 umols C

Table E.10. Complete list of OTUs designated putatively cellulolytic based on differential abundance between ^{13}C- and ^{12}C-pyrotag libraries.

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01BZTGT	FBP	-	KM391432	Min	380.4	86.46
IIKFCBR01BFFL6	FBP	-	KM391438	Min	138.8	63.08
HN7PONR02H7VJP	FBP	-	KM391441	None	95.8	52.25
IIKFCBR01AO3YU	FBP	-	KM391442	Min	86.0	50.83
HIGEX0001DHO1H	FBP	-	KM391496	Min	104.0	9.46
IIKFCBR01DEH19	FBP	-	KM391570	Min	13C-only	2.71
IIKFCBR01D1LXB	FBP	-	KM391612	Min	13C-only	1.5
IIKFCBR01D3UQS	FBP	-	KM391623	None	13C-only	1.21
IIKFCBR01EFACT	FBP	-	KM391624	Min	13C-only	1.21
IIKFCBR01CSGER	FBP	-	KM391628	Org	24.8	1.12
IIKFCBR01A1GBU	Chloroflexi	c__Anaerolineae	KM391569	Min	9.2	2.5
HN7PONR02GQXBE	Chloroflexi	c__Anaerolineae	KM391607	Min	33.0	1.5
IIKFCBR01BMT7K	TM7	c__SC3	NA	Min	9.7	8.33
IIKFCBR01B8I79	TM7	c__TM7-3	KM391459	Min	16.8	23.67
IIKFCBR01CC1CG	TM7	c__TM7-3	KM391535	Min	5.4	3.92
IIKFCBR01AVU5M	TM7	c__TM7-3	KM391552	Min	4.0	2.92
IIKFCBR01A3FLV	TM7	c__TM7-3	KM391555	Min	76.1	3.46
HIGEX0001AOCX6	TM7	c__TM7-3	KM391631	Min	23.8	1.08
IIKFCBR01EEO74	Acidobacteria	f__Acidobacteriaceae	KM391499	Org	8.3	7.88
IIKFCBR01DRU3P	Betaproteobacteria	f__Burkholderiaceae	KM391469	Org	8.6	16.33
IIKFCBR01CIYUN	Alphaproteobacteria	f__Caulobacteraceae	KM391466	Min	3.8	16.67
IIKFCBR01DYD22	Alphaproteobacteria	f__Caulobacteraceae	KM391477	Min	11.1	13.67
IIKFCBR01CIUIN	Verrucomicrobia	f__Chthoniobacteraceae	KM391501	Min	13C-only	8.08
IIKFCBR01ERXIS	Betaproteobacteria	f__Comamonadaceae	KM391426	Org	6.0	463
IIKFCBR01DS94Y	Betaproteobacteria	f__Comamonadaceae	KM391457	Org	8.9	24.12
IIKFCBR01C43J2	Betaproteobacteria	f__Comamonadaceae	KM391482	Min	32.0	13.08
IIKFCBR01A7C7S	Betaproteobacteria	f__Comamonadaceae	KM391506	Org	81.6	7.42
IIKFCBR01A1YGP	Betaproteobacteria	f__Comamonadaceae	KM391536	None	9.6	3.92
IIKFCBR01ANKGB	Betaproteobacteria	f__Comamonadaceae	KM391609	None	16.0	1.46
IIKFCBR01C5N8I	Betaproteobacteria	f__Comamonadaceae	KM391633	Org	11.5	1.04
IIKFCBR01EGBTI	Betaproteobacteria	f__Comamonadaceae	KM391634	None	11.5	1.04
IIKFCBR01BU6ZQ	Actinobacteria	f__Microbacteriaceae	KM391455	Org	4.9	23.17
IIKFCBR01C2U0N	Actinobacteria	f__Microbacteriaceae	KM391529	Org	9.3	4.62
IIKFCBR01B6QL0	Actinobacteria	f__Microbacteriaceae	KM391557	Org	37.6	3.42
IIKFCBR01APJE8	Actinobacteria	f__Microbacteriaceae	KM391584	Org	8.3	1.88
IIKFCBR01BYZFY	Actinobacteria	f__Micrococcaceae	KM391440	Min	7.4	49

Table E.10... *continued*

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01BCOEN	Actinobacteria	f__Micrococcaceae	KM391458	Min	9.4	24.04
IIKFCBR01DDYRU	Actinobacteria	f__Micrococcaceae	KM391474	Min	8.7	15
IIKFCBR01BJ93C	Actinobacteria	f__Micrococcaceae	KM391475	Min	11.7	14.88
HN7PONR01BR35E	Actinobacteria	f__Micrococcaceae	KM391480	Min	5.3	12.12
IIKFCBR01BDZM0	Actinobacteria	f__Micrococcaceae	KM391487	Min	6.9	10.92
IIKFCBR01BE44L	Actinobacteria	f__Micrococcaceae	KM391493	Min	6.1	8.62
IIKFCBR01C7YA6	Actinobacteria	f__Micrococcaceae	KM391494	Min	6.7	8.5
IIKFCBR01A57Z2	Actinobacteria	f__Micrococcaceae	KM391516	Min	11.9	5.96
IIKFCBR01B332I	Actinobacteria	f__Micrococcaceae	KM391527	Min	16.0	5.08
IIKFCBR01BLXQH	Actinobacteria	f__Micrococcaceae	KM391575	Min	5.7	2.08
IIKFCBR01B0YQ8	Actinobacteria	f__Micrococcaceae	KM391576	Min	16.2	2.21
IIKFCBR01AWDW0	Actinobacteria	f__Micrococcaceae	KM391579	Min	47.7	2.17
IIKFCBR01BFXK4	Actinobacteria	f__Micrococcaceae	KM391586	None	9.9	1.79
IIKFCBR01AEE5B	Actinobacteria	f__Micrococcaceae	KM391608	Min	10.4	1.42
IIKFCBR01BKECV	Actinobacteria	f__Micrococcaceae	KM391616	None	13C-only	1.42
IIKFCBR01CUHF7	Verrucomicrobia	f__Opitutaceae	KM391546	Min	13C-only	3.92
IIKFCBR01DZQMM	Betaproteobacteria	f__Oxalobacteraceae	KM391434	None	3.2	62.17
IAJCH1401CBT2J	Betaproteobacteria	f__Oxalobacteraceae	KM391436	Min	25.1	66.12
IIKFCBR01BAN8Z	Betaproteobacteria	f__Oxalobacteraceae	KM391449	Min	8.2	28.29
IIKFCBR01A76N2	Betaproteobacteria	f__Oxalobacteraceae	KM391451	Min	31.6	28.75
HNWQNZC02JYSX7	Betaproteobacteria	f__Oxalobacteraceae	KM391452	Min	7.6	26.04
IIKFCBR01BCCJU	Betaproteobacteria	f__Oxalobacteraceae	KM391484	Min	7.8	11.67
IIKFCBR01C2P23	Betaproteobacteria	f__Oxalobacteraceae	KM391497	Min	27.0	8.58
IIKFCBR01B6UNX	Betaproteobacteria	f__Oxalobacteraceae	KM391531	Min	8.7	4.33
IIKFCBR01CXGLN	Betaproteobacteria	f__Oxalobacteraceae	KM391532	Min	4.6	4
IIKFCBR01A2C6E	Betaproteobacteria	f__Oxalobacteraceae	KM391540	None	21.8	3.96
IIKFCBR01CHYHE	Betaproteobacteria	f__Oxalobacteraceae	KM391542	Min	43.5	3.96
IIKFCBR01EYBXG	Betaproteobacteria	f__Oxalobacteraceae	KM391549	None	9.1	3.29
IIKFCBR01B4BRK	Betaproteobacteria	f__Oxalobacteraceae	KM391581	Min	6.7	1.83
IIKFCBR01CHZ6D	Betaproteobacteria	f__Oxalobacteraceae	KM391615	None	31.2	1.42
IIKFCBR01B2HLT	Betaproteobacteria	f__Oxalobacteraceae	KM391625	None	13C-only	1.21
IIKFCBR01CPZBH	Betaproteobacteria	f__Oxalobacteraceae	KM391644	None	11.0	0.5
IIKFCBR01BXH5Y	Deltaproteobacteria	f__Polyangiaceae	KM391587	None	13.1	1.79
IIKFCBR01DEHMO	Deltaproteobacteria	f__Polyangiaceae	KM391594	Min	13C-only	1.75
IIKFCBR01DMDVI	Deltaproteobacteria	f__Polyangiaceae	KM391617	None	14.2	1.29
IIKFCBR01BYX3V	Alphaproteobacteria	f__Rhizobiaceae	KM391503	Min	9.2	7.12
IAJCH1401C9UV8	Alphaproteobacteria	f__Rhizobiaceae	KM391533	Org	4.5	3.71
IAJCH1401A7YMK	Alphaproteobacteria	f__Sphingomonadaceae	KM391488	None	6.7	10.29
IIKFCBR01C0549	Alphaproteobacteria	f__Sphingomonadaceae	KM391518	Org	20.8	5.67
IAJCH1401AEXIJ	Actinobacteria	f__Streptomycetaceae	KM391421	Min	10.9	1048.58
IIKFCBR01BYUBL	Actinobacteria	f__Streptomycetaceae	KM391422	Org	11.9	1039.96
IAJCH1401BBVVT	Actinobacteria	f__Streptomycetaceae	KM391425	Org	52.8	628.71
IIKFCBR01D5T95	Actinobacteria	f__Streptomycetaceae	KM391430	Org	15.9	101.46
IIKFCBR01AZO3D	Actinobacteria	f__Streptomycetaceae	KM391435	Org	20.1	75.75
IIKFCBR01ECL7T	Actinobacteria	f__Streptomycetaceae	KM391489	Org	4.8	9.58

Table E.10... *continued*

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01CGLNH	Actinobacteria	f__Streptomycetaceae	KM391490	Org	11.8	10.17
IAJCH1401BOAMR	Actinobacteria	f__Streptomycetaceae	KM391512	Org	5.0	5.62
IIKFCBR01CLDHG	Actinobacteria	f__Streptomycetaceae	KM391539	Org	90.8	4.12
IIKFCBR01AXDQT	Actinobacteria	f__Streptomycetaceae	KM391547	Org	41.7	3.79
IIKFCBR01E0MN1	Actinobacteria	f__Streptomycetaceae	KM391548	None	26.3	3.58
IIKFCBR01DMD0B	Actinobacteria	f__Streptomycetaceae	KM391554	None	76.1	3.46
IIKFCBR01B5BIG	Actinobacteria	f__Streptomycetaceae	KM391556	Org	12.1	3.29
IIKFCBR01DXW8W	Actinobacteria	f__Streptomycetaceae	KM391558	Org	6.7	3.04
IIKFCBR01B7AGX	Actinobacteria	f__Streptomycetaceae	KM391559	Org	74.3	3.38
IIKFCBR01CGADV	Actinobacteria	f__Streptomycetaceae	KM391566	Org	14.9	2.71
IIKFCBR01EPBWF	Actinobacteria	f__Streptomycetaceae	KM391567	Org	62.3	2.83
IIKFCBR01BOVHS	Actinobacteria	f__Streptomycetaceae	KM391573	Org	28.4	2.58
IIKFCBR01AU6O6	Actinobacteria	f__Streptomycetaceae	KM391574	Org	56.8	2.58
IIKFCBR01D7QZF	Actinobacteria	f__Streptomycetaceae	KM391597	Org	11.6	1.58
IAJCH1401BKC7U	Actinobacteria	f__Streptomycetaceae	KM391627	Org	24.8	1.12
IIKFCBR01AID29	Actinobacteria	f__Streptomycetaceae	KM391637	Org	13C-only	0.96
IIKFCBR01DVIF8	Actinobacteria	f__Streptomycetaceae	KM391638	Org	13C-only	0.79
IIKFCBR01CPO5D	Verrucomicrobia	f__Verrucomicrobiaceae	KM391614	Min	31.2	1.42
IIKFCBR01EWOZA	Alphaproteobacteria	g__Asticcacaulis	KM391431	Org	9.3	92.92
IIKFCBR01DL1OD	Alphaproteobacteria	g__Asticcacaulis	NA	Min	249.7	56.75
IIKFCBR01CMBQF	Alphaproteobacteria	g__Asticcacaulis	KM391445	Min	6.2	33.38
HS30DLI03GTYPT	Alphaproteobacteria	g__Asticcacaulis	KM391453	Org	11.2	26.92
HTIQ2CV02D5F0W	Alphaproteobacteria	g__Asticcacaulis	KM391486	Org	17.5	11.96
HTIQ2CV02EMOB8	Alphaproteobacteria	g__Asticcacaulis	KM391491	Min	3.6	8.54
IIKFCBR01B3UOM	Alphaproteobacteria	g__Asticcacaulis	KM391504	None	2.5	5.71
IIKFCBR01C57G0	Alphaproteobacteria	g__Asticcacaulis	KM391509	Org	10.0	6.38
IAJCH1401CIS09	Alphaproteobacteria	g__Asticcacaulis	KM391515	Org	2.8	4.88
IAJCH1401AWMYF	Alphaproteobacteria	g__Asticcacaulis	KM391596	Org	8.0	1.46
IIKFCBR01DHW5N	Alphaproteobacteria	g__Asticcacaulis	KM391604	Min	13C-only	1.58
IIKFCBR01DXYAY	Alphaproteobacteria	g__Bosea	KM391478	Min	9.7	13.25
IIKFCBR01ETHS5	Alphaproteobacteria	g__Bradyrhizobium	KM391485	None	2.9	9.71
HN5XJPU01A522S	Betaproteobacteria	g__Burkholderia	KM391468	Org	8.1	16.5
IAJCH1401C0K4M	Betaproteobacteria	g__Burkholderia	KM391476	None	6.0	13.46
IIKFCBR01C5DL9	Betaproteobacteria	g__Burkholderia	KM391498	Org	9.3	8.04
IIKFCBR01AX8JN	Betaproteobacteria	g__Burkholderia	KM391578	Org	22.9	2.08
IIKFCBR01DE6JJ	Alphaproteobacteria	g__Caulobacter	KM391429	None	10.6	101.71
IIKFCBR01DW3ZT	Alphaproteobacteria	g__Caulobacter	KM391463	None	10.7	20.88
IIKFCBR01D0O4W	Alphaproteobacteria	g__Caulobacter	KM391583	Org	8.3	1.88
IIKFCBR01DE6JJ	Alphaproteobacteria	g__Caulobacter	KM391429	None	13C-only	0.62
IIKFCBR01DWA8V	Verrucomicrobia	g__Chthoniobacter	KM391602	None	11.0	1.5
HNWQNZC01E81FU	Actinobacteria	g__Cryocola	KM391444	Min	3.0	33.83
IIKFCBR01CKQ7J	Actinobacteria	g__Cryocola	KM391461	None	5.0	20.04
IIKFCBR01DE9YX	Actinobacteria	g__Cryocola	KM391481	None	4.6	11.58
IIKFCBR01AYG8N	Actinobacteria	g__Cryocola	KM391483	None	13.1	12.54
IIKFCBR01ATR7O	Actinobacteria	g__Cryocola	KM391528	None	6.4	4.67

Table E.10... *continued*

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01BOI04	Actinobacteria	g__Cryocola	KM391551	None	3.0	2.75
IIKFCBR01AGJ26	Actinobacteria	g__Cryocola	KM391565	Min	5.5	2.5
IIKFCBR01D19UA	Bacteroidetes	g__Cytophaga	KM391534	Org	6.3	4
IIKFCBR01DRRUD	Alphaproteobacteria	g__Devosia	KM391525	Min	5.5	4.71
IIKFCBR01EWZMX	Betaproteobacteria	g__Janthinobacterium	KM391423	Min	5.7	853.88
IIKFCBR01CGTHF	Betaproteobacteria	g__Janthinobacterium	KM391427	Min	9.1	290.62
IAJCH1401AD6I5	Betaproteobacteria	g__Janthinobacterium	KM391428	Min	5.2	122.12
IIKFCBR01AZ0B7	Betaproteobacteria	g__Janthinobacterium	KM391433	None	6.1	72.54
IIKFCBR01EOLME	Betaproteobacteria	g__Janthinobacterium	KM391446	None	4.9	29.79
IIKFCBR01B7Z4O	Betaproteobacteria	g__Janthinobacterium	KM391454	Org	15.2	26.92
IIKFCBR01DKN65	Betaproteobacteria	g__Janthinobacterium	KM391471	None	7.6	15.83
IAJCH1401B0CT5	Betaproteobacteria	g__Janthinobacterium	KM391502	Min	2.7	5.88
HNWQNZC01AW5S8	Betaproteobacteria	g__Janthinobacterium	KM391537	Min	2.7	3.17
IIKFCBR01ATT4K	Betaproteobacteria	g__Janthinobacterium	KM391560	None	17.4	3.17
IIKFCBR01CSF10	Betaproteobacteria	g__Janthinobacterium	KM391563	Org	22.0	3
IIKFCBR01B7NTC	Betaproteobacteria	g__Janthinobacterium	KM391580	Org	10.8	1.96
IIKFCBR01DPHE6	Betaproteobacteria	g__Janthinobacterium	KM391595	Min	11.6	1.58
IIKFCBR01EL7PJ	Betaproteobacteria	g__Janthinobacterium	KM391598	Min	6.4	1.46
HNWQNZC01DF0MN	Betaproteobacteria	g__Janthinobacterium	KM391622	None	8.3	1.12
IIKFCBR01BHM2D	Actinobacteria	g__Kitasatospora	KM391437	None	10.8	62.71
IIKFCBR01A92N7	Actinobacteria	g__Kitasatospora	KM391450	None	35.2	30.38
IIKFCBR01CPH7B	Actinobacteria	g__Kitasatospora	KM391524	Org	29.3	5.33
IIKFCBR01BNMKB	Betaproteobacteria	g__Leptothrix	KM391424	None	8.8	644.5
IIKFCBR01CBLMK	Betaproteobacteria	g__Leptothrix	KM391460	None	17.8	22.62
IIKFCBR01DDITR	Betaproteobacteria	g__Leptothrix	KM391513	None	4.3	5.46
IIKFCBR01DBPL1	Betaproteobacteria	g__Leptothrix	KM391635	Org	21.1	0.96
IIKFCBR01D9FYH	Betaproteobacteria	g__Leptothrix	KM391640	None	13C-only	0.71
IIKFCBR01CTV7M	Alphaproteobacteria	g__Mesorhizobium	KM391517	Min	4.0	4.96
IIKFCBR01EIWNB	Alphaproteobacteria	g__Mesorhizobium	KM391541	None	3.2	3.17
IIKFCBR01BMHDJ	Gammaproteobacteria	g__Nevskia	KM391467	Org	6.4	17.71
IIKFCBR01D4HTE	Verrucomicrobia	g__Opitutus	KM391654	Min	115.7	21.04
IIKFCBR01BJI11	Betaproteobacteria	g__Paucibacter	KM391448	None	4.7	27.08
IAJCH1401CKQZM	Betaproteobacteria	g__Paucibacter	KM391456	None	10.6	24.5
IIKFCBR01BZE2Q	Betaproteobacteria	g__Pelomonas	KM391465	None	10.6	19.83
IIKFCBR01EBDC6	Betaproteobacteria	g__Pelomonas	KM391530	Min	105.4	4.79
IIKFCBR01AQLQI	Betaproteobacteria	g__Pelomonas	KM391639	None	13C-only	0.75
IIKFCBR01BJ8KD	Alphaproteobacteria	g__Phenylobacterium	KM391462	Min	5.8	19.83
IIKFCBR01DVJID	Alphaproteobacteria	g__Phenylobacterium	KM391585	Min	6.3	1.71
HVPQ45002GZ4LA	Actinobacteria	g__Phycicoccus	KM391505	Min	5.9	6.75
IIKFCBR01A5WQO	Betaproteobacteria	g__Polaromonas	KM391521	Min	5.6	4.88
HPPSXLA04JDLB2	Betaproteobacteria	g__Ralstonia	KM391470	Min	129.9	17.71
IIKFCBR01ENIXV	Betaproteobacteria	g__Rubrivivax	KM391508	Min	4.3	5.83
IIKFCBR01CS2O2	Actinobacteria	g__Salinibacterium	KM391514	Org	5.7	5.71
IIKFCBR01CF4HA	Alphaproteobacteria	g__Sphingomonas	KM391443	Min	3.8	41.04
IAJCH1401ANLMS	Alphaproteobacteria	g__Sphingomonas	KM391619	Org	14.7	1.33

Table E.10... *continued*

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01AP3GS	Alphaproteobacteria	g__Telmatospirillum	KM391561	Org	9.3	2.96
HNWQNZC01DTU7D	Betaproteobacteria	g__Variovorax	KM391492	Org	3.3	8.08
IIKFCBR01AK29F	Actinobacteria	o__Actinomycetales	KM391519	Min	17.7	5.62
HN7PONR02G9M87	Actinobacteria	o__Actinomycetales	KM391523	Min	11.4	5.17
IIKFCBR01DDQMW	Actinobacteria	o__Actinomycetales	KM391577	None	4.7	1.92
IIKFCBR01BDV50	Actinobacteria	o__Actinomycetales	KM391591	None	4.0	1.46
IIKFCBR01DKQLI	Betaproteobacteria	o__Burkholderiales	NA	Min	19.7	5.38
IIKFCBR01EGDAL	Betaproteobacteria	o__Burkholderiales	KM391522	None	8.5	5.04
IIKFCBR01DEFOY	Bacteroidetes	o__Cytophagales	KM391439	Min	142.1	58.12
IIKFCBR01AZQYV	Bacteroidetes	o__Cytophagales	KM391447	Min	21.3	33.83
HN5XJPU01C054O	Bacteroidetes	o__Cytophagales	KM391473	None	20.6	15.92
IIKFCBR01EUQP0	Bacteroidetes	o__Cytophagales	KM391495	Min	16.7	9.08
IIKFCBR01CGUSV	Bacteroidetes	o__Cytophagales	KM391510	Min	13C-only	6.96
IIKFCBR01D0ZL6	Bacteroidetes	o__Cytophagales	KM391562	Min	13C-only	3.25
IIKFCBR01BFRBV	Bacteroidetes	o__Cytophagales	KM391605	None	33.0	1.5
IIKFCBR01BID66	Bacteroidetes	o__Cytophagales	KM391620	None	29.3	1.33
IIKFCBR01DUERY	Bacteroidetes	o__Cytophagales	KM391621	None	27.5	1.25
IIKFCBR01CHWQH	Planctomycetes	o__DH61	KM391479	Min	13C-only	14.25
IIKFCBR01DSIAC	Planctomycetes	o__DH61	KM391572	Min	57.8	2.62
IIKFCBR01EN49F	Alphaproteobacteria	o__Ellin329	KM391511	Org	4.5	5.75
IIKFCBR01BGW6K	Alphaproteobacteria	o__Ellin329	KM391520	Org	5.1	4.83
IIKFCBR01DYEEF	TM7	o__EW055	KM391544	None	3.1	3.12
IIKFCBR01A2CCO	Armatimonadetes	o__FW68	KM391550	None	13C-only	3.58
IIKFCBR01ER26I	Armatimonadetes	o__FW68	KM391553	Org	8.8	3.21
IIKFCBR01DFO06	Armatimonadetes	o__FW68	KM391590	Min	12.5	1.71
IIKFCBR01BKF9Q	Verrucomicrobia	o__Methylacidiphilales	KM391646	None	13C-only	0.46
IIKFCBR01B9OL1	Deltaproteobacteria	o__MIZ46	KM391472	Min	37.9	17.21
IAJCH1401DPXDE	Deltaproteobacteria	o__MIZ46	KM391507	Min	19.7	7.17
IIKFCBR01C6K44	Deltaproteobacteria	o__MIZ46	KM391526	Min	8.2	4.83
IIKFCBR01BRR15	Deltaproteobacteria	o__MIZ46	NA	None	8.0	1.46
IIKFCBR01CBA0U	Deltaproteobacteria	o__MIZ46	KM391613	None	32.1	1.46
IIKFCBR01DMVF6	Deltaproteobacteria	o__MIZ46	KM391632	Min	23.8	1.08
IIKFCBR01BM6WY	Deltaproteobacteria	o__Myxococcales	KM391582	None	22.0	2
IIKFCBR01CAP7T	Deltaproteobacteria	o__Myxococcales	KM391599	None	11.3	1.54
IIKFCBR01BF99Q	Deltaproteobacteria	o__Myxococcales	KM391626	Min	13C-only	1.21
IIKFCBR01C50P4	Deltaproteobacteria	o__Myxococcales	KM391636	None	19.3	0.88
IIKFCBR01DXTPR	Deltaproteobacteria	o__Myxococcales	KM391643	None	13C-only	0.58
IIKFCBR01C17DJ	Deltaproteobacteria	o__Myxococcales	KM391645	None	13C-only	0.5
IIKFCBR01DRG3U	Alphaproteobacteria	o__Rhizobiales	KM391545	Min	20.4	3.71
IIKFCBR01EHNDT	Alphaproteobacteria	o__Rhizobiales	KM391589	Min	39.4	1.79
IIKFCBR01BEDM7	Planctomycetes	o__WD2101	KM391653	Min	11.0	2
IIKFCBR01DJ6HG	Planctomycetes	o__WD2101	KM391588	None	7.3	1.67
IIKFCBR01DVO2Q	Planctomycetes	o__WD2101	KM391593	Min	37.6	1.71
HN7PONR02HB4QW	Planctomycetes	o__WD2101	KM391601	Min	11.3	1.54
IIKFCBR01EHYT4	Planctomycetes	o__WD2101	KM391603	Min	7.8	1.42

Table E.10... *continued*

Representative Sequence ID	Phylum	Lowest taxonomic classification	GenBank Accession	Soil Layer Affiliation	Ratio (^{13}C:^{12}C)	Mean Counts ^{13}C Libraries
IIKFCBR01A4L7J	Planctomycetes	o__WD2101	KM391610	Min	10.4	1.42
IIKFCBR01DONLF	Planctomycetes	o__WD2101	KM391611	Min	13C-only	1.5
IIKFCBR01BPA0N	Planctomycetes	o__WD2101	KM391618	None	30.3	1.38
HN7PONR02FP5QD	Planctomycetes	o__WD2101	KM391629	None	23.8	1.08
IIKFCBR01C0JKL	Planctomycetes	o__WD2101	KM391630	None	11.5	1.04
IIKFCBR01DS8OU	Bacteroidetes	Unclassified	KM391464	Min	242.5	22.04
IIKFCBR01AELU7	Unclassified	Unclassified	KM391652	Min	47.1	10.71
IIKFCBR01BUBLL	Bacteroidetes	Unclassified	KM391500	Min	29.9	8.17
IIKFCBR01BHPNL	Bacteroidetes	Unclassified	NA	Min	29.2	7.96
IIKFCBR01E1K9Q	Alphaproteobacteria	Unclassified	KM391538	None	5.2	3.54
HN7PONR02JXI1W	Betaproteobacteria	Unclassified	KM391543	Org	13.9	3.79
IIKFCBR01BBGJU	Bacteroidetes	Unclassified	KM391564	Org	8.6	2.75
IIKFCBR01EI8BP	Unclassified	Unclassified	KM391568	Min	13C-only	2.79
IIKFCBR01ANT35	Bacteroidetes	Unclassified	KM391571	Min	58.7	2.67
IIKFCBR01CL9KO	Unclassified	Unclassified	NA	None	13C-only	2.25
IIKFCBR01D9WAN	Bacteroidetes	Unclassified	KM391592	Min	18.8	1.71
IIKFCBR01EPMN9	Verrucomicrobia	Unclassified	KM391600	Org	13C-only	1.67
IIKFCBR01CIOT3	Unclassified	Unclassified	KM391606	Org	33.0	1.5
IIKFCBR01BGI0P	Proteobacteria	Unclassified	NA	None	13C-only	0.71

Table E.11. Full details of draft genomes recovered from metagenomics assemblies. 'Completeness' is a measure of the total number of housekeeping genes present from a list of single copy 'essential' genes (Albertsen *et al.* 2013). 'Redundancy' refers to the number of times those house-keeping genes recurred. The final four columns correspond to the percentage of reads mapped to each draft genome from the respective metagenomic samples. Note: 'CheckM' was not used in this analysis, in contrast to Chapter 4.

Taxonomic Affiliation of Draft Genome Bin	MEGAN Classification (% of bases assigned)	Scaffold Accession Numbers (E.N.A.)	Size of Partial Genome (Mb)	Number of Contigs	Largest Scaffold (Kb)	% Complete	% Redundant	Control (%)	OM1 (%)	OM3 (%)	Reference (%)
Myceliophthora thermophila	Ascomycota (99%)	FJWA01000001-FJWA01010187	46.2	10187	32.1	100	24	0.47	14.07	9.67	7.97
Kitasatospora sp.	Actinobacteria (79%)	FJVZ01000001-FJVZ01001553	8.1	1553	31.7	100	21	0.08	0.54	0.28	6.05
Opitutaceae spp.	Verrucomicrobia (78%)	FJVV01000001-FJVV01001035	5.7	1035	46.9	100	18	0.00	0.03	0.03	1.71
Herbaspirillum sp.	β - Proteobacteria (100%)	FJVX01000001-FJVX01001352	4.3	1352	19.3	89	24	0.00	1.73	0.02	0.06
Chthoniobacter sp. 1	Verrucomicrobia (74%)	FJVU01000001-FJVU01000901	4.0	901	21.9	66	24	0.00	0.09	0.05	1.11
Caulobacteraceae spp.	α - Proteobacteria (96%)	FJVW01000001-FJVW01001105	3.3	1105	14.8	76	19	0.00	0.06	0.04	0.72
Heterogeneous Bin	Cand. Saccharibacteria (27%)	FJVY01000001-FJVY01000803	2.4	103	37.7	94	32	0.01	0.27	0.18	0.64
Arthrobacter sp.	Actinobacteria (60%)	FJVT01000001-FJVT01000548	0.8	548	5.3	10	0	0.01	0.07	0.15	0.06
Oxalobacteraceae spp.	β - Proteobacteria (96%)	FJVS01000001-FJVS01000283	0.4	283	3.4	0	NA	0.00	0.03	0.01	0.08
Candidatus Saccharibacteria	Cand. Saccharibacteria (56%)	FJVR01000001-FJVR01000103	0.4	803	16.0	38	34	0.00	0.01	0.02	0.15
Chthoniobacter sp. 2	Verrucomicrobia (65%)	NA	0.4	119	6.0	5	0	0.00	0.01	0.00	0.11

Table E.12. Complete list of OTUs designated putatively hemicellulolytic, cellulolytic and/or lignolytic based on differential abundance between ^{13}C- and ^{12}C-pyrotag or metagenomic libraries at ecozones across North America. Taxa identified exclusively in metagenomic libraries are denoted with an asterix (*).

Phylum	Class	Order	Family	Genus	Ecozones	Soil Assoc.	Substr.
Acidobacteria	Acidobacteria-6	iii1-15	Unclassified	Unclassified	BS;CA;TX	None	Lig
Acidobacteria	DA052	Ellin6513	unclassified	unclassified	TX	None	Lig
Acidobacteria	Solibacteres	Solibacterales	Unclassified	Unclassified	BS;CA;TX	None	Lig
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unclassified	unclassified	BS,TX	None	Lig
Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	Unclassified	JP	None	Lig
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	Unclassified	BS,TX	None	Lig
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Unclassified	All	None	Cell;Lig
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Salinibacterium	BC;BS;CA;TX	None	Hemi;Cell
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Unclassified	BC;CA	Min	Hemi;Lig
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	BC;CA	None	Hemi
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Unclassified	CA;BS;JP	None	Hemi;Cell
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Unclassified	TX	None	Lig
Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	Conexibacter	BS;TX	Org	Cell;Lig
Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	Unclassified	BS;TX	None	Lig
Armatimonadetes	Armatimonadia	FW68	Unclassified	Unclassified	BC;CA;BS;TX	None	Cell
Bacteroidetes	Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Unclassified	BC;CA;TX	None	Hemi;Lig
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	BC;CA;TX	None	Cell
Chloroflexi	C0119	Unclassified	Unclassified	Unclassified	CA	Org	Lig
Elusimicrobia	Elusimicrobia	FAC88	Unclassified	Unclassified	BS	None	Lig
FBP	Unclassified	Unclassified	Unclassified	Unclassified	All	None	Cell
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	BC	Min	Hemi
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Unclassified	CA	Min	Hemi
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	TX;CA;BS	None	Lig
Planctomycetes	vadinHA49	DH61	Unclassified	Unclassified	BC;CA	None	Cell
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC;CA;BS;TX	None	All
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	All	None	Hemi;Cell
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	All	None	Lig
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Woodsholea	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bosea	CA;TX	None	Cell;Lig
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	BC;CA;TX	Org	Hemi;Cell
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	BC;CA	None	Hemi
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Oceanicaulis	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Hyphomonas	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Henriciella	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Maricaulis	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	Hirschia	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Telmatospirillum	JP;TX	None	Cell;Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Porphyrobacter	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Unclassified	CA;BS	None	Hemi;Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Zymomonas	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Citromicrobium	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	BC;CA;TX	None	Lig
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Blastomonas	BC;CA;TX	None	Lig
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	BC;CA;BS;TX	None	Hemi;Cell
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	BC;CA;TX	Min	Lig
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Pelomonas	BC;CA;TX	None	All
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Leptothrix	All	None	Cell
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Unclassified	All	None	Hemi;Cell
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	BS;TX	None	Lig
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	CA;JP;TX	None	Cell
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	All	None	Hemi;Cell
Proteobacteria	Deltaproteobacteria	MIZ46	Unclassified	Unclassified	BC;CA	None	Cell

Table E.12... *continued*

Phylum	Class	Order	Family	Genus	Ecozones	Soil Assoc.	Substr.
Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	Unclassified	BS	Org	Lig
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	Unclassified	BC;BS;JP	None	Cell
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Cellvibrio	BC;CA	None	Hemi;Cell
Proteobacteria*	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Ectothiorhodospira	CA;BS	None	Lig
Proteobacteria*	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Nitrococcus	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Alkalilimnicola	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Spiribacter	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Halorhodospira	BC;CA;TX	None	Lig
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Morganella	BC;CA;TX		Lig
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Erythrobacteraceae	Unclassified	BC;CA;TX	None	Lig
Proteobacteria	Gammaproteobacteria	Nevskiales	Sinobacteraceae	Nevskia	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Nevskiales	Sinobacteraceae	Hydrocarboniphaga	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Nevskiales	Sinobacteraceae	Solimonas	BC;CA;TX	None	Lig
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	BC;CA	None	Hemi
Proteobacteria	Gammaproteobacteria	Salinisphaerales	Salinisphaeraceae	Salinisphaera	BC;CA;TX	None	Lig
Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	Unclassified	CA	Min	Lig
Proteobacteria*	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dyella	BC;CA;TX	None	Lig
Proteobacteria*	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Frateuria	BC;CA;TX	None	Lig
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Unclassified	CA	None	Lig
TM7	TM7-3	Unclassified	Unclassified	Unclassified	BC; CA	None	Hemi
Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Unclassified	BC;BS;JP	None	Cell
Verrucomicrobia*	Unclassified	Unclassified	Unclassified	Unclassified	BC;CA;BS;JP	None	Cell
Ascomycota*	Dothideomycetes	Incertae_sedis	Pseudeurotiaceae	Pseudogymnoascus	BC;CA	None	Cell
Ascomycota*	Leotiomycetes	Helotiales	Sclerotiniaceae	Botrytis	CA;BS;JP;TX	None	Cell
Ascomycota*	Orbiliomycetes	Orbiliales	Orbiliaceae	Arthrobotrys	CA;TX	Org	Cell
Ascomycota*	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	Unclassified	CA;BS	Min	Lig
Ascomycota*	Sordariomycetes	Glomerellales	Plectosphaerellaceae	Verticillium	CA;BS;JP;TX	Org	Cell
Ascomycota*	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	CA;BS;JP;TX	Org	Cell
Ascomycota*	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	BC;CA;BS;TX	None	Cell
Ascomycota*	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	CA;BS;JP;TX	Org	Cell
Ascomycota*	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	Ophiocordyceps	CA;BS;JP;TX	None	Cell
Ascomycota*	Sordariomycetes	Sordariales	Chaetomiaceae	Chaetomium	CA;TX	None	Cell
Ascomycota*	Sordariomycetes	Sordariales	Chaetomiaceae	Humicola	CA;TX	None	Cell
Ascomycota*	Sordariomycetes	Sordariales	Chaetomiaceae	Myceliophthora	BC;CA;TX	Org	Cell
Ascomycota*	Sordariomycetes	Sordariales	Sordariaceae	Neurospora	CA;BS;JP;TX	Org	Cell
Ascomycota*	Sordariomycetes	Incertae_sedis	Magnaporthaceae	Magnaporthe	CA;BS;JP;TX	Org	Cell
Basidiomycota*	Agaricomycetes	Agaricales	Pleurotaceae	Pleurotus	BC;CA;BS;JP	None	Cell
Basidiomycota*	Agaricomycetes	Agaricales	Psathyrellaceae	Coprinopsis	BC;CA	None	Cell
Basidiomycota*	Agaricomycetes	Agaricales	Schizophyllaceae	Schizophyllum	BC;CA;TX	Org	Cell
Basidiomycota*	Agaricomycetes	Agaricales	Tricholomataceae	Laccaria	BC;CA	None	Cell
Basidiomycota*	Agaricomycetes	Sebacinales	Sebacinales group B	Piriformospora	BC;CA	None	Cell
Basidiomycota*	mitosporic Basidiomycota	NA	NA	Trichosporon	BC;CA;BS;JP	Org	Cell
Basidiomycota*	Tremellomycetes	Filobasidiales	Filobasidiaceae	Cryptococcus	All	Org	Cell
Bilateria*	Polychaeta	Annelida	Siboglinidae	Osedax	BC;CA;TX	None	Lig
Chordata*	Mammalia	Bovidae	Antilopinae	Pantholops	BC;CA;TX	None	Lig

Table E.13. Complete list of draft genomes recovered from cellulose and lignin metagenomic libraries from across North America. Bins were made using both ^{12}C and ^{13}C-libraries and, therefore, not all bins correspond to taxa designated as cellulolytic or lignolytic, some correspond to generally abundant taxa.

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	26	0.41	76%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	18	0.25	81%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Org	10	0.36	97%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	38	0.39	92%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	TX	Min	34	0.32	75%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	5	0.42	77%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	TX	Min	47	0.63	98%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	52	0.35	73%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	9	0.67	69%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Org	13	1.50	98%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	46	6.15	35%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Org	36	1.91	96%
Cell	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	ON	Org	25	0.32	71%
Cell	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	ON	Min	33	0.20	90%
Cell	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	ON	Org	54	0.56	58%
Cell	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	ON	Min	29	0.19	93%
Cell	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	ON	Org	48	0.66	93%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	ON	Org	11	1.94	42%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	CA	Org	27	1.48	26%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	CA	Org	9	0.47	34%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	ON	Org	16	0.21	26%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	ON	Min	15	1.87	52%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	CA	Min	21	4.45	51%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	TX	Min	19	0.17	28%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	TX	Org	31	0.31	32%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	BC	Min	15	0.08	33%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Microbacterium	ON	Org	20	0.06	24%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	BC	Org	7	0.14	24%
Cell	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	TX	Org	20	0.37	23%
Cell	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	CA	Min	4	2.83	80%
Cell	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	ON	Min	22	2.34	79%
Cell	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter	TX	Min	6	3.82	78%
Cell	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	TX	Min	29	3.85	43%
Cell	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	BC	Min	16	1.22	56%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	1	1.21	56%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	22	0.25	34%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	7	0.23	75%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	55	0.17	60%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	42	0.25	52%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	CA	Org	7	0.16	71%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	37	0.18	23%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	35	2.34	33%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	17	6.00	33%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	27	1.48	55%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	38	0.11	21%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	ON	Org	34	0.07	23%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	CA	Org	3	2.61	57%
Cell	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	CA	Org	25	0.47	55%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	TX	Org	39	0.21	33%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	17	0.09	39%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Min	15	1.45	55%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	35	0.13	61%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Min	22	0.15	52%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	TX	Min	20	1.59	56%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Min	16	1.49	44%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	BC	Min	17	0.68	46%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	14	0.06	28%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	44	0.38	31%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	26	0.18	46%
Cell	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	BC	Org	3	0.28	27%
Cell	Armatimonadetes	Chthonomonadales	Chthonomonadaceae	Chthonomonas	TX	Min	25	1.05	23%
Cell	Bacteroidetes	Bacteroidales	Bacteroidaceae	Bacteroides	TX	Min	44	0.06	22%
Cell	Bacteroidetes	Cytophagales	Cytophagaceae	Cytophaga	ON	Org	21	0.16	32%
Cell	Bacteroidetes	Cytophagales	Cytophagaceae	Cytophaga	TX	Org	37	2.70	95%
Cell	Bacteroidetes	Cytophagales	Cytophagaceae	Cytophaga	TX	Min	10	0.14	26%
Cell	Bacteroidetes	Cytophagales	Cytophagaceae	Cytophaga	TX	Min	12	1.36	38%
Cell	Bacteroidetes	Cytophagales	Cytophagaceae	Cytophaga	ON	Org	22	0.26	54%
Cell	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	BC	Min	12	0.07	25%
Cell	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	ON	Org	24	0.21	63%
Cell	Chloroflexi	Anaerolineales	Anaerolineaceae	Anaerolinea	BC	Min	11	0.67	39%
Cell	Firmicutes	Clostridiales	Peptococcaceae	Unclassified	ON	Org	6	0.29	23%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Org	15	0.10	38%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	TX	Min	33	23.30	69%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Org	2	0.87	79%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	24	0.11	56%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Org	17	10.16	54%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Min	2	0.13	36%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Min	5	3.55	87%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Min	9	1.49	78%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Org	30	1.05	58%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	29	2.69	66%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Min	15	0.32	55%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	11	5.86	50%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	45	0.15	54%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	28	0.20	73%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Org	15	0.62	74%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	TX	Min	5	0.44	67%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Min	18	1.38	58%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Min	12	2.72	87%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	9	0.40	23%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	10	2.10	48%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	ON	Org	4	0.17	55%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Org	29	5.24	80%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	19	0.54	38%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	2	0.73	57%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Org	4	0.10	40%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	BC	Min	21	1.06	87%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	20	0.17	63%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	39	2.24	50%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Org	17	2.68	86%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	ON	Org	11	1.44	68%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	28	0.13	41%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Org	22	0.18	79%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	ON	Min	18	1.24	57%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	27	2.56	37%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Org	1	4.38	94%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	BC	Org	6	1.25	83%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	BC	Min	1	0.28	46%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	9	5.47	48%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	31	0.80	40%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	13	0.19	47%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	28	1.57	74%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	ON	Org	37	0.39	54%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	6	3.04	36%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	CA	Org	19	0.31	74%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	TX	Min	17	0.38	76%
Cell	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	28	0.22	60%
Cell	Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Pelagibacterium	TX	Min	53	0.09	32%
Cell	Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Pelagibacterium	BC	Org	18	0.17	40%
Cell	Proteobacteria	Rhizobiales	Hyphomicrobiaceae	Pelagibacterium	BC	Org	11	0.18	21%
Cell	Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	TX	Min	52	0.06	22%
Cell	Proteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium	CA	Org	4	0.08	35%
Cell	Proteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	CA	Org	16	0.05	23%
Cell	Proteobacteria	Rhodospirillales	Acetobacteraceae	Unclassified	ON	Org	40	1.82	43%
Cell	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Unclassified	TX	Org	18	0.06	25%
Cell	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Magnetospirillum	TX	Org	1	0.54	28%
Cell	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	TX	Min	39	0.75	44%
Cell	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	TX	Org	23	0.10	42%
Cell	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	TX	Org	38	0.15	42%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	TX	Min	41	0.32	72%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Unclassified	TX	Org	4	0.23	64%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	CA	Org	23	0.34	43%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	ON	Min	17	0.32	56%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	CA	Org	8	0.23	49%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	TX	Min	24	17.47	29%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	CA	Min	10	3.05	64%
Cell	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	TX	Org	10	1.19	61%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	15	7.52	63%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	5	0.57	81%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	TX	Org	21	0.05	23%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	TX	Min	38	1.00	56%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	43	0.32	98%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	ON	Min	14	1.15	27%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	ON	Min	19	0.17	31%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	TX	Min	18	0.15	52%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	32	1.11	83%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	TX	Org	16	0.66	24%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	CA	Min	30	0.20	22%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	39	7.35	59%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	TX	Min	37	1.16	54%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	ON	Org	31	0.10	24%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	TX	Org	11	0.10	20%
Cell	Proteobacteria	Burkholderiales	Burkholderiaceae	Unclassified	ON	Min	1	0.61	24%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	TX	Min	50	1.69	23%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Min	2	0.08	30%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	TX	Org	24	0.19	23%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	BC	Min	4	0.54	23%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Variovorax	BC	Org	16	0.42	77%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	CA	Min	23	0.38	78%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	CA	Org	2	1.32	22%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Min	10	0.19	34%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Min	14	0.20	25%
Cell	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	CA	Org	10	0.14	20%
Cell	Proteobacteria	Burkholderiales	incertae sedis	Rubrivivax	BC	Min	7	0.17	20%
Cell	Proteobacteria	Burkholderiales	incertae sedis	Rubrivivax	ON	Org	6	0.06	27%
Cell	Proteobacteria	Burkholderiales	incertae sedis	Rubrivivax	TX	Min	13	0.65	48%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Proteobacteria	Burkholderiales	Unclassified	Unclassified	CA	Org	5	0.92	49%
Cell	Proteobacteria	Burkholderiales	Unclassified	Unclassified	ON	Min	1	0.10	50%
Cell	Proteobacteria	Burkholderiales	Unclassified	Unclassified	TX	Min	1	8.20	49%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Org	23	1.63	41%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	BC	Min	19	0.23	59%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	ON	Min	21	0.19	68%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	ON	Org	3	0.79	81%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	ON	Org	40	0.19	70%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Org	23	0.18	30%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	CA	Min	19	0.48	46%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Org	13	0.22	43%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	ON	Min	30	3.20	63%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Org	8	0.33	47%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	ON	Min	7	0.39	24%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Min	16	0.14	40%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	CA	Org	12	0.54	61%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	ON	Min	12	7.15	76%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	TX	Org	34	0.24	33%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	TX	Min	49	0.31	62%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Unclassified	CA	Min	18	0.90	64%
Cell	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	ON	Org	21	0.08	32%
Cell	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	ON	Min	21	1.08	24%
Cell	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	TX	Org	35	0.99	30%
Cell	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	TX	Min	48	0.40	22%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	BC	Min	13	0.87	42%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	TX	Min	55	0.40	79%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	CA	Org	21	10.44	40%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	TX	Org	12	6.12	67%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	TX	Org	26	2.02	38%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	BC	Org	8	1.80	45%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	TX	Org	19	4.08	38%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	CA	Min	16	1.39	42%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	BC	Min	10	4.07	41%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	TX	Min	32	4.23	20%
Cell	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	ON	Min	18	1.12	33%
Cell	Proteobacteria	Myxococcales	Sorangiineae	Polyangiaceae	CA	Min	11	0.10	24%
Cell	Proteobacteria	Myxococcales	Sorangiineae	Polyangiaceae	BC	Org	14	5.90	76%
Cell	Proteobacteria	Cellvibrionales	Cellvibrionaceae	Cellvibrio	BC	Org	9	0.43	61%
Cell	Proteobacteria	Chromatiales	Ectothiorhodospiraceae	Unclassified	ON	Min	25	0.15	21%
Cell	Proteobacteria	Nevskiales	Sinobacteraceae	Unclassified	ON	Min	4	0.26	27%
Cell	Proteobacteria	Nevskiales	Sinobacteraceae	Unclassified	ON	Min	2	0.91	27%
Cell	Proteobacteria	Nevskiales	Sinobacteraceae	Hydrocarboniphaga	ON	Min	6	0.05	23%
Cell	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	CA	Org	24	0.08	24%
Cell	Proteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	ON	Org	3	0.45	29%
Cell	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Unclassified	ON	Org	33	0.28	79%
Cell	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Unclassified	ON	Org	20	0.72	91%
Cell	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Unclassified	ON	Org	47	2.02	88%
Cell	Verrucomicrobia	Opitutales	Opitutaceae	Opitutus	TX	Org	9	2.65	65%
Cell	Verrucomicrobia	Opitutales	Opitutaceae	Unclassified	CA	Min	13	0.10	30%
Cell	Verrucomicrobia	Opitutales	Opitutaceae	Unclassified	BC	Org	12	1.91	73%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	14	0.39	50%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	8	8.51	30%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	40	5.71	59%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	16	1.03	59%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	BC	Org	13	1.81	65%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	35	0.25	22%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	22	4.72	51%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	11	0.21	46%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	CA	Min	26	0.75	67%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	ON	Org	7	0.11	26%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	43	0.25	70%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	CA	Org	13	0.05	21%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	ON	Org	53	0.13	20%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	CA	Min	3	0.54	26%
Cell	Verrucomicrobia	Verrucomicrobiales	Subdivision 3	Pedosphaera	ON	Org	32	0.24	43%
Cell	Verrucomicrobia	Verrucomicrobiales	Subdivision 3	Pedosphaera	TX	Min	23	0.07	21%
Cell	Verrucomicrobia	Verrucomicrobiales	Verrucomicrobiaceae	Unclassified	TX	Min	9	4.00	48%
Cell	Ascomycota	incertae sedis	Pseudeurotiaceae	Pseudogymnoascus	CA	Min	24	0.56	91%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	8	0.31	25%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	2	0.47	91%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	1	0.14	57%
Cell	Ascomycota	Sordariales	Sordariaceae	Neurospora	ON	Org	36	0.23	22%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	17	0.37	21%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	8	0.24	45%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	22	27.82	44%
Cell	Ascomycota	Sordariales	Sordariaceae	Neurospora	TX	Org	33	0.39	21%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	9	0.09	36%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	4	18.64	48%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	3	0.16	58%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	6	1.29	48%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	28	0.32	53%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	6	0.30	90%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	9	0.62	79%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	5	0.37	96%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	23	1.03	76%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	30	0.66	97%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	50	0.15	62%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	7	3.30	100%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	2	0.80	25%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	27	0.68	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	34	3.61	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	32	0.76	76%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	18	0.17	43%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	28	0.10	22%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	12	1.93	78%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	54	0.41	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	25	0.16	77%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	14	0.05	20%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	8	2.86	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	2	2.11	55%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	1	0.06	28%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	14	7.78	88%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	49	0.20	94%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Min	20	0.09	38%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	25	1.81	95%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	12	8.14	96%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	7	1.59	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	29	4.13	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	7	0.22	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	5	3.52	97%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	18	0.99	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	24	0.24	71%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	10	0.22	62%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	15	0.41	58%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	24	0.25	84%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	1	4.50	27%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	19	1.21	98%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	17	0.47	98%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Org	9	0.14	23%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Min	30	0.22	20%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Min	27	0.30	30%
Lig	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	13	0.09	34%
Lig	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	CA	Min	26	0.37	29%
Lig	Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Chitinophaga	ON	Org	3	0.09	29%
Lig	Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	ON	Org	15	0.17	42%
Lig	Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Unclassified	ON	Org	10	0.84	57%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	3	3.36	47%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	32	0.60	37%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	33	0.40	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	14	0.67	26%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	19	0.64	36%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	ON	Min	29	0.92	26%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	2	2.49	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	4	6.66	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	38	0.15	45%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	1	4.58	48%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	TX	Min	2	0.18	53%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	4	0.37	54%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	33	0.88	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	9	1.06	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	7	2.38	58%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	24	1.29	39%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	5	1.19	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	6	1.94	47%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	4	0.25	56%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	20	0.98	35%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	11	1.45	42%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	25	0.44	48%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	21	0.38	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	13	0.86	40%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	36	0.81	41%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	12	3.09	33%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	8	2.78	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	9	0.95	35%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	ON	Min	6	0.76	24%
Lig	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	CA	Org	5	0.10	33%
Lig	Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	ON	Min	35	0.98	50%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	CA	Org	1	0.08	24%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Org	12	3.72	75%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	ON	Org	6	0.07	29%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Min	28	0.80	67%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	CA	Org	7	0.55	24%
Lig	Proteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	ON	Min	18	2.74	46%
Lig	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	ON	Min	11	1.36	30%
Lig	Parcubacteria	Unclassified	Unclassified	Unclassified	ON	Min	3	0.38	42%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	16	0.67	49%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	11	0.56	87%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	16	0.62	69%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	34	1.88	46%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	22	0.15	32%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	26	2.05	64%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Lig	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	8	0.41	97%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	CA	Min	26	0.75	67%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	ON	Org	7	0.11	26%
Cell	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	TX	Min	43	0.25	70%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	CA	Org	13	0.05	21%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	ON	Org	53	0.13	20%
Cell	Verrucomicrobia	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum	CA	Min	3	0.54	26%
Cell	Verrucomicrobia	Verrucomicrobiales	Subdivision 3	Pedosphaera	ON	Org	32	0.24	43%
Cell	Verrucomicrobia	Verrucomicrobiales	Subdivision 3	Pedosphaera	TX	Min	23	0.07	21%
Cell	Verrucomicrobia	Verrucomicrobiales	Verrucomicrobiaceae	Unclassified	TX	Min	9	4.00	48%
Cell	Ascomycota	incertae sedis	Pseudeurotiaceae	Pseudogymnoascus	CA	Min	24	0.56	91%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	8	0.31	25%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	2	0.47	91%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	1	0.14	57%
Cell	Ascomycota	Sordariales	Sordariaceae	Neurospora	ON	Org	36	0.23	22%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	17	0.37	21%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	8	0.24	45%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	22	27.82	44%
Cell	Ascomycota	Sordariales	Sordariaceae	Neurospora	TX	Org	33	0.39	21%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	CA	Min	9	0.09	36%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	4	18.64	48%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Min	3	0.16	58%
Cell	Ascomycota	Sordariales	Sordariaceae	Chaetomium	TX	Org	6	1.29	48%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	28	0.32	53%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	6	0.30	90%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	9	0.62	79%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	5	0.37	96%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	23	1.03	76%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	30	0.66	97%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	50	0.15	62%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	7	3.30	100%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	2	0.80	25%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	27	0.68	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	34	3.61	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	32	0.76	76%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	18	0.17	43%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	28	0.10	22%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	12	1.93	78%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	54	0.41	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	25	0.16	77%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	14	0.05	20%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	8	2.86	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	2	2.11	55%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	1	0.06	28%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	14	7.78	88%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	49	0.20	94%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Min	20	0.09	38%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	25	1.81	95%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	12	8.14	96%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Org	7	1.59	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	29	4.13	91%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	7	0.22	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	5	3.52	97%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	18	0.99	99%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	24	0.24	71%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	10	0.22	62%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	15	0.41	58%

Table E.13... *continued*

Substr	Phylum	Order	Family	Genus	Ecozone	Soil	Bin #	Total Bases (Mb)	Percent Classified
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	24	0.25	84%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	TX	Min	1	4.50	27%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	19	1.21	98%
Cell	Unclassified	Unclassified	Unclassified	Unclassified	BC	Org	17	0.47	98%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Org	9	0.14	23%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Min	30	0.22	20%
Lig	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Unclassified	ON	Min	27	0.30	30%
Lig	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	ON	Org	13	0.09	34%
Lig	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	CA	Min	26	0.37	29%
Lig	Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Chitinophaga	ON	Org	3	0.09	29%
Lig	Bacteroidetes	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	ON	Org	15	0.17	42%
Lig	Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Unclassified	ON	Org	10	0.84	57%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	3	3.36	47%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	32	0.60	37%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	33	0.40	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	14	0.67	26%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	19	0.64	36%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	ON	Min	29	0.92	26%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	2	2.49	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	4	6.66	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	38	0.15	45%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	1	4.58	48%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	TX	Min	2	0.18	53%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	4	0.37	54%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	33	0.88	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	9	1.06	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	7	2.38	58%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	24	1.29	39%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	5	1.19	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Org	6	1.94	47%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	4	0.25	56%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	20	0.98	35%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	11	1.45	42%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	25	0.44	48%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	21	0.38	44%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	13	0.86	40%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	CA	Min	36	0.81	41%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	12	3.09	33%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Org	8	2.78	49%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	ON	Min	9	0.95	35%
Lig	Proteobacteria	Caulobacterales	Caulobacteraceae	Unclassified	ON	Min	6	0.76	24%
Lig	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	CA	Org	5	0.10	33%
Lig	Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	ON	Min	35	0.98	50%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	CA	Org	1	0.08	24%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Org	12	3.72	75%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	ON	Org	6	0.07	29%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Unclassified	ON	Min	28	0.80	67%
Lig	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	CA	Org	7	0.55	24%
Lig	Proteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	ON	Min	18	2.74	46%
Lig	Proteobacteria	Myxococcales	Polyangiaceae	Sorangium	ON	Min	11	1.36	30%
Lig	Parcubacteria	Unclassified	Unclassified	Unclassified	ON	Min	3	0.38	42%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	16	0.67	49%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	11	0.56	87%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Org	16	0.62	69%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	34	1.88	46%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	CA	Min	22	0.15	32%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	ON	Min	26	2.05	64%
Lig	Unclassified	Unclassified	Unclassified	Unclassified	CA	Org	8	0.41	97%

Table E.14. List of contigs containing clusters of three or more hits based on BLAST or hmmscan to CAZymes. All CAZyme annotations are putative and have not been validated (i.e. most AA2 hits were to Class II catalase-peroxidases). Taxonomic classification was based on LCA for the full length of the cluster. Partial open-reading frames, as predicted by Prodigal, were not included. The following column names have been abbreviated: 'ecozone' (eco.), 'substrate' (subs.) and 'horizon' (hor.). Some CAZyme clusters may repeat if multiple lignin-modifying genes were present, or if the cluster appeared on multiple contigs within the same library.

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
dyp2	A8056	Assem.	BS	Cell	Min	CBM50;GH35;GH42;GH42;dyp2;AA3;GT4	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	scaffold-1518	514-18981
dyp2	TXA37	Assem.	Tex	Cell	Min	dyp2;GT2;GT2;GH36;GT4;GT4;GH63;GH19;GH88	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	k99_542889	4101-94289
dyp2	Bin.14	Draft	BS	Cell	Min	GH3;GT2;dyp2	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	k99_414325	2702-32487
dyp2	BR_Antib	Assem.	CA	Lig	Min	CE10;GT4;dyp2;GH74	Actinobacteria	Actinomycetales	Frankiaceae	Frankia	scaffold-147	1692-17328
dyp2	Bin.11	Draft	BS	Cell	Org	dyp2;GT2;GT2;AA3;2	Actinobacteria	Actinomycetales	Microbacteriaceae	Leifsonia	k99_262087	4089-13763
dyp2	A9086	Assem.	BS	Cell	Min	GT2;CBM2;dyp2	Actinobacteria	Actinomycetales	Microbacteriaceae	Leifsonia	scaffold-1717	4036-13615
dyp2	Bin.21	Draft	CA	Cell	Min	PL9;AA6;dyp2	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	k99_1594532	8107-18322
dyp2	DC578	Assem.	BC	Cell	Min	GT51;GT30;dyp2;CE1;GH16;CE14	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	scaffold-4838	1734-32891
dyp2	TXA_Antib	Assem.	Tex	Lig	Min	GT53;CE5;dyp2;CBM51;CE11	Actinobacteria	Actinomycetales	Nocardiaceae	Nocardia	scaffold-186	2012-27483
dyp2	JS080	Assem.	JP	Cell	Min	GH13;26;CBM48;dyp2;CE4;CE11	Actinobacteria	Actinomycetales	Streptomycetaceae	Kitasatospora	scaffold-250	1497-23907
dyp2	LH020	Assem.	CA	Cell	Min	CE14;AA5;dyp2;GH3	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-488	689-12473
dyp2	JS080	Assem.	JP	Cell	Min	dyp2;AA5;CE14;GH32	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-455960	5416-22058
dyp2	JS080	Assem.	JP	Cell	Min	dyp2;GH1;GT5;GT2;CBM50;CBM14;GH23;CBM13	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-3202	4767-40835
dyp2	A8055	Assem.	BS	Cell	Org	GH65;GH65;dyp2;GH92	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-5450	871-14240
dyp2	JS080	Assem.	JP	Cell	Min	GT51;GH16;dyp2;AA5	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-1901	5192-25468
dyp2	JS080	Assem.	JP	Cell	Min	GT51;GT2;dyp2;AA5	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-457701	4606-18455
dyp2	JS080	Assem.	JP	Cell	Min	GT51;GT2;dyp2;AA5	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-448968	9547-23396
dyp2	JE121	Assem.	JP	Cell	Org	GT41;dyp2;GT4	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-1555	1675-10729
dyp2	JE122	Assem.	JP	Cell	Min	CBM35;GH109;CE11;CBM48;GT2;GH32;GH32;GH1;CE11;GT2;GH39;GH36;CBM2;CE1;GH3;GT2;GH65;PL9;4;CE1;CBM2;GT2;GH128;GT2;GT2;GH3;GT2;GT51;CBM61;CBM50;GH35;GH42;dyp2;AA3;GT4;CBM50;GT2;GH13;26;GT4;CBM50;GT2;GT51	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	scaffold-798	1799-396092
dyp2	TXA37	Assem.	Tex	Cell	Min	dyp2;PL1;GT5	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	k99_562108	6054-14556
dyp2	Bin.12	Draft	JP	Cell	Min	dyp2;GT2;CBM50	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	k99_130551	1930-16536
dyp2	JE121	Assem.	JP	Cell	Org	CBM50;GT2;GT80;GT4;CBM50;GT2;dyp2;GT4;GH23	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	scaffold-1209578	12648-55728
dyp2	TXA37	Assem.	Tex	Cell	Min	GH125;GT4;dyp2	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	k99_282716	2677-48679
dyp2	A7M1_13C	Assem.	BS	Lig	Min	CE1;GH16;dyp2;GT4;GH78	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-895	219-63108
dyp2	DC578	Assem.	BC	Cell	Min	CE14;dyp2;GT4	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-191	1176-13442
dyp2	OC458	Assem.	BC	Cell	Min	GH37;dyp2;GT2;CE1;GH19;GT4;GH13;31	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-797	2837-70504
dyp2	Bin.11	Draft	BS	Cell	Min	GT2;dyp2;GH78	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	k99_450390	11800-49618
dyp2	A8056	Assem.	BS	Cell	Min	GT35;GH78;dyp2;GT2	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-462	13479-53223
dyp2	Bin.21	Draft	CA	Cell	Org	dyp2;GH16;CBM48;CBM2	Proteobacteria	Myxococcales	Cystobacteraceae	Stigmatella	k99_528071	216-12505
dyp2	DC578	Assem.	BC	Cell	Min	CE2;CBM13;dyp2	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	scaffold-1088	2732-15238
dyp2	A7026	Assem.	BS	Cell	Min	CBM61;CBM50;GH35;GH42;dyp2;AA3;GT4	Proteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	scaffold-256	5796-30437
dyp2	BRM2_13C	Assem.	CA	Lig	Min	dyp2;AA3;ary.oh.oxi;AA3	Proteobacteria	Rhodobacterales	Rhodobacteraceae	Thalassobacter	scaffold-1303	5267-10995
dyp2	DC578	Assem.	BC	Cell	Min	CE10;GT41;dyp2	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Nitrospirillum	scaffold-954	4536-24275
dyp2	Bin.50	Draft	Tex	Cell	Min	CBM13;GT2;CE10;CE1;CE10;GT5;PL1;dyp2	Proteobacteria	uncl. Burkholderiales	uncl.	Rubrivivax	k99_734139	11513-35776
dyp2	JS080	Assem.	JP	Cell	Min	PL9;dyp2;GT2;GH65	uncl.	uncl.	uncl.	uncl.	scaffold-5687	856-12441
laccase	Bin.14	Draft	BS	Cell	Org	CE10;CBM2;AA1;GH53	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Candidatus	k99_393656	806-22240
laccase	BRM2_13C	Assem.	CA	Lig	Min	AA4;AA1;AA1	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Candidatus	scaffold-8911	346-5363
laccase	Bin.14	Draft	BS	Cell	Org	GH105;GH109;AA1	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	k99_1926388	3111-7912
laccase	Bin.37	Draft	CA	Lig	Min	GT4;AA1;GT2;CBM5;CE1	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	k99_154957	17646-52187
laccase	TXA37	Assem.	Tex	Cell	Min	GT2;GH73;AA1	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	k99_433354	1-5823

Table E.14... *continued*

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
laccase	BRO1_13C	Assem.	CA	Lig	Org	AA1;AA1;sm.lacc	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	scaffold-909	66-1735
laccase	BRM2_13C	Assem.	CA	Lig	Min	AA1;sm.lacc;AA1	Acidobacteria	Acidobacteriales	uncl. Acidobacteriaceae	Acidobacteriaceae	scaffold-6478	1548-3484
laccase	BRM3_13C	Assem.	CA	Lig	Min	AA1;sm.lacc;AA1	Actinobacteria	Actinomycetales	Frankiaceae	Frankia	scaffold-357	1005-2848
laccase	BR_Antib	Assem.	CA	Lig	Min	AA1;lacc;sm.lacc;AA1	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	scaffold-336	586-2265
laccase	BRO1_13C	Assem.	CA	Lig	Org	AA1;AA1;lacc	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	scaffold-1020	498-2270
laccase	JS080	Assem.	JP	Cell	Min	CBM2;GH92;AA1	Actinobacteria	Actinomycetales	Streptomycetaceae	Kitasatospora	scaffold-3196	28554-36408
laccase	Bin.45	Draft	Tex	Cell	Min	GT2;AA1;GH18	Bacteroidetes	Cytophagales	Cytophagaceae	Emticicia	k99_70244	2923-9264
laccase	OC458	Assem.	BC	Cell	Min	GH30;1;GH43;AA1;AA1	Firmicutes	Bacillales	Paenibacillaceae	Paenibacillus	scaffold-71980	1776-14333
laccase	Bin.16	Draft	Tex	Cell	Min	AA1;GT2;CBM6;CBM6;GT4;CBM50	Planctomycetes	Planctomycetales	Planctomycetaceae	Gemmata	k99_772951	690-35445
laccase	Bin.12	Draft	JP	Cell	Min	GH13;GT41;AA1	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	k99_1039102	2419-19963
laccase	JE121	Assem.	JP	Cell	Org	GH65;GT41;AA1	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-138654	98-16625
laccase	TXA37	Assem.	Tex	Cell	Min	CBM13;AA1;AA1	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	k99_1267288	765-3222
laccase	A8055	Assem.	BS	Cell	Org	CE16;AA1;AA1	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-7250	10463-20720
laccase	A7026	Assem.	BS	Cell	Min	cohesin;CBM35;CBM5;AA1	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	scaffold-3510	15182-29445
laccase	BRM2_13C	Assem.	CA	Lig	Min	GH3;GH3;GH15;AA1;lacc;AA1	Proteobacteria	Burkholderiales	Comamonadaceae	Variovorax	scaffold-10184	7046-29625
laccase	A7026	Assem.	BS	Cell	Min	CE11;GT2;GT2;AA1;AA1	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	scaffold-349135	1932-10189
laccase	BRM3_13C	Assem.	CA	Lig	Min	GH1;GH1;GH1;AA1;AA1	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-2048	2458-9179
laccase	A9085	Assem.	BS	Cell	Org	GH27;CE6;GH55;GH97;GH39;GH120;GH11;GH9;GH51;GH43;GH95;GH3;CE6;CBM13;CE1;GH31;CE10;GH3;GH51;GH36;GH92;AA1;lacc;GH130;GH2;CBM32;CE1;GH43;GH5;13;CBM13;GT2	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-4490	11105-178981
laccase	Bin.11	Draft	BS	Cell	Min	GT2;GT2;AA1;GH2;GH53	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	k99_630852	5818-25520
laccase	TXA37	Assem.	Tex	Cell	Min	GT51;AA7;AA1;AA1;lacc	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	k99_410057	13757-20114
laccase	A9M2_13C	Assem.	BS	Lig	Min	AA1;AA1;lacc	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-1394	3598-5737
laccase	A8056	Assem.	BS	Cell	Min	GT2;GT2;CBM32;AA1;AA1	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-109	1830-14549
laccase	Bin.40	Draft	Tex	Cell	Min	CBM51;AA1;CBM48	Proteobacteria	Chromatiales	Ectothiorhodospiraceae	Nitrococcus	k99_1587968	3187-10845
laccase	Bin.10	Draft	BC	Cell	Min	GH19;GT51;lacc;CBM56;CBM2	Proteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacter	k99_2309801	1172-25857
laccase	BRM2_13C	Assem.	CA	Lig	Min	AA1;sm.lacc;AA1;CE11	Proteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacter	scaffold-4442	3422-7929
laccase	A8O2_12C	Assem.	BS	Lig	Org	CBM50;CE14;GT51;GH2;GT2;AA1;AA1	Proteobacteria	Legionellales	Legionellaceae	Legionella	scaffold-17	21640-83058
laccase	TXA37	Assem.	Tex	Cell	Min	AA1;AA1;AA1	Proteobacteria	Methylococcales	Methylococcaceae	Methylobacter	k99_802051	248-5945
laccase	Bin.21	Draft	CA	Cell	Org	GH65;GH65;AA1	Proteobacteria	Myxococcales	Cystobacteraceae	Stigmatella	k99_710181	1701-11075
laccase	A8M3_13C	Assem.	BS	Lig	Min	CE7;GT2;GT2;GH23;CE14;cohesin;AA1	Proteobacteria	Myxococcales	Myxococcaceae	Corallococcus	scaffold-139	697-30917
laccase	A8056	Assem.	BS	Cell	Min	GT2;CBM50;AA1	Proteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	scaffold-452078	764-24395
laccase	LHO3_13C	Assem.	CA	Lig	Org	AA1;AA1;AA1	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	scaffold-563	634-2779
laccase	A8055	Assem.	BS	Cell	Org	CE4;GT41;AA1	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	scaffold-156	735-10054
laccase	Bin.37	Draft	CA	Lig	Min	lacc;GH23;CE11;GT41;CBM2	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	k99_288891	8799-52380
laccase	TXA37	Assem.	Tex	Cell	Min	GT2;GT2;GT4;AA1	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	k99_51393	24292-38289
laccase	A7M2_13C	Assem.	BS	Lig	Min	CBM32;AA1;AA1	Proteobacteria	Rhizobiales	Methylocystaceae	Methylopila	scaffold-3314	1855-4974
laccase	Bin.12	Draft	JP	Cell	Min	AA1;AA1;lacc	Proteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	k99_4257	75-2310
laccase	JW014	Assem.	JP	Cell	Min	AA1;AA1;lacc	Proteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	scaffold-5331	1577-3794
laccase	JE122	Assem.	JP	Cell	Min	AA1;AA1;lacc;CBM16	Proteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	scaffold-489	75-7513
laccase	LHO3_13C	Assem.	CA	Lig	Org	AA1;lacc;AA1;lacc;AA1	Proteobacteria	Rhodospirillales	Acetobacteraceae	Acidiphilium	scaffold-1142	630-2906
laccase	A7026	Assem.	BS	Cell	Min	AA1;AA1;sm.lacc	Proteobacteria	Rhodospirillales	Acetobacteraceae	Gluconacetobacter	scaffold-1190	2748-4934
laccase	Bin.39	Draft	CA	Lig	Min	GH53;GH53;lacc;GT83	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella	k99_91797	848-22665
laccase	A7O2_13C	Assem.	BS	Lig	Org	AA1;AA1;AA1	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Rhodanobacter	scaffold-1779	971-2934
laccase	A7025	Assem.	BS	Cell	Org	CE3;GT1;GH78;AA1	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Rhodanobacter	scaffold-2524	7368-30232
laccase	Bin.1	Draft	CA	Lig	Min	CBM35;AA1;AA1	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	k99_696794	721-4014
laccase	Bin.16	Draft	Tex	Cell	Min	lacc;CBM32;GT51	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_44625	581-7869
laccase	TXA37	Assem.	Tex	Cell	Min	CE1;CE1;sm.lacc;sm.lacc	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_1318568	2262-24587
laccase	TXA37	Assem.	Tex	Cell	Min	GT2;GH8;CBM51;AA1;AA1	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_223923	558-14475
laccase	TXA37	Assem.	Tex	Cell	Min	sm.lacc;CBM32;GT2	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_137018	8120-20693
laccase	TXA37	Assem.	Tex	Cell	Min	GH53;GT35;AA1	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_181593	3893-9957
laccase	Bin.40	Draft	Tex	Cell	Min	GH53;GT35;AA1	Verrucomicrobia		uncl.	Chthoniobacter	k99_971100	4879-10943
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;lacc;AA1;3	Ascomycota	Pseudogymnoascus	Pseudeurotiaceae	Pseudogymnoascus	k99_1532366	4603-6745
laccase	JW013	Assem.	JP	Cell	Org	AA1;3;AA1;AA1	Ascomycota	Onygenales	Ajellomycetaceae	Blastomyces	scaffold-56842	290-1929

Table E.14... *continued*

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;AA1;3;AA1;3	Ascomycota	Onygenales	Arthrodermataceae	Trichophyton	k99_1558275	8285-10049
laccase	Bin.22	Draft	Tex	Cell	Org	CE1;CBM1;lacc;GH3;GH3	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_824021	7588-29529
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;2;sm.lacc;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_29332	438-2259
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;2;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_110531	1656-2937
laccase	Bin.22	Draft	Tex	Cell	Org	GH23;AA1;2;AA1;2;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_336935	385-6893
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;2;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_965275	707-1996
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;3;AA1;3;AA1;3	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_349944	1160-2814
laccase	TXA40	Assem.	Tex	Cell	Org	AA1;2;sm.lacc;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	scaffold-31345	89-2192
laccase	TXA40	Assem.	Tex	Cell	Org	AA1;2;sm.lacc;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	scaffold-20528	397-2009
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;2;AA1;2;AA1;2	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	k99_1360305	2801-4831
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;AA1;AA1	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	k99_1263514	843-2867
laccase	TXA40	Assem.	Tex	Cell	Org	AA1;AA1;AA1	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	scaffold-369338	9-2125
laccase	TXA37	Assem.	Tex	Cell	Min	AA1;AA1;AA1	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	k99_379059	2446-4578
laccase	TXA37	Assem.	Tex	Cell	Min	AA1;lacc;CBM2;CBM2;CBM2;GH55;GH55;CBM18	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	k99_122719	16637-44890
laccase	Bin.22	Draft	Tex	Cell	Org	AA1;3;AA1;3;AA1;3;AA1;3;AA1;3;AA1;3	Ascomycota	Sordariales	Sordariaceae	Neurospora	k99_125672	1667-4748
laccase	Bin.22	Draft	Tex	Cell	Org	PL1;10;PL1;10;PL1;10;CE5;CE5;AA1;3	Ascomycota	Sordariales	Sordariaceae	Neurospora	k99_1445522	2060-31698
laccase	TXA37	Assem.	Tex	Cell	Min	CBM2;CBM2;AA1;lacc	Ascomycota	Sordariales	Sordariaceae	Neurospora	k99_595818	56-9101
laccase	Bin.22	Draft	Tex	Cell	Org	GH43;AA1;3;AA1;3	Ascomycota	Sordariales	Sordariaceae	Sordaria	k99_440136	6533-26357
laccase	Bin.22	Draft	Tex	Cell	Org	GH13;5;GH13;5;GH43;AA1;3;AA1;3;AA1;3	Ascomycota	Sordariales	Sordariomycetidae	Melanocarpus	k99_524124	651-8035
laccase	JW013	Assem.	JP	Cell	Org	AA1;AA1;AA1	Ascomycota	Xylariales	Xylariaceae	Daldinia	scaffold-2085	1312-3222
laccase	A9085	Assem.	BS	Cell	Org	AA1;AA1;AA1;AA1	Ascomycota	Xylariales	Xylariaceae	Daldinia	scaffold-239	671-3409
laccase	Bin.9	Draft	CA	Cell	Min	AA1;3;AA1;3;AA1;3	uncl.	uncl.	uncl.	uncl.	k99_298110	239-2698
oxidase	A9085	Assem.	BS	Cell	Org	CBM32;AA3;CBM18;CBM50;GH43;GH10;GT2;GT22;GH27;CE10;van.oh.oxi;CE6;CE7;GT9;GH3;GH2;CE1;CE10;CBM32;GH108;GT2;GT19;GH23;GH23;GT2;CE4;GT41;CE9;GH20;GH1	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	scaffold-1955	2623-210198
oxidase	Bin.12	Draft	BS	Cell	Min	GT4;GH92;van.oh.oxi	Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium	k99_353450	11026-18644
oxidase	LH020	Assem.	CA	Cell	Min	AA3;GT2;ary.oh.oxi;GT2;GT2;GT2	Actinobacteria	Actinomycetales	Micromonosporaceae	Micromonospora	scaffold-581	1-18361
oxidase	TXA_Antib	Assem.	Tex	Lig	Min	GH1;GT9;AA2;GH15;van.oh.oxi;GT2	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	scaffold-2307	1358-70445
oxidase	TXA_Antib	Assem.	Tex	Lig	Min	GT8;GH23;GT4;van.oh.oxi;GT2	Actinobacteria	Actinomycetales	Nocardiaceae	Nocardia	scaffold-444	14052-81553
oxidase	A8M3_13C	Assem.	BS	Lig	Min	AA3;2;ary.oh.oxi;AA3;2	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	scaffold-8167	18-1436
oxidase	A7026	Assem.	BS	Cell	Min	GT2;GH33;CBM32;van.oh.oxi	Actinobacteria	Actinomycetales	Ruaniaceae	Ruania	scaffold-14	4518-16378
oxidase	Bin.16	Draft	JP	Cell	Min	CE1;CBM50;van.oh.oxi	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	k99_265676	1245-11720
oxidase	DC578	Assem.	BC	Cell	Min	CE1;CBM50;van.oh.oxi	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	scaffold-1323143	493-11106
oxidase	DC578	Assem.	BC	Cell	Min	CE1;van.oh.oxi;CBM32;GT9;GT2;GT2;GT2;GT2;GT2;GT4;GT4;GT2;GT83;GT2;GT2;GT4;CBM50;GT2;GT2;GT4;GT2;GT4;GT34;GT2;GT4;GT2;GT2;GT32;GT32;GT2;GT2;GT2;GT2;AA11	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	scaffold-4847	11682-96970
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM50;van.oh.oxi;GT51	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	k99_1401118	280-12942
oxidase	DC578	Assem.	BC	Cell	Min	van.oh.oxi;GT9;GT4;GT4	Actinobacteria	Solirubrobacterales	Patulibacteraceae	Patulibacter	scaffold-11046	27326-68977
oxidase	A8_Antib	Assem.	BS	Lig	Min	GH29;CBM9;ary.oh.oxi	Bacteroidetes	Cytophagales	Cyclobacteriaceae	Mariniradius	scaffold-3548	1042-8993
oxidase	BRO3_13C	Assem.	CA	Lig	Org	CBM51;CE1;van.oh.oxi	Bacteroidetes	Cytophagales	Cytophagaceae	Spirosoma	scaffold-402	7662-10934
oxidase	BRO3_13C	Assem.	CA	Lig	Org	CE9;ary.oh.oxi;CE4;CBM6	Bacteroidetes	Sphingobacteriales	Chitinophagaceae	Segetibacter	scaffold-765	5327-12507
oxidase	Bin.11	Draft	CA	Cell	Org	GH2;CE3;CE1;CE10;CE1;van.oh.oxi	Chloroflexi	Ktedonobacterales	Ktedonobacteraceae	Ktedonobacter	k99_271091	144-10487
oxidase	JS080	Assem.	JP	Cell	Min	CBM14;CE1;GH2;CE3;van.oh.oxi;CE1;CE1	Chloroflexi	Ktedonobacterales	Ktedonobacteraceae	Ktedonobacter	scaffold-3182	2048-25260

Table E.14... *continued*

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
oxidase	BLM1_13C	Assem.	CA	Lig	Min	AA3;AA3;ary.oh.oxi	Cyanobacteria	Nostocales	Rivulariaceae	Calothrix	scaffold-406	503-2947
oxidase	Bin.15	Draft	BS	Cell	Org	ary.oh.oxi;GT2;GH16	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	k99_805993	2307-10761
oxidase	JE121	Assem.	JP	Cell	Org	AA3;AA3;ary.oh.oxi	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-2058	15-2128
oxidase	TXA37	Assem.	Tex	Cell	Min	CE10;GH95;CE1;van.oh.oxi	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	k99_70196	1142-6851
oxidase	TXA37	Assem.	Tex	Cell	Min	AA4;van.oh.oxi;CBM32	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	k99_309180	11601-19514
oxidase	A7O2_13C	Assem.	BS	Lig	Org	AA4;AA4;van.oh.oxi;AA4	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-2132	3865-6110
oxidase	A9085	Assem.	BS	Cell	Org	GT4;CE1;AA6;van.oh.oxi	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-109	835-14080
oxidase	Bin.11	Draft	CA	Cell	Org	CE1;CE1;CE1;van.oh.oxi;CE1	Proteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	k99_127628	5142-13440
oxidase	A8M3_13C	Assem.	BS	Lig	Min	GH39;AA3;2;van.oh.oxi	Proteobacteria	Burkholderiales	Burkholderiaceae	Pandoraea	scaffold-1186	1016-8680
oxidase	JS080	Assem.	JP	Cell	Min	CE1;CE1;CE1;GT2;van.oh.oxi	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	scaffold-72749	3719-22575
oxidase	JW014	Assem.	JP	Cell	Min	AA3;AA3;2;ary.oh.oxi	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	scaffold-2826	2110-3374
oxidase	A8056	Assem.	BS	Cell	Min	CE1;CE1;GT2;van.oh.oxi	Proteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	scaffold-452765	6108-21565
oxidase	JE122	Assem.	JP	Cell	Min	GH18;GT2;GH51;CBM5;CBM50;GH53;GT4;AA6;van.oh.oxi;GT9;AA1;AA1;GH2;GT2;GT2;GT2;GT2;GT4;GH73;GH53;GT26;CBM5;CBM13;CBM32;GT51;GH28;GT2;CBM3;GT41;GT4;GT4	Proteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	scaffold-6	13429-216098
oxidase	JW014	Assem.	JP	Cell	Min	GT51;GT2;ary.oh.oxi;GH109	Proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	scaffold-388931	3321-21104
oxidase	Bin.12	Draft	JP	Cell	Min	CE1;GH18;AA7;van.oh.oxi;GH84;GT9	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	k99_973203	11698-105574
oxidase	Bin.12	Draft	JP	Cell	Min	GT9;van.oh.oxi;AA6	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	k99_798008	711-4629
oxidase	JW013	Assem.	JP	Cell	Org	GT2;GH28;CE11;GT4;GT51;GH2;GH3;GH78;GT2;AA6;van.oh.oxi;GT2;GH23;GH39;GT2;GT2;GH17;GH6;CE11;CBM48;GT2;CE11;GT2;CBM2;GH2;GT4;AA3;2;GT1;GT1;PL9;2;GT2;GT2;GT2;CBM50;CBM50;GH28;GH53	Proteobacteria	Burkholderiales	Oxalobacteraceae	Herbaspirillum	scaffold-843346	13569-320703
oxidase	BRM2_13C	Assem.	CA	Lig	Min	CE1;CE8;PL9;AA3;2;AA3;2;ary.oh.oxi	Proteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	scaffold-156	328-16328
oxidase	Bin.17	Draft	CA	Cell	Org	van.oh.oxi;GH3;GH5;4;GH17	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	k99_528430	1228-18527
oxidase	TXA40	Assem.	Tex	Cell	Org	AA3;GH3;ary.oh.oxi;GT4;CE1;GT19;GT2;GT2;CE16	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-751922	1239-43941
oxidase	BL043	Assem.	CA	Cell	Org	CBM35;ary.oh.oxi;GH130;CE12;GH109;CE6	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-217	2874-16284
oxidase	DC575	Assem.	BC	Cell	Org	CBM35;ary.oh.oxi;CE12;GH109	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-1120	5835-13449
oxidase	TXA37_12C	Assem.	Tex	Cell	Min	CBM35;ary.oh.oxi;CE12;GH109	Proteobacteria	Caulobacterales	Caulobacteraceae	Asticcacaulis	scaffold-1121	5835-13449
oxidase	Bin.7	Draft	BS	Lig	Org	van.oh.oxi;GT41;CBM50;GT4	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	k99_17159	219-19680
oxidase	OC458	Assem.	BC	Cell	Min	CBM51;GT28;GT4;AA7;GT4;GT51;CBM50;van.oh.oxi;GT2	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-381	7222-62784
oxidase	A7M1_13C	Assem.	BS	Lig	Min	GT26;AA3;ary.oh.oxi;AA3	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-7	1436-4774
oxidase	A7M2_13C	Assem.	BS	Lig	Min	AA3;ary.oh.oxi;AA3	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-3812	101-2110
oxidase	DC578	Assem.	BC	Cell	Min	van.oh.oxi;GT2;GT83	Proteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	scaffold-1323141	2248-13994
oxidase	TXA37	Assem.	Tex	Cell	Min	GT51;GH103;van.oh.oxi;GT41;CE14;CBM32;GH72;AA3	Proteobacteria	Chromatiales	Ectothiorhodospiraceae	Alkalilimnicola	k99_1136228	30147-103107
oxidase	Bin.10	Draft	BC	Cell	Min	GT2;GT4;ary.oh.oxi;GT2;GT2;GT4	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	k99_629962	2290-18595
oxidase	TXA37	Assem.	Tex	Cell	Min	GT26;van.oh.oxi;CE11;CBM13;GH18;GH1	Proteobacteria	Myxococcales	Myxococcaceae	Myxococcus	k99_616522	6810-54880
oxidase	BRM2_13C	Assem.	CA	Lig	Min	GH2;GT2;van.oh.oxi;GH109	Proteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	scaffold-10837	319-11365
oxidase	Bin.9	Draft	BC	Cell	Org	ary.oh.oxi;GH26;GT30;CE1	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Cellvibrio	k99_433757	1-11744
oxidase	BRM2_13C	Assem.	CA	Lig	Min	GT4;AA3;ary.oh.oxi	Proteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	scaffold-8160	5603-9762
oxidase	TXA37	Assem.	Tex	Cell	Min	GT4;van.oh.oxi;CBM50	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	k99_813141	26128-35395
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM14;van.oh.oxi;AA4;GT51;CE4	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	k99_160060	3195-16649
oxidase	BRM2_13C	Assem.	CA	Lig	Min	van.oh.oxi;CE10;AA2	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	scaffold-3102	4263-15793
oxidase	A7O2_13C	Assem.	BS	Lig	Org	AA3;2;AA3;2;AA3;2;ary.oh.oxi	Proteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	scaffold-3261	2368-4695
oxidase	Bin.1	Draft	CA	Lig	Min	GT2;PL1;van.oh.oxi	Proteobacteria	Rhizobiales	Rhodobiaceae	Lutibaculum	k99_798473	5956-16637

Table E.14... *continued*

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
oxidase	BRO3_13C	Assem.	CA	Lig	Org	CE10;GH39;GH3;GH3;van.oh.oxi	Proteobacteria	Rhizobiales	Rhodobiaceae	Parvibaculum	scaffold-1106	4272-10273
oxidase	TXA37	Assem.	Tex	Cell	Min	GT4;GT2;van.oh.oxi;GH31;CBM13	Proteobacteria	Rhizobiales	Xanthobacteraceae	Starkeya	k99_269685	6840-17977
oxidase	BRM2_13C	Assem.	CA	Lig	Min	dyp2;AA3;ary.oh.oxi;AA3	Proteobacteria	Rhodobacterales	Rhodobacteraceae	Thalassobacter	scaffold-1303	5267-10995
oxidase	A7M1_13C	Assem.	BS	Lig	Min	GH75;van.oh.oxi;GH53	Proteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	scaffold-353206	1772-12716
oxidase	TXA37	Assem.	Tex	Cell	Min	GT2;GT2;CE11;CBM13;ary.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Blastomonas	k99_727174	5169-11347
oxidase	TXA37	Assem.	Tex	Cell	Min	ary.oh.oxi;GH3;AA6;GH92	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	k99_166369	1184-15132
oxidase	TXA37	Assem.	Tex	Cell	Min	GH92;GH109;CBM35;ary.oh.oxi;GH2	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	k99_248725	1147-22803
oxidase	A7M1_13C	Assem.	BS	Lig	Min	GH2;van.oh.oxi;AA3;2	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-112	11595-36855
oxidase	A7O1_13C	Assem.	BS	Lig	Org	AA4;AA4;van.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-693	171-2081
oxidase	BRM2_13C	Assem.	CA	Lig	Min	AA3;2;AA3;2;ary.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-1298	1785-4135
oxidase	BRM2_13C	Assem.	CA	Lig	Min	AA3;2;ary.oh.oxi;AA3;2	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-9300	305-2347
oxidase	A9M2_13C	Assem.	BS	Lig	Min	AA4;AA4;van.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-1897	1174-3131
oxidase	A9M2_13C	Assem.	BS	Lig	Min	van.oh.oxi;AA3;2;AA3;2;ary.oh.oxi;GT9	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-1228	674-32478
oxidase	A9086	Assem.	BS	Cell	Min	AA3;AA3;ary.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	scaffold-574	3027-5011
oxidase	Bin.7	Draft	CA	Cell	Min	GH32;GH109;ary.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	k99_522714	5152-14423
oxidase	OC458	Assem.	BC	Cell	Min	GH74;GH74;GT2;GT2;GT4;GT4;ary.oh.oxi	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	scaffold-1416	962-15150
oxidase	DC578	Assem.	BC	Cell	Min	GT4;GT28;van.oh.oxi;GT41	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	scaffold-1845	11006-28720
oxidase	OC458	Assem.	BC	Cell	Min	AA11;CE14;GT51;van.oh.oxi;CBM13;GT2	Proteobacteria	uncl. Burkholderiales	uncl.	Rubrivivax	scaffold-713	2854-81867
oxidase	DC578	Assem.	BC	Cell	Min	CBM50;CBM51;CBM20;van.oh.oxi	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Dyella	scaffold-1306171	2190-24584
oxidase	BRM2_13C	Assem.	CA	Lig	Min	AA3;2;ary.oh.oxi;AA3;2;GT35	Proteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	scaffold-11	22109-28583
oxidase	DC578	Assem.	BC	Cell	Min	GT4;GT2;van.oh.oxi;GT83	uncl. Bacteria	uncl.	uncl.	uncl.	scaffold-1323154	12358-25225
oxidase	Bin.40	Draft	Tex	Cell	Min	CBM14;GT9;ary.oh.oxi	Verrucomicrobia	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	k99_1017246	3011-9565
oxidase	Bin.45	Draft	Tex	Cell	Min	CBM27;van.oh.oxi;CBM32;CBM48	Verrucomicrobia	Opitutales	uncl. Opitutaceae	Opitutaceae	k99_116819	2087-13205
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM32;CBM32;CBM35;CBM13;CBM26;CBM40;GH18;GT2;GH97;PL11;2;PL4;1;GH93;GT41;PL8;CBM47;CBM47;PL4;1;GH39;van.oh.oxi;GH109;CBM32;PL11;2;CBM32;GH38;CBM32;GT83;CE11;GT2;CBM6;CBM62;CBM62;CBM62;GH10;GH109;CE1;GH10;GH43;GH25;GT2;GT2;GT2;GT2;GT2;GT2;CBM66;GH127;CBM35;CBM5;GH43;GT2	Verrucomicrobia	Verrucomicrobiales	uncl.	Pedosphaera	k99_260195	2693-310068
oxidase	TXA37	Assem.	Tex	Cell	Min	GT2;van.oh.oxi;CBM13;GH13;CE11	Verrucomicrobia	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	k99_193390	10277-48642
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM48;van.oh.oxi;GT83	Verrucomicrobia	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobium	k99_1062264	763-6837
oxidase	TXA37	Assem.	Tex	Cell	Min	PL4;PL11;2;ary.oh.oxi	Verrucomicrobia		uncl.	Chthoniobacter	k99_383562	22-13016
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM32;CBM6;CBM6;GT19;van.oh.oxi;GH13;16	Verrucomicrobia		uncl.	Chthoniobacter	k99_797249	4272-40194
oxidase	TXA37	Assem.	Tex	Cell	Min	CBM14;GT9;ary.oh.oxi	Verrucomicrobia		uncl.	Chthoniobacter	k99_227527	3011-9565
oxidase	TXA40	Assem.	Tex	Cell	Org	AA3;2;AA3;2;ary.oh.oxi;AA3;2;AA3;2	Ascomycota	Glomerellales	Plectosphaerellaceae	Verticillium	scaffold-87	3589-5618
oxidase	TXA37	Assem.	Tex	Cell	Min	AA3;2;AA3;2;AA3;2;ary.oh.oxi	Ascomycota	Glomerellales	Plectosphaerellaceae	Verticillium	k99_1426652	1842-3647
oxidase	JW013	Assem.	JP	Cell	Org	AA3;2;AA3;2;AA3;2;ary.oh.oxi	Ascomycota	Hypocreales	Hypocreaceae	Trichoderma	scaffold-767073	1-1925
oxidase	JW013	Assem.	JP	Cell	Org	AA3;2;AA3;2;ary.oh.oxi	Ascomycota	Hypocreales	Hypocreaceae	Trichoderma	scaffold-456930	3794-5630
oxidase	Bin.22	Draft	Tex	Cell	Org	AA4;AA4;van.oh.oxi;AA4;AA4	Ascomycota	mitosporic Onygenales	Eurotiomycetidae	Coccidioides	k99_78246	433-2339

Table E.14... *continued*

CAZyme	SampleID	Source	Eco.	Subs.	Hor.	Cazyme Cluster	Phylum	Order	Family	Genus	Contig	Location
oxidase	Bin.22	Draft	Tex	Cell	Org	AA3;2;AA3;2;AA3;2;ary.oh.oxi;AA3;2;GH31;GH31	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_469555	2585-14254
oxidase	TXA37	Assem.	Tex	Cell	Min	GH76;van.oh.oxi;GT2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_1289398	8931-38076
oxidase	TXA37	Assem.	Tex	Cell	Min	GH109;GH51;GH51;GH51;van.oh.oxi	Ascomycota	Sordariales	Chaetomiaceae	Thielavia	k99_414188	29216-45953
peroxi	DC578	Assem.	BC	Cell	Min	CBM32;CBM32;CBM32;AA2	Actinobacteria	Actinomycetales	Frankiaceae	Frankia	scaffold-354	70-6228
peroxi	TXA_Antib	Assem.	Tex	Lig	Min	GH1;GT9;AA2;GH15;van.oh.oxi;GT2	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	scaffold-2307	1358-70445
peroxi	TXC148	Assem.	Tex	Cell	Org	ver.perox;GH35;GT2	Actinobacteria	Actinomycetales	Promicromonosporaceae	Xylanimonas	scaffold-103129	410-8446
peroxi	Bin.1	Draft	JP	Cell	Org	GH32;AA2;GT2;GT2;CE4	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	k99_423361	2688-18835
peroxi	Bin.17	Draft	BS	Cell	Org	GH13;26;CBM48;GH20;AA2	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	k99_178235	3568-13274
peroxi	BR_Antib	Assem.	CA	Lig	Min	GT4;GT2;CBM32;AA2;AA2;CBM4;CBM50	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-179	30319-63022
peroxi	DC578	Assem.	BC	Cell	Min	GT4;AA2;GH2;GH53;CE14	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-4846	3278-34214
peroxi	A8M3_13C	Assem.	BS	Lig	Min	AA2;AA2;AA2	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	scaffold-5768	240-2770
peroxi	DC578	Assem.	BC	Cell	Min	GT83;CE8;GT4;GT2;GT83;AA2;CBM50	Firmicutes	Bacillales	Alicyclobacillaceae	Alicyclobacillus	scaffold-5408	172-33467
peroxi	A7026	Assem.	BS	Cell	Min	AA2;CE14;GH65	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-224	1714-27222
peroxi	OC458	Assem.	BC	Cell	Min	PL11;2;GH128;GH128;AA2	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-414	4410-16251
peroxi	JE121	Assem.	JP	Cell	Org	GH28;GT51;CBM5;AA2;GH3;CBM53;CE10	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-47	328-75445
peroxi	A7M2_13C	Assem.	BS	Lig	Min	CE1;GH39;CBM2;AA2	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-401	777-20133
peroxi	A8055	Assem.	BS	Cell	Org	AA2;GH23;GH23	Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	scaffold-5535	4597-9679
peroxi	Bin.11	Draft	JP	Cell	Min	CBM13;CBM13;CBM5;GH5;AA2;CE10;GT35;GH78;CBM50	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	k99_366491	20251-57297
peroxi	JS080	Assem.	JP	Cell	Min	GH23;AA2;GH12	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	scaffold-3043	4363-12183
peroxi	TXA37	Assem.	Tex	Cell	Min	AA2;AA2;GH102;CBM14;GT1;GT4;GT4;GT1;GT1	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	k99_373252	7468-44019
peroxi	JW014	Assem.	JP	Cell	Min	GH3;GH3;GH78;AA2	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	scaffold-3189	10878-24752
peroxi	A8056	Assem.	BS	Cell	Min	GT4;GH12;AA2;GH51	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	scaffold-295	3692-16860
peroxi	JE122	Assem.	JP	Cell	Min	CBM50;GH78;GT35;CE10;AA2;CBM13;CBM13;CBM5;GH5	Proteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	scaffold-407992	5342-42388
peroxi	OC458	Assem.	BC	Cell	Min	GH92;GH92;AA3;GT2;AA2;CBM50;CBM50	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-1346	11375-80365
peroxi	TXA37	Assem.	Tex	Cell	Min	GH3;GT2;GH3;GH78;GT35;AA2;GH43;GH43;GH32	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	k99_279297	11487-47415
peroxi	A9085	Assem.	BS	Cell	Org	GH24;AA2;GH132;CBM50;GH13;23;CE6;GH4;CE1;GT51;CE1;CBM50;CBM18;AA10;CBM13;GT4;CE1;GT41;GT2;CE6;GT2;GH18;GT41;GT32;GT32;GH28;GT2;GH3;GT4;CBM57;CE10;GT2;GH29;GH1	Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	scaffold-9	133-263677
peroxi	BRM2_13C	Assem.	CA	Lig	Min	van.oh.oxi;CE10;AA2	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	scaffold-3102	4263-15793
peroxi	TXA_Antib	Assem.	Tex	Lig	Min	AA3;AA2;AA2	Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Nitrobacter	scaffold-398	52-5641
peroxi	TXA37	Assem.	Tex	Cell	Min	GT90;AA2;GT4;GH3	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	k99_978139	384-31846
peroxi	TXA37	Assem.	Tex	Cell	Min	GH12;GT51;AA2;AA12;CBM2;AA9;CE14;GH3	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	k99_444936	8022-76017
peroxi	A9086	Assem.	BS	Cell	Min	GH2;AA2;AA2	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	scaffold-73	4353-12388
peroxi	A7M2_13C	Assem.	BS	Lig	Min	AA2;AA2;GT2	Proteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis	scaffold-810	5285-8158
peroxi	TXA37	Assem.	Tex	Cell	Min	AA2;CBM32;CBM50;GH128;PL11;2	Verrucomicrobia	Verrucomicrobiales	uncl.	Pedosphaera	k99_680169	1678-32259
peroxi	Bin.22	Draft	Tex	Cell	Org	ver.perox;AA5;1;AA5;1	Ascomycota	Glomerellales	Plectosphaerellaceae	Verticillium	k99_231870	2465-4926
peroxi	JW013	Assem.	JP	Cell	Org	GH88;GH28;ver.perox	Ascomycota	Hypocreales	Hypocreaceae	Trichoderma	scaffold-843429	2919-11503
peroxi	Bin.22	Draft	Tex	Cell	Org	GT4;AA2;CBM50;CBM50	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_1470342	5281-15115
peroxi	Bin.22	Draft	Tex	Cell	Org	CE10;GT4;CBM1;AA2;CBM50;CBM50	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_414950	20866-38194
peroxi	TXA37	Assem.	Tex	Cell	Min	GH13;40;CBM50;CBM50;AA2;GT4;CE10;AA7;GT4;GT4	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_204014	8277-61013
peroxi	TXA37	Assem.	Tex	Cell	Min	AA5;1;AA5;1;AA5;1;AA2	Ascomycota	Sordariales	Chaetomiaceae	Chaetomium	k99_253334	1-3086
peroxi	TXA37	Assem.	Tex	Cell	Min	AA2;AA2;AA2	Ascomycota	Sordariales	Sordariaceae	Neurospora	k99_638427	491-1782