FUNCTIONAL AND COMPOSITIONAL RESPONSES OF MICROORGANISMS TO RECLAMATION OF SURFACE-MINED BOREAL FOREST SOILS

by

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ABSTRACT

Over the last four decades, surface mining of oil sands in the boreal forest of western Canada has created large areas of disturbed land. The current regulatory framework requires that derelict land be reclaimed to pre-disturbance conditions. This has prompted the need to assess the effectiveness of reclamation, which relies on the use of salvaged materials (e.g., tailings sand and overburden), on key ecosystem components such as soil microorganisms. In this thesis, I examined landscape-scale changes in soil microbial community composition and function in response to different reclamation amendments and in natural sites comprising a regional environmental gradient. Using molecular fingerprinting (phospholipid fatty acids and denaturing gradient gel electrophoresis) and phylogenetic analyses of 16S rRNA genes, I found that microbial communities in natural soils differed from those of reclaimed soils. This dissimilarity was driven by increasing abundances of fungal and actinomycetal biomarkers in natural soils. After 30 years, however, reclamation did not place soil microbial communities on a predictable recovery path. The composition of microorganisms was particularly affected by tailings sand-based amendments. Functional potential, determined with assays targeting the activities of enzymes responsible for macromolecule degradation, was mainly impacted by prescriptions containing overburden. Variance partitioning analyses indicated that microbial responses to reclamation were partially determined by vegetation cover development, soil pH, and the fungal-to-bacterial biomass ratio. pH effects on bacterial composition were partly driven by the abundance of Acidobacteria. The relative abundances of several bacterial biomarkers covaried with individual enzyme activities, suggesting certain sub-sets of the microbial communities were functionally relevant. I tested this idea experimentally by assembling a laboratory-scale reciprocal transplant of microorganisms

sourced from two distinct peat types. My main finding was that differences in initial microbial community composition were functionally significant for lignin depolymerization, while the activities of nutrient-acquiring enzymes (a more ubiquitous function) were mostly influenced by peat type. Overall, my results indicate that the responses of abundant microbial populations to reclamation were largely accounted for by abiotic properties of reclamation materials and, indirectly, by the effects of reclamation on plant growth.

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CO-AUTHORSHIP STATEMENT

Chapter 2 was co-authored with Cindy Prescott, Sylvie Quideau, and Susan Grayston. I performed most of the research, analyzed the data, and wrote the manuscript. Cindy Prescott, Sylvie Quideau, and Susan Grayston identified and designed the original research program and assisted with data analysis and manuscript revision. Sylvie Quideau was responsible for the PLFA analysis.

Chapters 3 and 4 were co-authored with Sue Grayston and David Lee. David Lee and I performed the research. I designed the experiments, analyzed the data, and wrote the manuscript. Susan Grayston assisted with experimental design and manuscript revision.

1 INTRODUCTION

1.1 General context

Over the last four decades, surface mining of oil sands in mixed-wood boreal forest of northeastern Alberta, Canada has created large areas of disturbed land. Bituminous sand reservoirs contain an estimated 1.6 trillion barrels of oil, of which 311 billion are allegedly recoverable with current mining technologies (Alberta Government, 2007). The three main companies operating in the region —Albian Sands, Syncrude Canada, and Suncor Energy— are exploiting a combined ~150 km^2 of land (Alberta Government, 2007). Typically, access to oil sands entails removing the vegetation cover, topsoil, and deep geological overburden overlying the oil deposits. The bitumen is then separated from the sand using a hot water extraction process, which results in nutrient-poor, high-pH tailings sand containing residual bitumen (Fung and Macyk, 2000; MacKinnon et al., 2001).

Current regulations require that disturbed areas be reclaimed to diverse, self-sustaining boreal forest communities similar to those in the surrounding region (Oil Sands Vegetation Reclamation Committee, 1998; Fung and Macyk, 2000). The different steps involved in reclamation of oil-mined land impose unique constraints on microbial components of the soil decomposer community. For instance, stockpiling of salvaged soils used for reclamation selects for certain bacterial guilds and reduces fungal biomass (Harris, 1993), which may have as-yet unexplored impacts on nutrient cycling rates once the material has been applied onto disturbed land. The type of reclamation treatment (prescription) often influences the rates of seedling emergence and the establishment of plant communities following revegetation (Gretarsdottir et al., 2004; Mackenzie and Naeth, 2009); similarly, bacterial community structure can respond more clearly to amendments than plant density at

early stages of reclamation (Machulla et al., 2005). These considerations are important, because microorganisms contribute to the maintenance of plant diversity and productivity, indirectly through their influence on the availability of soil nutrients or directly via root-associated organisms that form mutualistic relationships with plants (van der Heijden et al., 2007). If at broad (e.g., regional) scales plant and microbial responses to biotic and abiotic changes are coupled, then the identification of abundant microbial groups and the processes they mediate in reclaimed soil may be essential to understanding how a management framework intended to recover a productive upland boreal forest is affecting plant communities.

1.2 Microbial ecology of boreal forest soils

In boreal forests, fungi play crucial roles in the cycling of carbon and nutrients through the ecosystem. Saprotrophic fungi are the principal decomposers of wood and litter (Rayner and Boddy, 1988) and obtain energy by degrading dead organic matter. Mycorrhizal fungi, in contrast, obtain energy mainly as photosynthates supplied by symbiotically associated plants and in return provide their plant hosts with soil-derived nutrients (Smith and Read, 1997). Boreal forest soils are typically acidic with episodic availability of mineral nutrients (N and P) and high C:N ratios due to the surface accumulation of recalcitrant organic matter resulting from incomplete oxidation of plant material (Prescott et al., 2000; Allen et al., 2003). The forest floor is the most metabolically active fraction of these soils and is heavily colonized by ectomycorrhizae (ECM) and ericoid mycorrhizae (ERM) root systems of trees and understory vegetation (Lundström et al., 2000). Indeed, ITS sequencing has pointed to a strong positive correlation between overstory tree composition and ECM fungal species

composition in organic horizons of mixed-wood forests (Kernaghan et al., 2003; De Bellis et al., 2006).

While the forest floor contains a large proportion of the microbial biomass (up to one third of which is composed of ECM extraradical mycelium; Högberg and Högberg, 2002), changes with depth in soil chemistry and mineralogy (e.g., soluble Al-organic-acid complexes) also create suitable habitats for fungi—for instance, Rosling et al. (2003) documented clear ECM compositional shifts down a soil profile, with about 50% of the taxa restricted to mineral horizons. This vertical stratification of ECM is interrelated to the depth distribution of saprotrophic fungi and appears to underpin a spatial separation of function: saprotrophic fungi are primarily confined to recently-shed litter and upper portions of the forest floor, where organic carbon is typically mineralized and nitrogen is retained; ECM dominate in the underlying layers, where presumably they mobilize N and make it available to their host plants (Lindahl et al., 2007; Luis et al., 2005).

While functional and taxonomic aspects of fungal communities in boreal forests are well understood, the distribution of bacterial communities has not been extensively documented. In boreal environments, the primary method for studying below-ground microorganisms—phospholipid fatty acid (PLFA) profiling—has enough resolution to provide information on the presence of broad taxonomic groups such as Gram-negative bacteria, Gram-positive bacteria, actinomycetes, and fungi (Leckie, 2005). Using PLFA profiling, several studies have provided evidence for direct links among site productivity parameters (e.g., tree growth and soil nutrient concentrations), vegetation composition, and microbial community composition.

Pennanen et al. (1999) and Myers et al. (2001) examined phospholipid fatty acid PLFA patterns of microbial populations across fertility gradients in northern Scandinavia. While microbial biomass was constant among sites, changes in the community structure — e.g., enrichment of signatures corresponding to Gram-positive bacteria— and a decrease in the relative proportion of fungal to bacterial PLFAs were found in sites that were more productive, had higher nitrogen mineralization rates, and nitrogen availability. These changes were tentatively attributed to tree-specific effects via different litter qualities and quantities incorporated into soil, likely in the form of litter and rhizodeposits. In laboratory incubations, Saetre (1998) found that soil microbial communities from birch and spruce forests differed and that mixtures of the two soils yielded assemblages expected from the pure soils alone. Similarly, Saetre and Bååth (2000) observed that the PLFA differences between spruce and birch soils were the same as differences between soils sampled in the vicinity of trees from the same species in a mixed stand. Again, these patterns lent support to the idea that microbial community composition may be related to the tree species' influences on soil organic matter, rather than the effect of tree species on soil physical properties.

Although it appears that tree species influence the microbial community, these effects may not occur in a predictable manner and likely interact with other site characteristics. In microcosms containing mineral soil planted with Scots pine, Norway spruce, and silver birch, Priha et al. (1999) detected no differences in the PLFA and CLPP profiles among tree species. When the experiment was repeated with forest floor materials, there was an added effect of tree species: the proportions of Gram-positive PLFAs and fungal PLFAs were higher in the birch microcosm than in the other treatments. In a study using monospecific stands of the same species as above, Priha et al. (2001) found species-specific PLFA profiles,

but their concentration patterns differed with respect to the pot study, suggesting that the quantities and qualities of soil organic materials are also regulated by the availability and biochemical composition of the litter derived from the dominant tree species' understory vegetation, as well as from root exudates (Grayston and Campbell, 1996).

A recent report linked quality and heterogeneity, as well as diversity, of organic substrates found in boreal forest soils to plant growth and activity and structure of the microbial community. In a greenhouse experiment, Dehlin et al. (2006) added six organic substrates (charcoal, berries, sporocarps, vertebrate faeces and leaf litter) singly or in mixtures of up to six resource types to microcosms containing forest floor (with or without silver birch seedlings). The largest positive effects of single substrates on microbial basal respiration, substrate-induced respiration, and microbial metabolic quotient (qCO_2) were found for nutrient-rich substrates or substrates with high sugar content; greater diversity of resources had no influence on catabolic diversity. In contrast, plant growth was inhibited by single-substrate additions, perhaps due to microbial competition for nutrients. Heterogeneous mixtures of substrates, especially those containing sporocarps and leaf litter —i.e., N- and P-rich— enhanced seedling nutrient uptake. In a similar laboratory set-up using mineral soil from the boreal forest, Orwin et al. (2006) used combinations of up to eight synthetic C substrates of varying complexity. Their results supported those by Dehlin et al. (2006): the identity of the compounds significantly affected microbial function (decomposition of cellulose paper and respiration) and structure (PLFA composition), while C substrate diversity effects saturated at low levels of diversity. Thus, there appear to be landscape-level patterns of microbial community composition in forest ecosystems harboring different plant species and soil process rates. These patterns seem to be driven by macroclimate and soil

type differences; the different vegetation communities, in turn, largely dictate the quality and quantities of substrate inputs.

1.3 Functional implications of microbial community diversity

Over the last 15 years, accelerated rates of species loss have stimulated extensive research addressing the functional significance of diversity erosion, mainly of terrestrial plants (Loreau et al., 2002). In this sense, acquiring knowledge on the relationship between soil microbial diversity[1] and function may provide insight into how key ecosystem processes (e.g., decomposition and biogeochemical cycling) respond to environmental change. Because of their overwhelming diversity, however, microorganisms are rarely explicitly considered in ecosystem process models (Schimel and Gulledge, 1998). Soil disturbance can result in the loss of abundant microbial groups (Degens et al., 2000; Gómez et al., 2004; Nsabimana et al., 2004). However, the extent to which microbial diversity (both richness and composition) affects terrestrial process rates and influences ecosystem responses to disturbances such as N addition and land use change is unclear (Nannipieri et al., 2003).

A question that has been raised regarding the decline in soil microbial diversity is whether it will affect the ability of the resulting communities to continue performing their functions. One body of opinion holds that species richness is not important, because the diversity of the soil microbial genebank is generally so high that microorganisms can always play their full part in ecosystem functions (Andrén and Balandreau, 1999). Indeed, the use of

[1] *Microbial diversity* is a multifaceted concept that includes genetic diversity within microbial species (more generally, *o*perational *t*axonomic *u*nits—OTUs), diversity of microorganisms within communities, and ecological diversity, that is, variation in community composition, complexity of interactions, number of trophic levels, and number of guilds (Nannipieri et al., 2003). For practical purposes, the diversity of a community is commonly described with two metrics: species richness (the number of species) and evenness (the relative abundance of species) (Magurran, 2004).

molecular techniques has shown that the number of microbial species in soil is much higher than revealed by cultivation-based studies. Current soil bacterial species richness estimates range from < 100 (Kemp and Aller, 2004) and ~10^3 (Hong et al., 2006) to almost 10^6 (Gans et al., 2005) in a ~1-g sample; about 350 and 8000 distinct units can be found in sandy soil and organic soils, respectively (Øvreås and Torsvik, 1998).

Conversely, it has been speculated that high biodiversity may be vitally important in structurally diverse ecosystems such as soil because it may promote stability of this environment (Grime, 1997; van Bruggen and Semenov, 2000). In soil, establishment of an unfavorable environmental condition can result in the inhibition of some populations that perform essential functions. In highly diverse communities, however, there is a higher probability for the co-occurrence of microorganisms that perform the same function but have different ecophysiological strategies and tolerances to environmental insults (van Bruggen and Semenov, 2000). However, the central question is how widely specific ecological functions are distributed among the soil microbial community. In fact, some functions are more general and performed by a greater number of species than others. For example, one could expect that many soil microbial species are capable of degrading cellulose due to its abundance and importance as source of carbon and energy in soil environments. In contrast, fewer species are expected to degrade specific anthropogenic-made compounds such as pesticides. This idea has been corroborated by studies demonstrating that the effect of stress or disturbance on the functional stability of soils depends on the level of specificity of the function. For example, functions performed by a broad array of microorganisms (e.g., decomposition of organic matter) are generally not affected by declines in microbial diversity (Wertz et al., 2006), whereas more specialized niche functions such as nitrification,

denitrification, methane oxidation, and mineralization of xenobiotics tend to decrease as biodiversity decreases (Griffiths et al., 2000; Girvan et al., 2005; but see Wertz et al., 2006). A few studies have also demonstrated that cultivated soils that have undergone losses of soil organic matter have reduced catabolic diversity (Degens et al., 2001) or functional stability (Griffiths et al., 2001) compared to paired undisturbed sites when exposed to further physical and chemical stresses simulated in laboratory.

While the microbial biomass mediates key functions such as nutrient cycling and energy flow, it is not clear whether microbial community composition is functionally relevant. Changes in the soil environment, for example, often lead to alterations in community composition, declines in microbial biomass, and changes in extracellular enzyme activity levels (Waldrop et al., 2000; Bossio et al., 2005). In these correlative studies, composition effects cannot be completely separated from the abiotic differences in the altered habitats. Also, this approach assumes that the environment ultimately controls ecosystem process rates; that is, changes in microbial community composition exert proximate controls on process rates but the environment ultimately structures the community. Reed and Martiny (2007) discuss two additional experimental models that can be used to address whether community composition matters for ecosystem functioning: common gardens and reciprocal transplants. In the former, distinct communities are reared in a uniform environment, such that differences in ecosystem functioning can be directly attributed to differences in microbial composition (Langenheder et al., 2005, 2006). Reciprocal transplants combine correlative and common garden experiments, simultaneously testing for interactive effects of the environment and compostion on process rates. Reciprocal transplants and common gardens under controlled conditions are indeed yielding evidence that microbial communities

and the environment interactively influence functioning, both for taxonomically-broad (Strickland et al., 2009a; Strickland et al., 2009b) and for narrowly-defined (Reed and Martiny, 2007) functions. The suitability of these hypotheses-driven approaches, however, has not been assessed in the context of

1.4 Mining of the Athabasca oil sands

1.4.1 Reclamation materials and prescriptions

Reclaimed soil profiles or prescriptions are derived from a variety of materials that are either salvaged to aid soil restoration or are by-products of the mining process. These include (i) peat deposits from northern Alberta (usually of the humic and mesic types) mixed with the underlying mineral material creating a peat-mineral mix (P/M), (ii) forest-floor material salvaged from upland forest sites or lowland material high in organic matter (direct placement, DP), and (iii) Pleistocene deposits (secondary, 2^0); underlying materials are (i) Cretaceous overburden (OB) and (ii) tailing sands (TS), a fine sand that constitutes one of the final products of the hydrocarbon removal process (Yarmuch, 2003). (See Appendix A, Table A.1 for a detailed physico-chemical description of reclamation materials.) Current reclamation practices comprise the following prescriptions (i.e., combinations of different materials named with capital letters): *M*: TS amended with a 70-cm layer of 2^0 capped with a 20-cm layer of P/M; *F*: OB covered with a 50-cm layer of DP; *E*: a 20-cm stratum of P/M underlain by 30 cm of 2^0 and OB; *I*: 20 cm of P/M capping on OB; *H*: TS covered with 20 cm of P/M; *B*: a 50-cm layer of DP on TS; and *A*: TS capped with 50 cm of 2^0 and 20 cm of P/M. Table 1 shows a schematic representation of the amendments, and highlights the depth variability of the capping materials.

1.4.2 Revegetation

According to soil remediation standards, a reclaimed growing medium should support a healthy plant community that will evolve toward an ecosystem comparable to that of neighboring natural areas, collectively termed target *ecosites*[2]. Under the Environmental Protection and Enhancement Act, the objective of reclamation and conservation is to return disturbed land to a land capability equal to that found before surface mining. The industrial developers must restore the native ecosites at the mining sites within 15 years of oil extraction in order for the provincial government to assume responsibility for the area (Oil Sands Vegetation Reclamation Committee, 1998). To achieve this goal, the following steps are typically taken after mining. Topsoil spreading generally occurs in the winter and early spring seasons. Revegetation of all reclaimed areas involves planting a primary cover crop of barley followed by the immediate planting of a variety of native herbaceous and woody plant species to restore the native ecosite phases (Yarmuch, 2003). The choice of barley to aid in reclamation reflects its ability, particularly in the presence of peat, to stabilize soil and facilitate leaching of ions by root penetration into the substrate (Renault et al. 2003). Examples of woody species include white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*), jackpine (*Pinus banksiana*), and paper birch (*Betula papyrifera*). Some of the main woody shrub species include green alder (*Alnus crispa*), red-osier dogwood (*Cornus stolonifera*), low-bush cranberry (*Viburnum edule*), and common blueberry (*Vaccinium myrtilloides*) (Fung and Macyk, 2000; Yarmuch, 2003). Forest management governmental

[2] Ecosites are ecological units that develop under similar environmental influences –climate, moisture, and nutrient regime. Each ecosite is designated with a small letter: letter "a" represents the driest and most nutrient-poor ecosite; the last letter in the series –k— is the wettest and most nutrient-rich. Ecosites are frequently named after plant species that are typical of the ecosite (e.g., a, lichen). Ecosite *phases* are subdivisions of main ecosites based on the dominant canopy species (e.g., a1, lichen jack-pine) (Beckingham and Archibald, 1996).

regulation stipulates that the seed collection for revegetation must come from within a 80-km radius (Yarmuch, 2003). In general, fertilization at Suncor operations occurs during the first four years after revegetation; in the case of Syncrude, only in the first year.

1.4.3 Microbial communities

Most research concerning the effects of mining and ensuing reclamation on the Athabasca oil sands region has revolved around fungal communities. Danielson and colleagues (1983) and Abbott and Robson (1991) determined that the level of occurrence of arbuscular mycorrhizal and ectomycorrhizal fungi in highly disturbed mine spoils is low; comparable results were obtained for ascomycetous fungi (Visser, 1985). More recently, Bois et al. (2005) found that pure reclamation materials (TS, CT, and OB) planted with red clover (*Trifolium pratense* L.), hybrid poplar (*Populus deltoides*), and jack pine (*Pinus banksiana*) supported low levels of ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) propagules. In contrast, reclaimed sites (*I* series) of different ages harbored higher inoculum potential of ECM and AM, with older sites (14 and 20 yrs) supporting the highest inoculum potential of ECM. The low levels of mycorrhization in pure reclamation materials were attributed to a lack of key nutrients – e.g., Ca, Mg, K— essential for plant growth and fungal colonization, but residual bitumen may have had a negative impact as well.

Another important biotic component of reclamation materials is the prokaryotic microbial community. Thus far, little has been advanced with regard to this aspect in the context of the Athabasca oil sands region. Comparing reclaimed oil sands tailings sites with undisturbed oil sand sites, Visser (1985) found significantly more culturable bacteria in the 0-15cm surface soils of undisturbed sites than in any sub-surface soils and the 0-5cm surface

soil in the reclaimed site. In addition, considerable research has been conducted to elucidate microbial degradation mechanisms of naphtenic acids — naturally occurring, linear and cyclic carboxylic surfactants associated with the acidic petroleum fraction obtained during the oil extraction process— in laboratory microcosms (Holowenko et al., 2001; Clemente and Fedorak, 2005; Hadwin et al., 2006). Using molecular fingerprinting (DGGE) and chemotaxonomic (PLFA signatures) techniques, Hadwin et al. (2006) were able to group microbial communities retrieved from wetland sediments according to the levels of naphtenates they were exposed to, and revealed that actinomycete lipid signatures increased in localities with high naphtenate concentrations. In the first study directly addressing the effects of oil sands reclamation on microbial dynamics, McMillan et al. (2007) reported lower nitrogen mineralization and microbial biomass in peat-mineral-mix-based amendments, but provided no taxonomic information on putative microorganisms involved in nutrient fluxes.

1.5 Thesis objectives

My thesis addresses the following overarching question: Does a suite of reclaimed sites with different developmental histories place the microbial community on a putative recovery path toward a “natural” state, and, if so, are some prescriptions more appropriate than others for the re-establishment of the microbial community? I focus on the following three objectives:

i. To evaluate changes in soil bacterial and fungal community composition and function in response to different prescription/age-since-reclamation combinations in post-mining boreal forest soils.

ii. To assess broad-scale phylogenetic patterns of bacteria in soils of natural boreal forest sites characterized by contrasting moisture and nutrient regimes.

iii. To test the functional significance of bacterial communities sourced from distinct peat types.

1.6 Thesis overview

In Chapter 2, I assess the composition and function of microbial communities across a chronosequence of soils reclaimed with different prescriptions in the Athabasca oil sands region. For comparative purposes, I also examine microbial community patterns in a series of adjacent sites representing a natural fertility gradient**.** I tested the hypotheses that (i) microbial community structure and function in older reclaimed sites would be more similar to natural sites than to younger reclaimed sites, and (ii) microbial community composition and function in reclamation prescriptions covering tailings sand would be distinct from communities found in natural forests**.** Additionally, I statistically examine the relative contribution of plant cover, spatial variability, and soil chemical properties in explaining shifts in the composition and function of soil microorganisms.

I use PLFA and bacterial and fungal denaturing gradient gel electrophoresis (DGGE) fingerprinting to assess microbial community composition. To examine functional shifts, I measure the potential activities of nutrient-acquiring and lignin-degrading extracellular enzymes. The rationale for using enzymatic activities as measures of functional potential is two-fold: (i) at the community level, the distribution of extracellular enzyme activities can be interpreted as a resource allocation strategy for the decomposer community and thus can be indirectly linked to soil resource quality and availability (Sinsabaugh et al., 2002); (ii) the

estimation of activities related to the turnover of C, N, and P, as well as lignin degradation, should enable the calculation of integrative diversity indices that in turn allow direct comparison of enzyme functional diversity with the taxonomic diversity of the soil microbial community and the above ground plant community (Caldwell, 2005). Additionally, while enzyme activities and nutrient cycling rates in boreal peatlands are well studied (Thormann, 2006), the distribution of extracellular enzymes in upland boreal forest soils is incompletely understood.

In Chapter 3, I use a clone-library-based approach to quantify soil bacterial β-diversity (i.e., inter-site compositional turnover) patterns of 16S rRNA genes across natural boreal forest sites belonging to two distinct fertility classes and contrast them to the composition of active and total bacterial populations of two disturbed sites, one of them bare and the other supporting a diverse plant community. I hypothesized that nutrient-poor sites would harbor a higher fractional abundance of oligotrophic, slow-growing groups (e.g., Actinobacteria), while nutrient-rich soils would tend to select for relatively fast-growing taxa (e.g., Acidobacteria). The hypothesis enabled me to examine the prediction of greater compositional similarity among soils sourced from productive sites. I apply both species- and divergence-based metrics to describe site-to-site community variation and evaluate if patterns of compositional variability can be accounted-for by key edaphic properties.

The molecular ecological approaches (fingerprints and clone libraries) I apply in Chapters 2 and 3 fill two gaps: first, they help resolve little-known patterns of microbial community variation across natural boreal forest gradients; and second, they provide a sensitive means for testing hypotheses about potential relationships between soil community

structure and environmental (biotic and abiotic) parameters (Fromin et al., 2002) within the little-explored context of a reclamation chronosequence.

Chapter 4 examines the functional significance of bacteria sourced from two distinct peat types using a microcosm-scale transplant experiment. I tested the hypothesis that both the inoculum source and the peat receiving the inoculum would affect potential enzyme activity rates, with the inoculum source becoming more important for the activities of enzymes that catalyze oxidative reactions and the peat type mainly affecting the activities of nutrient-acquiring enzymes. With this set-up I also explore potential relationships between bacterial diversity gradients—achieved by serial dilutions of peat samples—and functional attributes, namely, respiration and extracellular enzyme activities. I hypothesized that diversity erosion would have a minor effect on respiration rates but would affect the production of extracellular enzyme activities.

Finally, in Chapter 5, I summarize the main points of the thesis with general conclusions. I also discuss limitations of my research and suggest future research directions.

Table 1.1 Post-mining reclamation treatments from the Athabasca oil sands region used in this thesis*

Depth (cm)	A	B	E	F	H	I	M	SYN§	WA5§	ALB§
10	Peat-Mineral	Direct placement	Peat- Mineral	Subsoil	Peat-Mineral	Peat-Mineral	Mesic peat: Mineral	Fibric peat-Mineral	Peat	Peat-Mineral
20			Subsoil							
30										
40	Subsoil				Tailings sand	Overburden (Shales)	Subsoil	Subsoil		Tailings sand
50			O'burden (Shales)							
60										
70				O'burden (Shales)			Clearwater shales	Clearwater shales		Lean oil sand
80	Tailings sand	Tailings sand								
90										
100										
....										

*Data from AMEC Earth & Environmental and Paragon Soil and Environmental Consulting Inc. (2005).
§Treatments SYN, WA5, and ALB were also included in my study; see Chapters 2 and 3.

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2 Impact of reclamation of surface-mined boreal forest soils on microbial community composition and function[3]

2.1 Introduction

With an estimated reserve of 1.6 trillion barrels of bitumen (Government of Alberta, 2009), the Athabasca oil sands in northern Alberta represent the largest single oil deposit in the world. Surface mining of bituminous sand in this area generates extensive areas of disturbed land that are not conducive to bioremediation and require reclamation (Johnson and Miyanishi, 2008). The companies extracting the oil are required by law to reclaim this land with commercial forest within the natural range of variability found in this region of the boreal forest (Government of Alberta, 1999). If selected judiciously, reclamation strategies should bring about an improvement in soil quality, the development of pedogenic processes, and the restitution of soil organic C (SOC) pools, all of which should ultimately support revegetation (Bradshaw, 1997; Munro, 2006).

Existing restorative strategies in the oils sands and other drastically disturbed systems have been studied with respect to their effects on soil properties such as bulk density, organic matter content, and microbial biomass (Harris et al., 1993; Mummey et al., 2002). Little is known, by contrast, about the response of the soil microbial community to different reclamation prescriptions applied after oil extraction (McMillan et al., 2007); to date most reclamation studies have focused on the aftermath of other types of mining,e.g., for coal (Coyne et al., 1998; Frouz and Nováková, 2005).

[3] A version of this chapter will be submitted for publication. Dimitriu, P.A., Prescott, C.E., Quideau, S.A., Grayston, S.J. Impact of reclamation of surface-mined boreal forest soils on microbial community composition and enzyme activities.

In part due to a need to streamline assessment methods, historical attempts to describe the microbial ecology of nascent and regenerating sites have centered on broad estimates of microbial biomass and a few "universal" measures of microbial activity, such as dehydrogenase activity and respiration (cf. Harris, 2003). In Alberta, for instance, dehydrogenase activity discriminated between undisturbed and industrially disturbed soils, in contrast to the measured physical and chemical parameters (Rowell and Florence, 1993). Yet these generic approaches do not address how other essential soil processes, such as rates of organic matter turnover and biological interactions, are affected in restored soils, nor do they take into account potential changes in the diversity of the microbial community driving these functions. Molecular methodologies have expanded the knowledge base on how microbes respond to reclamation (Harris, 2003; DeGrood et al., 2005). However, our understanding of the potential interplay between the microbial community composition and function, crucial "indicator" variables (plant community development and organic matter build-up, for instance), and soil edaphic factors in recovering ecosystems remains rudimentary.

Most studies concerning the effects of land reclamation on microorganisms in the Athabasca oil sands region have revolved around fungi, particularly mycorrhizae (cf. Quoreshi, 2008). Not surprisingly, field observations (Visser, 1985; Danielson, 1991) and laboratory microcosm studies (Bois et al., 2005) have demonstrated a trend of low numbers of mycorrhizal fungi in early reclaimed soils with increasing abundance in older sites (i.e., > 15 yrs). In comparison, the composition and function of prokaryotes in soils reclaimed in the oils sands region has received little attention (McMillan, et al., 2007). Indeed, the majority of the research has focused on the effects of naphtenate exposure in both laboratory enrichments (Clemente and Fedorak, 2005; Biryukova et al., 2007) and wetland sediments (Hadwin et al.,

2006). These surveys, however, have not attempted a concomitant molecular assessment of fungal and bacterial populations dwelling in reclaimed soils. Moreover, published research has overlooked the influence of natural environmental gradients, useful to compare potential site trajectories derived from reclamation prescriptions (Johnson and Miyanishi, 2008), on the distribution of microbial communities.

In this study, we examined the composition and function of microbial communities across a sequence of reclaimed sites of differing prescription type and age in the Athabasca oil sands region. We also assessed microbial responses along a series of adjacent replicated 'target ecosites', i.e., sites representing a typical range of natural variability in the area (Johnson and Miyanishi, 2008). We used fingerprinting (DGGE and phospholipid fatty acid profiling) and sequencing-based approaches to identify compositional differences in microbial communities, whereas functional potential was evaluated by measuring extracellular enzyme activities. Our objective was to evaluate whether certain prescription-age combinations have promoted the development of microbial community composition and/or function that are similar to microbial properties found in undisturbed sites. We hypothesized, as suggested by the observed recovery trajectory of vegetation and edaphic properties at the same sites (Rowland et al., 2009), that microbial community structure and function in older reclaimed sites would be more similar to natural sites than to younger reclaimed sites. We also hypothesized that microbial community structure and function in reclamation prescriptions covering tailings sand would be the least similar to communities found in natural forests, as materials containing tailings appear to be less conducive to the reestablishment of plant communities and decomposition processes within ranges of natural variability (Rowland et al., 2009). Previous research on upland forests suggests a key

influence of dominant tree species on the composition of microbial communities (e.g., Pennanen et al., 1999; Myers et al., 2001). Thus, we also explored whether plant cover and soil properties could explain the variability in microbial community composition and function.

2.2 Methods

2.2.1 Site description and soil sampling

The study area lies in the Mid Boreal Mixed-wood Ecoregion of northern Alberta, Canada. It extends over approximately 6700 km^2 in the Athabasca oil sands region near Fort McMurray (57º 00' N, 111º 28' W). Luvisolic soils with eluvial (Ae) and illuvial (Bt) horizons are the typical medium to fine-textured soils of the area, while Brunisols are found on coarser substrates (Fung and Macyk 2000). The dominant tree species are white spruce (*Picea glauca* (Moench) Voss], black spruce [*Picea mariana* (Mill.) BSP], trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), and white birch (*Betula papyrifera* Marsh.); jack pine (*Pinus banksiana* Lamb.) is dominant on well-drained sandy areas. The representative understory vegetation consists of berries (*Vaccinium* sp., bluejoint reed grass [*Calamagrostis canadensis* (Michx.) Nutt.], sedge (*Carex* sp.), feathermosses, and lichens. Details of the regional climate and plant community characteristics can be found elsewhere (Fung and Macyk, 2000; Rowland et al, 2009).

Soil samples were collected in July 2005 from a system of 10- × 40-m geo-referenced (UTM coordinates) long-term monitoring plots (46 in total) established in 2000 on both reclaimed and natural sites (Johnson and Miyanishi, 2008). The reclaimed plots represent different times since the onset of reclamation, ranging from less than 5 to over 30 years

(average = 14.4 ± 6.2 years), and seven reclamation prescriptions, typically involving a ~15-30-cm-thick layer of mineral material (~40%) and peat (~60%) mixes capped on tailings sand or geological parent material to a total depth of ~100 cm (Table 1). Plot *WA5*, a re-colonized peat waste area established for an associated study (Rowland et al, 2009), was also sampled. Reclaimed soils had an average pH of 6.9 ± 0.2, a carbon concentration of 71 ± 11 g kg^{-1} and a nitrogen concentration of 3 ± 0.5 g kg^{-1}. Representative forest ecotypes —*sensu* Beckingham and Archibald's (1996) ecosystem classification system— were sampled to account for the range of natural variability (Table 1). Natural ecosites were characterized by soils with acidic pH (5.0 ± 0.2), low C (16 ± 2 g kg^{-1}) and N (0.9 ± 0.2 g kg^{-1}), and a stand age of 72.2 ± 7.8 years. Additional features of reclamation prescriptions and revegetation procedures are provided in Johnson and Miyanishi (2008) and Rowland et al. (2009). From each site, 10 soil cores (7 cm diameter × 20 cm long) were randomly collected from the upper portion of the soil profiles and separated into organic and mineral fractions. At four sites (one from treatment *I*, one from treatment *A*, site *ALB*, and site *SYN*; see Table 1) a recognizable organic layer had not developed and therefore only mineral soil was obtained. After being bulked to produce composite samples, the samples were transported in cooled boxes to the laboratory, sieved (< 2 mm), and stored at either 4ºC or –20ºC.

2.2.2 Soil properties and plant community characterization

The basic chemical and physical properties of the soils, as well as the plot plant community characteristics, were obtained from Rowland et al. (2009). Carbon, nitrogen, pH (H_20), and moisture content were determined with standard methods (Mulvaney, 1996) (Appendix A, table A.2). Nutrient availability (also in Rowland et al., 2009) was determined with Plant

Root Simulator (PRS™) probes (Western Ag Innovations Inc., Saskatoon, SK, Canada). The PRS™-probes consist of cation- and anion-exchange resin membranes encased in a plastic holding device, which are inserted into soil to measure nutrient supply *in situ* with minimal disturbance (Qian and Schoenau, 2002). Briefly, four probes per site were inserted into the soils so that the midpoint of the resin window was at the organic/soil interface. After a field incubation period of 65 d spanning the peak of the growing season, probes were removed and sent to Western Ag. The PRS™-probes were washed with deionized water, bulked according to treatment plot, and then eluted for one hour using 0.5 N HCl/2 M KCl. The eluate was analyzed for levels of ammonium (NH_4^+) and nitrate (NO_3^-) with automated colourimetry. Inductively-coupled plasma (ICP) spectrophotometry/Atomic absorption spectrometry (AAS)/flame emission spectrometry (FES) was used to measure concentrations of phosphorous (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), aluminum (Al), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and lead (Pb) in the 0.5 N HCl/2 M KCl eluate. Nutrient supply rates generated with the PRS™-probes are reported as the amount of nutrient adsorbed per amount of adsorbing surface area per time or burial in soil (i.e., μg nutrient 10 cm^{-2} time of $burial^{-1}$) (Qian and Schoenau, 2002) (Appendix A, table A.4).

Vegetation structure was estimated by assessing the presence of ten ground-cover types, namely, pine, spruce, broadleaf, woody shrubs, forbs, grasses, mosses, lichens, woody debris (>5 cm long or >1 cm diameter), and bare ground. In short, 1-m × 1-m quadrats were laid systematically around each plot starting with one corner and at 10-m intervals along each long side and at the 5-m mid-point of the short sides, for a total of ten quadrats per plot. Within each quadrat a visual estimate of all layers of ground-cover < 2 m above ground level

was scored as % cover (one 100-cm^2 = 1%) of class vegetation and other materials. Detailed plant cover percentages can be found in Rowland et al. (2009) and in Appendix A, Table A.3.

2.2.3 Soil function: potential soil enzyme activities

A range of extracellular enzyme activities involved in C, N, and P cycling processes was investigated using microplate assays as described by Stursova et al. (2006). The activities of β-glucosidase (BGLUCO; E.C. 3.2.1.21), N-acetyl-β-D-glucosaminidase, a proxy for chitinase (NAG; E.C. 3.2.1.30), β-xylosidase (XYLO; E.C. 3.2.2.37), and acid phosphatase (PASE; E.C. 3.1.3.2) were measured with 200mM of 4-methylumbelliferyl (MUB)-linked substrates and 0.2 ml soil suspensions (1 g soil homogenized in 100 ml 0.1 M acetate buffer, pH 5); reference standards (10 mM 4-methylumbelliferone) and quench controls were added to each plate. After incubation for 2-5 h at 20ºC, fluorescence was measured with a spectrofluorometer using an excitation of 365 nm and an emission of 460 nm. The activity of urease (URE; E.C. 3.5.1.5) was determined after soil incubation with urea by quantifying NH_4^+ production with salicylate and cyanurate and measuring color development at 610 nm (Sinsabaugh et al., 2000). Activity rates (μg NH_4^+-N g^{-1} soil h^{-1}) were obtained by comparing color development to a standard NH_4^+ curve. The activities of lignin-depolymerizing enzymes, phenoloxidase (POX; E.C. 1.10.3.2) and peroxidase (PER; E.C. 1.11.1.7), were determined in microplates containing 25 mM L-DOPA and, for peroxidase, each well also received H_2O_2 (0.3%). Color development was measured at 460 nm after incubation at 20ºC. Assay and control wells were replicated 16 times. Activity rates (nmol [hydrolases] or μmol [oxidases] of converted substrate g^{-1} soil h^{-1}) were calculated on an oven dry mass (105 ºC) basis.

To gain insight into soil functional diversity, we condensed all activity rates into a single 'functional evenness' index (*E*). Proposed by Degens et al. (2000), *E* is calculated from the soils' respiration response profiles after individual substrate additions as $E = 1/\Sigma p_i^2$, where p_i is the respiration response of each substrate as a proportion of total respiration responses summed over all substrates. Thus, the index gives an indication of the variability in substrate use, or functional diversity. Though infrequently, evenness indices have been applied to enzymes by replacing respiration responses with activity rates (Tscherko et al., 2004; Rodríguez-Loinaz et al., 2008), so that the theoretical maximum value of *E* equals the total number of enzymes being assayed (seven in this case).

2.2.4 Enumeration of culturable soil bacteria

Numbers of culturable soil bacteria were determined by the plate-count method for viable cells. The soils were diluted 10-fold in a sodium pyrophosphate solution (0.1% w/v). The suspensions were vigorously vortexed for 2 min, allowed to settle for 5 min, and inoculated onto R2A agar plates containing cycloheximide (400 mg mL^{-1}) using a Spiral System Model D (Spiral System Inc., Bethesda, MD) spiral plater (Fernández-Canigia and Coyne, 2001). Numbers of heterotrophic bacteria (CFU) were determined after incubation for 5 days at 21ºC.

2.2.5 Phospholipid fatty acid (PLFA) analysis

PLFAs were extracted from the soil samples (3 g frozen soil) and identified with gas chromatography, according to Frostegård et al. (1993). The fatty acid concentrations (nmol g^{-1} dry soil) were estimated using 19:0 as internal standard. Total microbial biomass was

estimated by summing the concentrations of fatty acids with less than 20 carbons. The PLFAs specifically attributed to bacteria were 14:0, i15:0, a15:0, i16:0, 16:1ω9, 16:1ω7c, 10Me16:0, cy17:0, i17:0, a17:0, 18:1ω7, 10Me18:0, and cy19:0, while PLFAs 18:2ω6,9, 18:3 ω6c, and 18:1ω9c were attributed to fungi (Frostegård and Bååth, 1996). Because in some sites the organic layer was either absent or not deep enough to sample, we only determined the PLFAs of mineral horizons from the long-term monitoring sites.

2.2.6 Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial and fungal communities

We extracted DNA from 0.25-g soil samples with a PowerSoil Mobio DNA extraction kit (MoBio, Carlsbad, CA, USA). The bacterial community structure was characterized by PCR-DGGE of 16S rRNA genes. Amplicons were generated with primers 338F and 518R, which target the V3 region (Øvreås et al, 1997), with a GC clamp attached to the 5' end of primer 338F. Polymerase chain reactions contained (final concentrations) 1 μM primers, 250 μM of each dNTP, 1 U of *Taq* polymerase (New England Biolabs), in 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and deionized water to a final volume of 25 μl. Amplification was conducted under the following conditions: an initial denaturation at 94ºC for 5 min; 30 cycles of 94ºC for 1 min., 55ºC for 1 min, and 72ºC for 1 min; a terminal elongation at 72ºC for 7 min. Amplification products were loaded on an 8% acrylamide gel with a 35-55% denaturing gradient (100% denaturing solution is 7M urea and 40% formamide). Gels were run for 15.5 h at 60 V and 60ºC in 1× TAE buffer. After staining with SYBR Green I (Molecular Probes, Eugene, OR) for 1 h, the gels were visualized with a Typhoon 9400 variable mode imager (Amersham Biosciences, Piscataway, NJ) and their

digitized images analyzed with GelCompar II (Applied Maths, Belgium). Band detection was accomplished under the default band-searching parameters. Following band matching, a presence-absence matrix with fingerprints from all samples was constructed and analyzed as described in the 'Statistical analysis' section.

To characterize the fungal community structure, DNA extracts were first PCR-amplified with primers ITS1-F and ITS4, which amplifies the two internal transcribed spacer (ITS) regions and the 5.8S gene plus 22 bp from the forward primer and a section of the 28S rRNA gene (Kennedy et al., 2005). Amplification conditions were as described by Kennedy et al. (2005). Polymerase chain reaction products were diluted 10-fold and subjected to a second round of PCR with primers ITS1-F-GC and ITS2 (Anderson et al. 2003), under conditions outlined by Anderson et al. (2003). Amplification products were loaded on a 6% acrylamide gel with a 15-50% denaturing gradient. Gels were run for 15 h at 65 V and 60ºC in 1× TAE buffer, and gel staining, visualization, and analysis was performed as described for bacteria. However, the complexity of the fingerprints (the inherent sensitivity of DGGE was compounded by the natural variability in ITS length) precluded a matrix-based analysis. Thus, we favored the approach of excising representative bands and subjecting them to a sequence-based analysis (see below).

2.2.7 Sequence analysis

The DNA from representative DGGE bands from at least one reclaimed and one natural site, for both mineral and organic horizons, was excised with a sterile scalpel, eluted in 20 µl of sterile deionized water overnight at 4ºC, and amplified with primers ITS1-F and ITS4. To confirm that the eluted DNA had originated from a single phylotype (i.e., was free of co-

migrating DNA), the amplification products were re-amplified with primers ITS1-F-GC/ITS2 and loaded on denaturing gels alongside lanes with amplified DNA from the original sample. Sequencing was performed at the McGill University and Genome Québec Innovation Centre using BigDye Terminator technology. Species-level sequence clusters were delineated using a 97% sequence similarity cut-off (Atkins and Clark, 2004). Putative taxonomic affiliations were obtained by comparing our sequences to DDBJ/EMBL/GenBank sequences using BLAST searches.

2.2.8 Statistical analysis

The composition of the soil microbial community was summarized using a principle components analysis (PCA) on the relative mole abundances (%mol) of PLFAs in each sample. PCA was chosen as it helps identify PLFAs whose abundance profiles covary positively, negatively or neutrally with respect to each other. In the ordination biplot, PLFA marker vectors that are orthogonal to each other may be considered as behaving independently, whereas the ones that are collinear may be seen as positively or negatively covarying, depending on the angles between the vectors being compared (Ramette, 2007).

The bacterial presence/absence matrix was transformed into a distance matrix using Bray-Curtis dissimilarity coefficients (Legendre and Legendre, 1998). Enzyme activities were converted into Bray-Curtis inter-sample distances after $\log_{10}(x+1)$ transformation. General bacterial community patterns were first visualized with non-metric multidimensional scaling (NMS) as implemented, using the default parameters, in PC-ORD v. 5. We applied a Canonical Analysis of Principal coordinates (CAP), which provides a constrained ordination that maximizes the differences among *a priori* groups (Anderson and Willis, 2003), to

classify bacterial fingerprints into age class (i.e., the time since prescription application) and prescription type. We defined the following age classes, in years: class 1, 1-9; class 2, 10-18; class 3, 19-27; and class 4, 27-35 (natural sites were classified into a separate class). To test whether interactions between age and prescription affected the enzymatic and community fingerprints (DGGE and PLFA), we used DISTLM in the context of an ANOVA for unbalanced designs (treatments with one replicate were excluded from the analysis) (McArdle and Anderson, 2001). Design matrices of orthogonal codes were generated with XMATRIX (Anderson, 2003). Significance ($P < 0.05$) was tested with 9999 unrestricted permutations of raw data. A multi-response permutation procedure (MRPP) was used to compare multivariate Sorensen (Bray-Curtis) distances among sites belonging to different age classes and prescription types. The statistical significance of groups was calculated by comparing within-group homogeneity, *A*, with the random expectation (Mielke and Berry, 2007). MRPP was also used in a manner akin to univariate ANOVA (Mielke and Berry, 2007) to determine the treatment effects on individual enzyme activities. Relationships between community fingerprints and explanatory variables (standardized to remove effects of different measurement units) were analyzed using non-parametric multivariate regression (DISTLM; McArdle and Anderson, 2001). We evaluated the significance of soil attributes (19 in total), plant-cover types (ten), and spatial position. Spatial effects were modeled from *X*-*Y* (i.e., UTM) geographical coordinates augmented by the terms of a third-order polynomial function: X, Y, XY, X^2, Y^2, X^2Y, Y^2X, X^3, and Y^3. Borcard et al. (1992) have applied polynomial functions of spatial coordinates to elucidate the spatial structuring of microbial processes. Significant variables were selected with a step-wise forward selection procedure. *P* values were obtained with 9999 permutations of residuals under the reduced

model (McArdle and Anderson, 2001). To test for 'pure' spatial effects, the statistical significance of spatial terms retained by forward selection was reassessed with DISTLM after entering the significant environmental variables as covariables. This procedure is conceptually analogous to a partial Mantel test (Borcard et al., 1992; McArdle and Anderson, 2001). Finally, Mantel tests were performed to test the null hypothesis of no association between microbial community structure and enzyme activity profiles.

2.3 Results

2.3.1 Extracellular enzyme activity and functional evenness patterns

While oxidative enzyme activities were higher, on average, in natural than in reclaimed sites, this was not always the case for hydrolytic activities. There were a few exceptions; for instance, in the organic horizon of treatment *M* the activity of β-glucosidase was up to 15 times lower than in the other prescriptions and that of peroxidase was 2-5 times higher (Table 2). A peak in hydrolytic, though not oxidative enzyme activities was apparent for age class 3 (19-27 y) (Table 3). In organic layers, there was a trend of increasing, non-significant β-glucosidase, chitinase, peroxidase, and phosphatase activities along the a1-d3 gradient, which was reflected in a gradual increase of functional evenness (Table 2). Enzyme activities in organic layers were significantly affected by age (MRPP: $A = 0.18$, $P<0.001$) and prescription ($A = 0.21$, $P<0.001$), but not their interaction [DISTLM (ANOVA), $P>0.05$]. To identify which enzymes were potentially driving the significant outcomes, we also analyzed the effects of prescription type on individual activities. The activity of β–glucosidase was affected by age, this was due to significant dissimilarities between activities in age class 3 (i.e., 19-27 yrs) and in natural sites ($A = 0.21$, $P<0.001$), and by prescription type, as a result

of a significant increase in prescription *I* compared to ecotype *a1* (A = 0.41, P<0.001). Chitinase activity was significantly higher in prescription *I* compared to ecotype a1 (A = 0.15, P<0.01), but was not affected by age.

Activities in the mineral horizons were highly variable— for instance, there was no discernible pattern (increase or decrease) along the fertility gradient of natural soils (Table 2). Consequently, the aggregate enzyme activities in mineral soils were not significantly affected by age, prescription, or their interaction [DISTLM (ANOVA) and MRPP: $P>0.05$]. However, the activity of phenoloxidase in mineral soils from sites belonging to prescription *I* was significantly lower than that in ecotype *d1* ($A = 0.35$, $P<0.01$).

2.3.2 Numbers of culturable bacteria and microbial biomass

Numbers of culturable soil bacteria (logCFU ± SEM g^{-1} soil) were significantly (*t*-test: $P < 0.001$) higher in reclaimed soils (4.31 ± 0.14) than in natural soils (3.41 ± 0.13). Overall, we found higher numbers ($P < 0.01$) in organic than in mineral horizons, for both reclaimed (organic = 4.64 ± 0.19; mineral = 3.98 ± 0.21) and natural (organic = 3.76 ± 0.16; mineral = 3.10 ± 0.18) sites (Table 2). Total PLFA biomass (nmol PLFA ± SEM g^{-1} soil) was significantly higher in natural (232 ± 25) than in reclaimed (132 ± 15) sites. Exceptions were prescriptions E and WA5, which contained higher concentrations of PLFA biomarkers than the average in natural ecosites (Table 2). Within the reclaimed sites, total PLFA biomass peaked at 19-27 y after reclamation (Table 3).

2.3.3 Microbial community compositional patterns

PCR amplification of bacterial 16S rRNA genes generated reproducible products of 215 bp. DGGE profiles clearly separated the reclaimed and natural sites (CAP, $P<0.05$), with no clear distinction between organic and mineral horizons (NMS, Fig. 1). However, bacterial community structure in the different prescription age classes and among prescription types could not be discriminated (CAP, $P>0.05$). The interaction of age and prescription was not significant, nor was either factor alone [DISTLM (ANOVA), MRPP, $P>0.05$].

We were able to obtain re-amplifiable fungal DNA from 55 of the 80 DGGE bands selected for sequencing. Distributed as shown on Table 4, the sequences comprised 15 unique species-level phylotypes with an average ITS sequence length of 225 ± 8 bp. There were no evident age- or prescription-specific sequence types. Natural sites, however, were dominated by sequences related to putative ectomycorrhizal *Basidiomycota*, as opposed to sequences with low similarity to sequences belonging to the *Zygomycota* and *Ascomycota* in reclaimed sites.

In total, 60 out of the 72 PLFAs identified in the samples were used in the PCA; 12 PLFAs were excluded from the analysis because they were found in fewer than 5% of the samples; a PCA with the 16 microbial-specific markers yielded similar ordinations (Mantel test: $r = 0.98$, $P<0.001$). The indicators 18:1ω9c (fungi) and18:1ω7c (Gram negative) were most influential in the separation of samples of natural and reclaimed origin, as indicated by the direction of the arrows on the bi-plot (Fig. 2); biomarkers 10Me16:0 (actinomycetes) and 16:0 (general) emerged as equally important for segregating microbial communities from reclaimed and natural samples (Fig. 2). DISTLM (ANOVA) revealed a non-significant effect of age-prescription interactions on PLFA composition. However, age and prescription were

significant (A = 0.20, P<0.001 and A = 0.40, P<0.0001, respectively). According to pairwise comparisons, age effects were driven by significant differences in the PLFAs from age class 2 (10-18 y) and those from natural sites (A = 0.19, P<0.001), whereas reclamation effects were due to significant dissimilarities between samples from prescriptions *H* and ecotype d3 (A = 0.40, P<0.01), *B* and d1 (A = 0.41, P<0.001), and *A* and d3 (A = 0.31, P<0.01).

2.3.4 Relating enzyme activities and microbial community composition to environmental variables

To address the relationship between the multivariate data sets (enzyme, DGGE, and PLFA profiles) and the environmental variables, sequential (linear) models were built with forward selection (DISTLM) for each variable set— soil parameters, plant cover percentage, and spatial terms (Tables 5 and 6). The parameters that explained the greatest amount of variation in the enzyme activities were, among reclaimed sites, soil N concentration (27.2%; mineral horizon) and broadleaves percent cover (26.8%; organic layer); among natural sites, it was pH (24.9%) and pine percent cover (19.4%) (Table 5).

The highest proportion in the variance of bacterial communities as determined by DGGE was explained by spatial term X^3 in mineral soils from natural forest ecosites, followed by Al^{3+} (11.5%) and BO_3^{3-} (11%) (Table 6). Among natural sites, the highest proportion of variance, 7.4%, was explained by spatial term *Y* (organic horizon) (Table 5). No spatial terms were significant after controlling for the effects of biotic and abiotic variables (data not shown).

Thirty-two percent of the variance in the PLFA data of reclaimed sites was explained by the fungal-to-bacterial-biomass ratio (F:B) (Table 6). In the natural forest ecotypes, soil N

concentration and pine cover explained 36 and 27% of the variability in PLFA composition (Table 6), followed by the micronutrient Zn^{2+} (15.4%). No spatial terms were significant, even after controlling for the effects of biotic and abiotic variables. When PLFA profiles in natural and reclaimed soils were analyzed together, the F:B ratio explained the highest proportion of variance (23.3%; pseudo-$F_{19, 59}$ = 11.96, P<0.001), followed by pH (13.1%; pseudo-$F_{19, 59}$ = 8.04, P<0.001) and the percent cover of woody debris (10.1%; pseudo-$F_{10, 59}$ = 4.51, P<0.01).

2.3.5 Linking microbial community composition to function

Mantel tests between enzyme activities and PLFA profiles were significant for both reclaimed (r = 0.23, P<0.01) and natural (r = 0.38, P<0.05) sites. We also calculated Pearson's correlations between individual PLFA abundances (nmol g^{-1} soil) and enzyme activities (Table 7). Across natural soils, the activities of peroxidase and phenoloxidase were significantly correlated to one fatty acid each [18:2ω6 (fungal) and 15:1ω6c, respectively], while those of phosphatase and chitinase were correlated to two fatty acids (P<0.05), one of them represented by fungi (18:2ω6); the activity of β-glucosidase was correlated to three fatty acids. The activities of xylosidase and urease were correlated to 7-9 primarily saturated fatty acids (characteristic of Gram-positive bacteria) (P<0.05 – P<0.01). Across reclaimed soils, the activities of oxidative enzymes were correlated to 7-10 biomarkers of various origins. No biomarkers significantly correlated to β-glucosidase activity, and only three correlated with urease activity (P<0.01). A large number of biomarkers representing Gram-positive and Gram-negative bacteria, actinomycetes, and fungi correlated significantly with the activities of phosphatase, chitinase, and xylosidase (P<0.05 – P<0.001).

2.4 Discussion

Microbial community function and structure differed in reclaimed and natural sites. As was previously observed for plant cover and nutrient data on our study sites (Rowland et al., 2009), we found enhanced dissimilarity among microbial communities of reclaimed sites where tailings had been used —*A*, *B*, and *H*— and those of productive ecotypes (e.g., series d1 and d3)—and this was independent of the material used to cap the tailings. Where a diverse plant community dominated by spruce and moss has been restored —as is the case for sites belonging to prescription *I*, characterized by a peat-mineral mix covering overburden (Rowland et al., 2009)— the development of an organic layer may decouple the deleterious influence of toxic materials potentially migrating upward into the topsoil (Jurinak et al., 1987). Indeed, the rates of enzymes that hydrolyze relatively labile macromolecules in organic horizons of treatment *I* sites were comparable to those of ecotype d3. Furthermore, the decline of phenoloxidase activity in mineral layers of treatment *I* sites relative to d1 sites suggests that soil organic matter of overburden-based prescriptions is depleted in lignin derivatives (Grandy et al., 2007; Grandy and Neff, 2008).

The influence of time since the onset of reclamation was overshadowed by the influence of prescription type. For instance, age class 2 (i.e., 10-18 yrs) was dominated by prescription A, B, and H sites, and in age class 3 half of the samples belonged to treatment I. In contrast, vegetation cover, particularly that developing on treatments E, H and I, rapidly recuperates, resulting in plant communities that resemble the preexisting community within 25 years of reclamation (Rowland et al., 2009). The lack of a clear age effect, however, was not unexpected, as the inherent heterogeneity of reclamation materials, different rates of fertilization application (both across and within reclamation practices), and uneven capping

depths, among other confounding factors, likely affect the distribution of microorganisms—for instance, the composition and concentration of each fatty acid depends on the soils' nutritional status and other environmental parameters such as pH (Frostegård and Bååth, 1996). The overwhelming effects of certain types of reclamation materials on microbial communities —as Bois et al (2005) observed for mycorrhizae— may be attributable to nutrient limitation, but residual bitumen in tailings sand may have had a negative impact as well.

Our molecular survey of fungi revealed a presumed dominance of ectomycorrhizal sequence types in natural forest stands, with preliminary indications that *Piloderma* sp., and perhaps some saprotrophs like *Hydnellum* sp., might be abundant. This is in agreement with what is known about the distribution of fungal taxa in mixed-wood boreal forests (Jonsson et al., 1999; DeBellis et al., 2006). In reclaimed sites, many sequences were related (albeit with low similarity) to putatively endophytic fungi, including *Preussia* sp., *Giberella* sp., and zygomycetes found in association with ericaceous plants. These occurrences were expected given the incidence of grasses and forbs in reclaimed sites (Rudgers and Clay, 2005). Additionally, revegetation practices, which involve planting barley after the amendments, could have favored arbuscular mycorrhizae (and associated yeasts such as *Dioszegia*; Renker et al., 2004) and restricted ectomycorrhizal inoculum potential. Bois et al. (2005) reported a high frequency of *Laccaria* sp. associated to jack pine in oil sands reclaimed sites. We found no *Laccaria*-type sequences, probably because our sample size was too small to capture the influence of pine trees, which were not abundant at reclaimed sites (Rowland et al., 2009).

Forest management practices such as clear-cut harvesting and prescribed burning are often linked to declines in the abundance of ectomycorrhizal sporocarps (Durall et al., 2005).

Bååth et al. (1995) and Hamman et al. (2007) attributed burning effects on microbial community composition (e.g., decreases in fungal biomarkers) to soil chemical factors, primarily pH and carbon. In our study, the F:B ratio in mineral soils was the major predictor of microbial community composition (PLFA signatures) both at reclaimed sites and along the reclamation-to-natural transition (i.e., when all the sites were analyzed together), suggesting that reclamation imposes qualitative shifts in microbial community composition by affecting the relative contributions of fungal and bacterial components to microbial biomass. Pine cover was equally effective for explaining the variance in microbial function and composition at natural sites. While this indicates that pine affected the distribution of enzyme activities by selecting for microbial populations that produce them, a pine effect may be ecologically relevant solely in nutrient-poor, dry ecosites (a1-b3), where pine dominates.

Our results indicate that, in addition to the F:B ratio, pH and the presence of woody debris (which can comprise up to 20% of the plant cover in natural sites but is virtually absent in reclaimed sites) were important factors explaining microbial community composition when all samples were analyzed together. The influence of pH on soil microbial community structure is well documented (e.g., Fierer and Jackson, 2006; Högberg et al., 2007), and in our case likely reflects higher pH values in reclaimed sites. A greater incidence of woody debris along the recovery chronosequence (Rowland et al., 2009) may have promoted the growth of fungal mycelia, allowing the proliferation of fast growing, chitin-degrading bacteria and microfungi (Ingham et al, 1989; Durrall et al., 2005). Thus, an increased fungal component in natural sites, as suggested by the PCA, together with the F:B ratio as a key predictor of microbial community shifts, may point to the suitability of fungal

markers (and other abundant groups such as actinomycetes) as indicators of ecosystem recovery.

We found a positive correlation ($r = 0.48$, $P<0.01$) between cumulative plant cover and microbial functional evenness in organic horizons of reclaimed sites, which may indicate that enzyme production in developing (reclaimed) stands parallels the accrual of plant biomass (cf. van der Heijden et al., 2008; Tscherko et al., 2004). Indeed, 27% of the potential enzyme activity in organic horizons of reclaimed sites was explained by the abundance of broadleaves. This scenario is consistent with previous research in boreal forests that documented a crucial influence of tree species (and associated understory vegetation) on microbial community composition of organic horizons (Priha et al., 1999; Priha et al., 2001). Broadleaves, which in boreal forests can be positively associated to calcium (Brais et al., 1995), may play an important, if indirect role on the regulation of soil enzyme rates. For instance, Hobbie et al. (2007) found greater nitrification:mineralization ratios under species with high exchangeable soil calcium, suggesting some tree-induced controls on N dynamics (and likely on microbial community composition) occur via soil cation chemistry. Whether tree species influence microbial community composition and function through effects on soil chemistry (for example, via root exudation) or by influencing cation concentrations may be context-dependent. Indeed, as illustrated by both PLFA and DGGE data, explained variability patterns differed in organic and in mineral horizons of reclaimed and natural sites.

The fact that enzyme activities and microbial community composition (PLFA) responded differently to distinct reclamation treatments initially implied little congruence between the overall abundance of PLFA biomarkers and individual enzyme rates. However, significant (but weak) Mantel test results pointed to putative links between microbial

community composition and function, both in reclaimed and in natural sites. While these relationships may have been the result of parallel changes in biodegradable C or microbial biomass, a large number of PLFAs were correlated with individual enzyme activities, suggesting extracellular enzymes were produced by specific subsets of the microbial community independently of whole-community changes, such as microbial biomass reductions. Because enzyme assays are conducted under optimal conditions, which include both free and stabilized enzymes pools (Wallenstein and Weintraub, 2008), significant correlations may not provide an accurate representation of enzyme activity and microbial composition (or abundance) covariation. However, similar relationships have been inferred in agriculturally-managed tropical soils (Waldrop et al., 2000) and, through quantification of fungal ITS pools, in burned boreal forest soils (Waldrop and Harden, 2008). Thus, changes in certain process rates may be related to abundance shifts in specific members of the microbial community rather than to ubiquitous taxa.

While PLFA profiling was a sensitive tool for detecting treatment differences, significant landscape-scale spatial effects were only observed for DGGE fingerprints. Boreal forest sites with similar vegetation have been noted to harbor microbial communities that display spatial dependency at regional (≤ 350 km) (Bach et al., 2008a) and within-plot (≤ 1 km) (Bach et al., 2008b) scales. The putative spatial structuring of microbial communities in our sites was not caused by measured environmental factors: when the influence of plant cover and soil properties were partialled out, the spatial models were not significant. Therefore, at least one potentially important aspect of the environmental gradient was not determined (Legendre and Legendre, 1998). In our study, substantial topographic variability, which is especially prevalent among reclaimed sites and has been shown to supersede the

influence of stand type on the structure of microbial communities of upland boreal forests (Swallow et al., 2009), may have accounted for this unexplained spatial variation. About half of the variance in bacterial composition (DGGE) at reclaimed sites (at least in organic layers) was explained by spatial terms, which may also be indicative of a plant-driven underpinning to this spatial structuring: the identity and diversity of C compounds commonly found in the forest floor as a result of litter inputs and root exudation may affect microbial function and structure (Dehlin et al., 2006).

Phospholipid fatty acid profiling had greater power than DGGE fingerprinting to resolve treatment effects, and this may explain why variability in their response profiles were not correlated to the same factors. Many other studies that used DGGE have detected no dissimilarities in fingerprints among different soils or treatments (e.g., Smit et al., 2001; Leckie et al., 2004). However, our results were somewhat unexpected, as PCR fingerprinting approaches tend to document shifts in bacterial community composition after large-scale perturbations (Kang and Mills, 2004; Smith et al., 2008). Although it is reasonable to expect that a PCR-based fingerprinting method would offer greater potential for characterization of underlying population-level changes, the inherent low resolution of DGGE fragments (in the sense that they represent a minor fraction of soil microbial diversity) may be responsible for the difficulty in detecting changes in the microbial community following a perturbation (Ramsey et al., 2006).

In contrast to our initial hypothesis that microbial composition and function in older reclaimed sites would be more similar to natural sites, we found that time-since-reclamation effects were overridden by the influence of reclamation material. Presumably, tailings sand and overburden materials were not suitable for the compositional and functional convergence

of microbial communities in reclaimed and natural sites. In addition, the distribution of microorganisms in reclaimed sites and natural ecosites appeared to be constrained by different factors. This was reflected in distinct patterns of enzyme activity expression, independently of biomass fluctuations. Compositional shifts in microbial communities were accompanied by changes in their abilities to degrade macromolecules, especially in reclaimed soils where microbial biomass was generally lower. The F:B ratio, pH and, to a lesser extent, the presence of woody debris explained a combined 47% in the variability of mineral soil PLFA data. This highlights an indirect consequence of plant cover development in rehabilitated sites, which with consequent litter and woody debris production may create conditions conducive to the growth of microbial communities. Although the spatial structuring of microbial communities may be substantial, the re-establishment of abiotic factors such as pH, and provided the use of tailings sand is avoided, is likely more important for returning the soil microflora to a state that resembles pre-disturbance conditions.

Figure 2.1 Non-metric multidimensional scaling (NMS) ordination plot of DGGE fingerprints from soil of reclaimed and natural sites at the Athabasca oil sands region in northern Alberta.

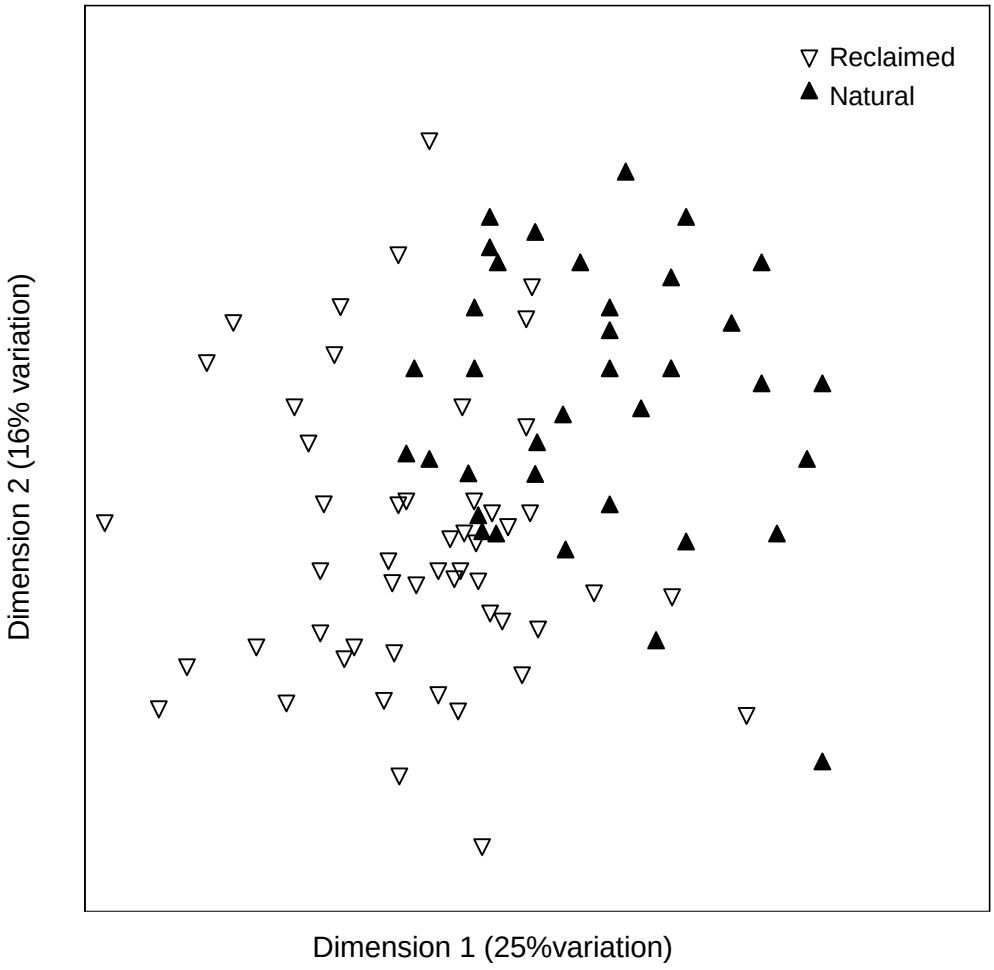

Figure 2.2 Principal components analysis (PCA) ordination bi-plot of the signature PLFA markers used for assessing the general soil microbial community composition in mineral soil layers of reclaimed and natural sites at the Athabasca oil sands region.

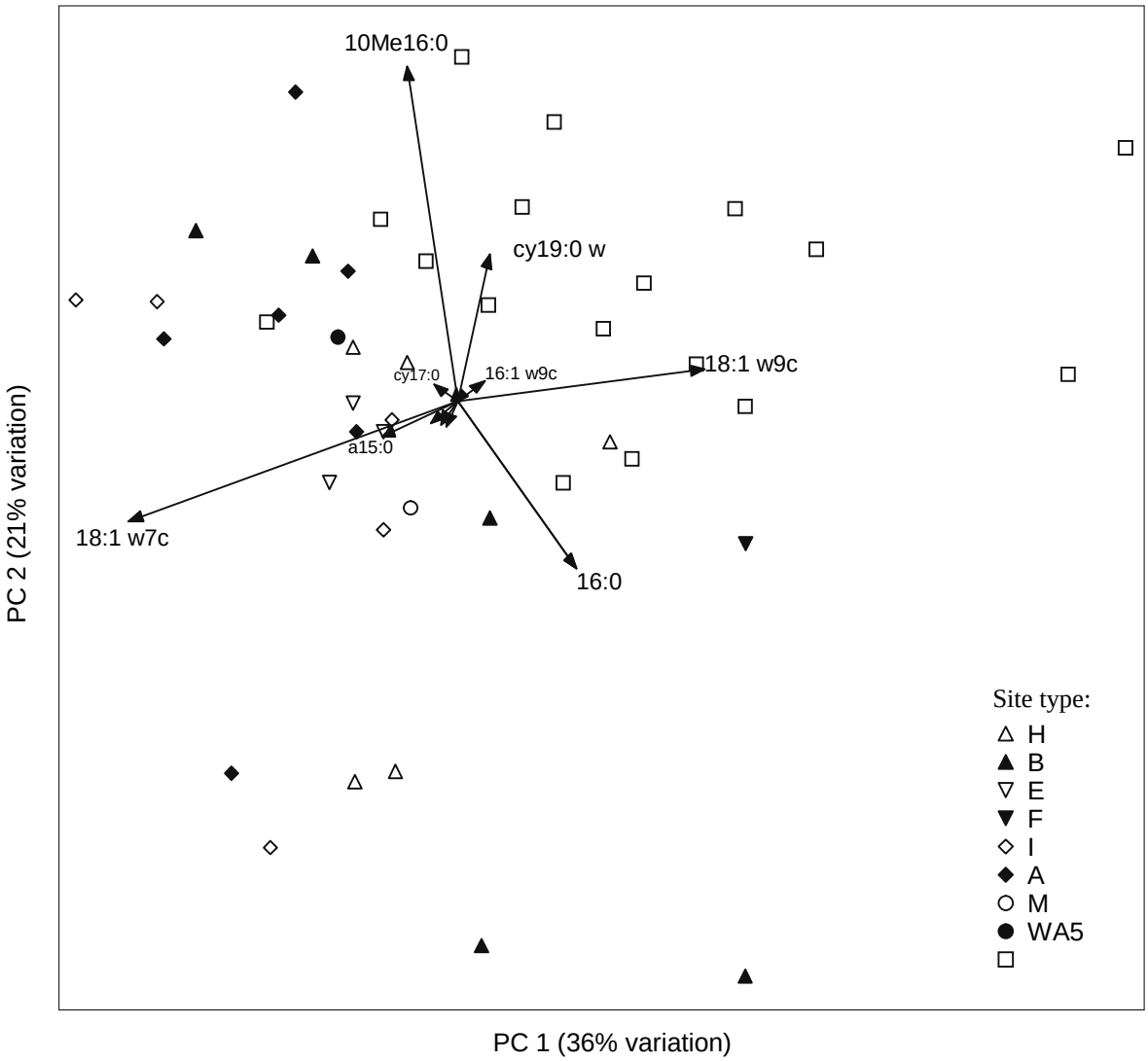

Table 2.1 Characteristics of the 47 reclaimed and natural sites sampled in the Athabasca oil sands region

Reclamation prescription or ecotype (*number of replicates*)	Prescription* or ecosite characteristics[†]	Age[‡], y (*plot ID*)
Reclaimed sites		
A (*6*)	P:M-SS-TS	5 (*89*), 11 (*36*), 14 (*40*), 5 (*87*), 4 (*88*), 24 (*37*)
B (*5*)	DP-TS	14 (*3*), 9 (*39*), 11 (*42*), 19 (*38*), 10 (*46*)
E (*3*)	P:M-SS-OB	12 (*7*), 10 (*14*), 22 (*43*)
F (*1*)	SS-OB	14 (*12*)
H (*5*)	P:M-TS	14 (*1*), 13 (*17*), 34 (*24*), 34 (*30*), 9 (*16*)
I (*5*)	P:M-OB	3 (*75*), 21 (*25*), 24 (*34*), 21 (*28*), 21 (*32*)
M (*1*)	Mesic P:M-SS-CWS	6 (*86*)
SYN (*1*)	Fibric P:M-SS-CWS	4
WA5 (*1*)	Peat	29
ALB (*1*)	P:M-TS-LOS	3
Natural sites (ecosites)		
a1 (*3*)	lichen, Pj	50 (*10*), 80 (*26*), 87 (*27*)
b1 (*3*)	blueberry, Pj-Aw	51 (*29*), 66 (*61*), 66 (*63*)
b3 (*3*)	blueberry, Aw-Sw	105 (*2*), 52 (*49*), 81 (*64*)
d1 (*3*)	low-bush cranberry, Aw	79 (*4*), 61 (*8*), 58 (*61*)
d2 (*3*)	low-bush cranberry, Aw-Sw	70 (*19*), 77 (*50*), 73 (*57*)
d3 (*3*)	low-bush cranberry, Sw	167 (*20*), 109 (*21*), 107 (*23*)

*DP, direct placement; P:M, peat:mineral mix; SS, subsoil; CWS, clearwater shales; OB, overburden; TS, tailings sand; LOS, lean oil sands.

[†]Aw = *Populus tremuloides*; Pj = *Pinus banksiana*; Sw = *Picea glauca*.

[‡]For reclaimed sites this represents the time since prescription application; for natural stands it is the age at breast height of the oldest tree from off-site tree data.

Table 2.2 Enzyme activities, functional evenness, and PLFA biomass in organic and mineral horizons in each reclaimed or natural site type

	BGLUCO	NAG	PER	POX	PASE	XYLO	URE	*E*	PLFA	logCFU
Organic										
a1	1252.2	1633.2	6805.3	7529.9	3349.2	498.3	3.8	2.86	ND	3.4
b1	5953.3	2799.2	14173.3	11122.1	5856.4	328.9	0.5	2.93	ND	4.0
b3	8446.3	2470.6	7120.7	1944.5	6574.0	653.4	1.5	2.98	ND	3.8
d1	4768.4	3939.3	6859.6	12055.3	3839.4	764.2	4.6	3.07	ND	3.8
d2	13164.2	5548.3	5835.4	4271.7	8514.5	376.0	1.8	3.28	ND	3.9
d3	22637.6	8722.9	5482.2	6019.8	13891.9	1178.3	2.3	3.51	ND	3.5
Average	**9370.3**	**4185.6**	**7712.7**	**8507.2**	**7004.2**	**633.2**	**2.4**	**3.1**		**3.7**
A	32422.7	5365.3	3822.2	1012.5	2888.2	195.1	4.2	1.92	ND	5.2
B	30395.4	5284.0	6831.1	1936.6	2431.5	793.9	13.0	2.19	ND	4.7
E	35754.1	9377.7	2856.9	4340.2	10610.3	839.5	4.8	2.29	ND	5.0
F	11373.1	9338.7	2378.1	3296.4	5279.2	260.3	7.0	3.90	ND	4.7
H	13956.9	2714.9	6909.8	1827.0	1826.5	344.8	2.7	2.74	ND	4.5
I	37897.8	16292.0	3025.6	4556.1	11336.3	1007.5	3.4	2.96	ND	4.6
M	2490.0	1140.9	12902.9	4826.8	1184.9	990.2	13.9	2.77	ND	5.6
WA5	0.0	10054.7	0.0	4144.7	8020.7	1056.8	9.7	2.95	ND	3.6
Average	**22411.2**	**7446.0**	**4840.8**	**3242.5**	**5447.2**	**686.0**	**7.3**	**2.7**		**4.7**
P-value[†]	**	*	NS	*	NS	NS	**	*		*

	BGLUCO	NAG	PER	POX	PASE	XYLO	URE	*E*	PLFA	logCFU
Mineral										
a1	6209.3	2684.6	7944.5	7624.7	1506.8	19.4	0.2	2.88	60.0	2.7
b1	2878.5	1052.0	8999.9	6605.9	4058.3	48.8	0.4	3.66	107.1	3.2
b3	2939.8	3054.5	16246.9	1552.8	6094.1	127.1	1.1	2.10	211.0	3.4
d1	301.8	869.7	12836.2	13554.4	2179.3	367.3	0.0	2.32	282.2	2.9
d2	12314.1	924.3	7863.5	4150.9	880.9	62.7	0.2	1.56	406.7	3.4
d3	2799.8	1566.9	5011.2	5346.5	1315.7	217.5	1.0	2.46	325.2	2.7
Average	**5023.9**	**1642.0**	**9817.0**	**6472.5**	**2672.5**	**140.5**	**0.5**	**2.5**	**232.0**	**3.1**
A	3244.0	2152.8	10738.5	4198.2	3129.4	152.4	0.4	2.69	114.0	3.8
B	7414.6	2762.5	5562.6	4348.9	5686.6	165.0	2.2	2.69	54.6	4.6
E	2939.2	2896.4	3531.8	7185.3	9800.2	557.2	1.1	2.64	254.2	3.6
F	57.4	126.2	4455.8	0.0	6.5	0.0	0.0	1.08	37.7	3.9
H	973.6	576.4	8552.9	3152.1	1441.5	172.2	0.9	2.31	62.9	3.9
I	13680.9	7199.2	6854.7	2915.7	4967.0	452.2	2.1	2.65	186.9	3.7
M	8338.3	6208.8	4685.6	6781.1	12006.2	180.5	2.4	4.55	84.1	3.6
ALB	48.2	50.2	12024.0	1324.6	0.0	45.1	2.4	1.24	-	4.1
SYN	541.9	289.8	9426.7	1340.2	916.8	22.5	0.0	1.71	-	3.8
WA5	0.0	4647.9	1303.9	10470.4	16740.3	438.6	0.0	2.73	266.7	4.7
Average	**3743.8**	**2691.0**	**6713.7**	**4871.7**	**5469.4**	**218.6**	**1.1**	**2.4**	**132.6**	**4.0**
P-value	NS	NS	*	NS	NS	NS	*	NS	**	*

* P < 0.05; ** P < 0.01.

† *P*-value based on comparing mean activities of reclaimed and natural sites (t-test).

Activities represent averages and are expressed as nmol g^{-1} soil h^{-1} or, for urease, as µg NH_4^+-N g^{-1} soil h^{-1}. Abbreviations: ND, not determined; NS, not significant (P>0.05); POX, phenoloxidase; PER, peroxidase; URE, urease; BGLUCO, β–glucosidase; NAG, N-acetyl-β-D-glucosaminidase (chitinase); XYLO, β-xylosidase; PASE, phosphatase. Values are the means of 3-6 values per site type. Where applicable, standard errors of the means were less than 15%.

"PLFA" represents the sum of biomarker concentrations (nmol PLFA g^{-1} soil) with less than 20 carbons; "logCFU" is the $\log_{10}$-CFU g^{-1} soil.

Table 2.3 Enzyme activities and PLFA biomass in organic and mineral horizons of soils of each prescription age class (time since reclamation; y) and natural soils

Age class (y)	BGLUCO	NAG	PER	POX	PASE	XYLO	URE	PLFA
Organic								
1 (1-9)	7143.8	1578.2	5987.4	3712.3	1406.6	550.60	6.21	ND
2 (10-18)	23339.5	4024.2	6627.7	2435.3	2903.5	647.3	4.80	ND
3 (19-27)	44046.2	14208.8	2867.7	4313.9	10452.8	777.5	10.30	ND
4 (28-36)	3713.1	5250.8	4131.2	1965.3	3584.2	445.7	3.52	ND
5 (natural)	9370.3	4185.6	7712.7	6990.6	7004.2	633.2	2.44	ND
Mineral								
1 (1-9)	2566.0	1993.5	9038.1	5080.1	3229.0	145.6	1.59	93.2
2 (10-18)	4318.3	1517.7	6288.9	4007.6	2702.5	135.4	0.80	100.5
3 (19-27)	11774.4	7113.9	6598.4	6643.9	8876.8	606.7	1.69	194.9
4 (28-36)	1449.6	2139.9	5979.6	5515.0	7620.3	244.4	0.75	120.4
5 (natural)	4573.9	1542.0	9817.0	6472.5	2672.5	140.4	0.47	242.7

See Table 2 for abbreviations and definitions.

Values are the means of 4-8 values per age class. Where applicable, standard errors of the means were less than 15%.

Table 2.4 Fungal ITS sequence affiliations to closest database relatives

Prescription- or ecotype-specific distribution of sequences (n = 55)	Closest BLAST relative	Accession	Identity (%)	NCBI description (origin)
	Basidiomycota			
1B[§] , 1A, 3d1	Uncultured clone 11.45	AY971685	85	Endophyte (pine)
1b1, 2b3	Uncultured clone 138-17	DQ421228	99	NA
2d1, 3d3, 1E	*Piloderma fallax* EL202	AY010281	96	ECM (Alaska forest)
2b1, 2b3, 1d2	*Piloderma fallax* HJA2138	AY534198	98	ECM (Douglas-fir)
2a1, 4b1,1d2	*Macrolepiota konradii*	AJ617494	99	NA
1b3, 2d2	*Hydnellum aurantiacum*	AY569022	98	Epiparasitic
3B, 1H, 2a1, 1b1	*Dioszegia crocea*	AJ581070	83	(Arbuscular mycorrhiza root)
	Zygomycota			
3d1, 1A, 2F	*Mortierella* sp. aurim1202	DQ093726	98	Ericacea root (afforested clearcut)
1I, 1F, 2a1	Uncultured fungus isolate RFLP-143	DQ309182	95	Ericacea root
2I, 1B, 1E	Uncultured fungus clone OTU37	EF521239	92	NA
1A, 3B, 1b1	Uncultured soil fungus clone 32-33	DQ420966	94	NA
	Ascomycota			
2I, 3H	*Preussia africana* 28/1.6.1	DQ865095	86	Root endophyte (sclerophyll forest)
3F, 1H	*Gibberella avenacea* K981	AY147281	86	Pathogen
2H, 1M	*Fusarium* sp. F13	EF055302	76	Pathogen

Abbreviation: NA, not available.

[§] Number of occurrences, i.e., individually sequenced DGGE bands, per ecotype or prescription.

Table 2.5 Proportion of aggregate enzyme activity variation explained by significant environmental variables based on DISTLM analysis

	Reclaimed		Natural	
Variable	Organic	Mineral	Organic	Mineral
Soil				
pH				24.9**
total C	17.6*			
total N		27.2**		11.9*
Mg	14.7*			
Ca			14.3*	
B				8.8*
Zn			8.6*	
S			11.5*	
Plant cover				
Bare			15.8*	
Broadleaves	26.8*			
Pine				19.4*

* $P < 0.05$; ** $P < 0.01$.

Table 2.6 Proportion of microbial community variation explained by significant soil, plant and spatial variables based on DISTLM analysis

	DGGE				*PLFA*	
	Reclaimed		Natural		Reclaimed	Natural
Variable[§]	Organic	Mineral	Organic	Mineral		
Soil						
F:B					31.8***	
C:N	7.1*					
total N						36.3***
Mg	6.5*					
K			10.0*			
P		6.8*	8.9*			
Al			8.6*	11.5**		
B				11.0*		
Fe				8.9*		7.7*
Zn						13.4**
Plant cover						
Broadleaves			10.6*			
Forbs		6.6*				
Spruce		6.1*				
Pine						27.1**
Space						
X^3				12.5**		
Y	7.4*					
XY^2	5.5*					

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; blank spaces indicate lack of significance.

[§] Sequential models were built by entering each variable set (e.g., "soil") separately.

Abbreviation: F:B, fungal-to-bacterial-biomass ratio.

Table 2.7 Significant (P<0.05) bivariate correlations between PLFA abundances and enzyme activities in reclaimed and in natural sites

	BGLUCO	NAG	PER	POX	PASE	XYLO	URE
Saturated							
12:0						0.41	(0.52)[§]
13:0				0.43		0.52	
14:0					0.42	0.71	
15:0		0.54			0.55		
16:0		0.44	-0.40		0.54	0.83	
17:0		0.46	-0.41		0.63	0.84	
18:0					0.51	0.68	
20:0						0.58	
Fungi							
18:1 ω9c		0.42	-0.42		0.54		
18:2 ω6		0.71 (0.69)	(0.57)		0.43	0.61	0.59 (0.55)
18:3 ω6c		0.42				0.69	
Gram -							
10:0 2OH		0.70	-0.34		0.47	0.82	0.43 (0.57)
11:0 2OH							(0.64)
10:0 3OH		(0.69)		0.42		0.52	(0.55)
12:0 2OH				0.41		0.52	
12:0 3OH		0.50	-0.39		0.53	0.81	(0.68)
15:0 2OH				0.46		0.52	
15:0 3OH							(0.49)
16:1 2OH						0.42	
16:0 2OH	(0.47)			0.40	0.45 (0.64)	0.71 (0.60)	
18:1 2OH		0.48	-0.46		0.50	0.78	
18:0 2OH						0.44	
18:0 3OH						(0.53)	
i14:0 3OH							(0.55)
i15:0 3OH			-0.49	0.48		0.66	
i17:0 3OH					0.45	0.67	
i15:1AT5					0.42	0.69	
i15:1G					0.41	0.72	
a15:1A						0.63	
i16:1G		0.43				0.76	
a17:1A				0.41		0.52 (0.53)	
i17:0 3OH					0.45	0.66	(0.52)
15:1 ω6c				(0.55)	0.42	0.70 (0.51)	
16:0 N alcohol	(0.50)				0.54	0.47	
16:1 ω11c		0.41			0.48	0.76	
16:1 ω5c					0.45	0.53	
17:1 ω9c						0.65	
17:1 ω8c					0.48	0.74	-
17:1 ω7c	(0.51)						
18:1 ω7c		0.43			0.47	0.79	
18:1 ω5c						0.65	
18:1 ω9t		0.51			0.50	0.88	
cy19:0 ω8c		0.49			0.47	0.83	

	BGLUCO	NAG	PER	POX	PASE	XYLO	URE
Gram +							
i12:0				0.45		0.52	
i13:0						0.52	
a13:0			-0.43		0.44	0.70	
i14:0					0.48	0.79	
a14:0				0.41		0.52	
i15:0		0.39			0.48	0.77	
a15:0					0.46	0.77	
i16:0		0.45			0.48	0.84	
a16:0		0.52			0.54	0.82	
i17:0						0.74	
a17:0		0.49			0.50	0.84	
i18:0				0.43		0.52	
a19:0						(0.57)	
cy17:0					0.46	0.82	
Actinomycetes							
10M 16:0						0.60	
10Me 17:0		0.41			0.46	0.82	
10Me 18:0						0.74	

[§]Numbers in parentheses are correlations between PLFA abundances and enzyme activities for natural sites.

Abbreviations: BGLUCO, β–glucosidase; NAG, N-acetyl-β-D-glucosaminidase (chitinase); PER, peroxidase; POX, phenoloxidase; PASE, phosphatase; XYLO, β-xylosidase; URE, urease.

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3 Relationship between soil properties and patterns of bacterial β-diversity across boreal forest soils of differing fertility[4]

3.1 Introduction

Boreal ecosystems comprise about 25% of the world's closed-canopy forest as well as vast expanses of open transitional forest; therefore, changes in their productivity levels due to disturbance may have significant effects on the global climate (Chapin et al. 2000). In these systems, much research to date has focused on assessing the effects of anthropogenic disturbances either on microbial populations driving relevant biogeochemical cycles (e.g., methane production: Metje and Frenzel, 2005; Mohanty et al., 2006) or on fungal communities required for mycorrhizal symbioses (Treseder et al., 2007; Allison et al., 2008). Considering the critical roles the bacterial communities may play in boreal forest soils (Thormann et al., 2004), studies on their composition and distribution and response to disturbance from a molecular-phylogenetic perspective are surprisingly scarce (e.g., Neufeld and Mohn, 2005).

Although several studies have recognized the importance of forest type as a driver of microbial community composition (e.g., Bach et al., 2008; Leckie et al., 2004), we have a poor understanding of how regional environmental gradients affect the abundance of specific bacterial taxa. In boreal forests, differences in slope position and soil parent material create moisture gradients along which upland vegetation is arranged (Bridge and Johnson, 2000). Across the gradients, plant species can influence forest floor microbial communities though organic matter quality (e.g., C:N ratio) effects (Priha et al., 2001), though non-specific

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changes in bacterial composition have also been attributed to other site factors such as pH and, indirectly, moisture (Pennanen et al., 1999; Hogberg et al., 2007). In mineral horizons, evidence for the direct impact of dominant tree species is less clear (Priha et al., 1999), and is likely linked to soil type differences rather than to plant species *per se* (Hackl et al., 2004, 2005).

Culture-independent techniques such as PLFA and FAME profiling have proven useful for ascertaining links between inter-site variation in edaphic properties and microbial community structure of forest soils (Bååth and Anderson, 2003; Hackl et al., 2005). Using DGGE and RISA fingerprinting, Smith et al. (2008) revealed how variation in the composition of bacteria across disturbed (clear-cut harvested and burned) boreal forest sites were mainly explained by changes in pH. With these relatively coarse techniques, however, taxa that are relevant for discerning treatment effects may remain undetected (Elshahed et al., 2008). In this sense, clone-library-based methods provide increased sensitivity as they allow both a more comprehensive sampling of key bacterial groups (Axelrood et al., 2002) and the estimation of divergence-based measures of community similarity, which account for the fact that not all detected phylotypes are equally related to each other (Lozupone and Knight, 2007; Lauber et al., 2008).

Soil microbial responses to forest management have been widely examined, though few studies have explored fine-resolution bacterial community shifts in post-reclamation landscapes (Tsai et al., 2009). Large-scale mining for oil extraction in the Canadian boreal forest has stimulated interest in assessing the success of reclamation in terms of its ability to recreate conditions suitable for the re-establishment of soil microbial components, which are vital for forest nutrient cycling processes (Johnson and Miyanishi, 2008). The majority of

research in this area has focused on the potential of mycorrhiza for re-colonizing mine spoils (Bois et al., 2005; Quoreshi et al., 2008), with research on the shifts in the relative abundance of bacteria that may occur following reclamation being relatively overlooked (McMillan et al., 2007).

Here, we used a 16S rRNA-sequence-based approach to quantify bacterial phylogenetic and compositional diversity patterns in natural boreal forest sites belonging to two contrasting fertility classes (xeric-poor and mesic-rich) in the Athabasca oil sands region, where most of the oil mining activity within the boreal forest is concentrated. We also analyzed two adjacent disturbed sites, one that had been capped with peat after surface mining and did not support plant growth ('ALB') and another that had been used to store peat and eventually developed a plant community similar to that found in productive natural sites ('WA5'; Rowland et al., 2009). Our goal was to compare the distribution of dominant bacterial taxa both within natural sites and among these and disturbed sites. Across natural sites, this was addressed by sampling mineral and organic horizons. Additionally, we examined whether variation in the composition of bacteria, independently of origin or horizon, corresponded to changes in key environmental factors—total carbon and nitrogen concentrations, moisture content, and pH. Based on the growth-strategy classification scheme proposed by Fierer et al. (2007), we hypothesized that nutrient-poor boreal forest soils would favor the dominance of slow-growing groups such as Actinobacteria; conversely, soil conditions from mesic sites would tend to favor Acidobacteria and other fast-growing taxa (e.g., Hackl et al., 2004). This led us to predict that (i) WA5 would bear a stronger compositional resemblance to natural sites than to barren ALB; (ii) the communities from xeric sites would be more similar to each other than to soils sampled from mesic sites; and

(iii) bacterial community similarity among natural sites would be larger than that between natural and disturbed sites. Using soils sampled from these sites, we also evaluated the suitability of 3-bromo-deoxyuridine (BrdU), a nucleotide analog, for assessing the composition of active bacterial taxa (as in, e.g., Hanson et al., 2008). Because we expected that active bacterial communities would be less diverse than total bacteria, this approach allowed us to determine if reduced diversity would be characterized by changes in the relative abundance of specific taxa or by evenly distributed shifts across all taxa.

3.2 Methods

3.2.1 Study sites and soil sampling

Our six study sites were selected from a system of long-term monitoring plots established in 2000 on reclaimed and natural sites within the Athabasca oil sands region near Fort McMurray, Alberta, Canada (57º 00' N, 111º 28' W) (Johnson and Miyanishi, 2008). Two sites (10 and 26) were represented by nutrient-poor, xeric, sandy Dystric Brunisols composed of fluvial material. Lichens and *Pinus banksiana* are the indicator species of the sites, and as such belong to the ecological unit (or ecotype) 'a1' [(following the 'ecotype' classification system of Beckingham and Archibald (1996)]. Two sites (50 and 57) have nutrient-rich, mesic, loam Gray Luvisols dominated by morainal-like material; low-bush cranberries and *Populus tremuloides* are their representative species (i.e., they were classified into ecotype 'd2'). Site ALB had been surface-mined and had recently (3 y prior to the time of sampling) been capped with a ~50 cm layer of a 60%:40% peat-mineral mix covering tailing sands. Finally, we sampled site WA5, 29-year-old peat waste area with a plant community also containing *P. tremuloides* as the main tree species. For all analyses, the two reclaimed sites

were regarded as “disturbed” and the other four sites as “natural”. Additional details of site characteristics (edaphic properties and dominant plant types) and reclamation procedures are found in Johnson and Miyanishi (2008), Rowland et al. (2009), and Table 1. The organic horizon and the upper 10 cm of the mineral horizon was sampled in June 2005 from ten random locations at each natural site (at the reclaimed sites only the top 10 cm of capping material was sampled), composited, sieved to < 2 mm, and stored at -80ºC.

3.2.2 BrdU labeling and isolation of BrdU-labeled DNA

Three soil sub-samples (0.5 g wet weight) from the disturbed sites, ALB and WA5, were placed in 5-ml vials and amended with 5 µl of 100 mM BrdU. Following 24 h of exposure to the analog in the dark at ~21ºC, DNA was extracted from each replicate as described in the ‘Clone library construction’ section. The extracts were pooled and the BrdU-labeled DNA was isolated with immunocapture according to the method of Yin et al. (2000). This yielded two distinct DNA fractions: one containing those populations that had actively incorporated the nucleotide analog and one containing the total bacterial diversity. The “total” fraction represents the diversity that would be recovered from samples incubated in the absence of BrdU (Yin et al., 2000). The procedure did not produce visible PCR amplification products following incubations with BrdU-free water, demonstrating that the BrdU was selectively incorporated into active (i.e., synthesizing) DNA.

3.2.3 Clone library construction

DNA was extracted from 0.25-g soil samples with Power Soil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA, USA). Triplicate PCR reactions were carried out on each soil

extract with bacterial primers 27F and 907R (Lane, 1991) under the conditions described by Dimitriu et al. (2008). The 25-µl PCR mixtures contained (final concentrations) 1 µM of primers, 250 µM of each dNTP, 1 U of *Taq* polymerase (New England Biolabs), in 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and deionized water. Pooled amplicons were purified using Qiagen QIAquick kits (Qiagen, Valencia, CA, USA) and cloned into *E. coli* JM109 cells with the Promega pGEM-T Easy cloning kit (Promega, Mississauga, ON, Canada) following the manufacturer's instructions. From each library, white colonies were picked and inoculated in LB with ampicillin (50 µg/mL). After an overnight incubation at 37ºC, cloned inserts were amplified from cultures using primer pair T7/SP6 and sequenced with one-directional reads at the McGill University and Genome Québec Innovation Centre.

3.2.4 Sequence analysis

DNA sequences were visualized and manually edited using BioEdit (Hall, 1999). Sequences were checked for chimeras with MALLARD (Ashelford et al., 2006). After removing short (i.e., <400 bp) and low-quality sequences, the remaining sequences were assigned to taxonomic groups using the RDP-II Classifier with a confidence threshold of 80% and aligned using MAFFT v. 5 (Mieszkin et al., 2009). Ambiguous assignments, i.e., those falling below the specified threshold at the class level, were compared with *blastn*-based affiliations and discarded if the ten best-scoring target database sequences were not assigned to the same bacterial group as determined by the Classifier. Aligned sequences were imported into *dnadist* and used to construct Jukes-Cantor-corrected distance matrices, further analyzed with DOTUR (Schloss et al., 2005) to bin sequences into *o*perational *t*axonomic

*u*nits (OTUs). DOTUR was also used to derive diversity parameters—Chao1 and Shannon-Weaver indices—from clone libraries. Phylogenetic trees were inferred with maximum likelihood as implemented in RAxML BlackBox (Stamatakis et al., 2008). When necessary, trees were rooted with a *Haloferax volcanni* 16S rRNA sequence (accession no. AY425724).

To evaluate the extent to which taxa were shared between communities from different soils (β-diversity), we used phylogenetic (divergence-based) and taxonomic (species-based) approaches. The best-scoring tree was used for a parsimony (P) test (Martin, 2002) as implemented in the program TreeClimber (Schloss and Handelsman); this allowed us to assess whether phylogeny significantly covaried with sample type (i.e., organic or mineral horizon for each ecotype, disturbed, and BrdU-labeled). Using the same maximum likelihood tree as input, we calculated the UniFrac metric to test for phylogenetic differentiation ('UniFrac test'; Lozupone and Knight, 2005). To qualitatively assess the environment-specific clustering of communities, we computed pairwise weighted (normalized) UniFrac distances and constructed an unweighted pair group method with arithmetic mean (UPGMA) dendrogram (Lozupone et al., 2007). We used SONS (Schloss and Handelsman, 2006) to compute abundance-based Jaccard similarities ($\hat{J}_{abd}$) among communities at sequence similarity thresholds of 97 and 90%, which correspond approximately to species- and order-level 16S groupings (DeSantis et al., 2007). Pairwise $\hat{J}_{abd}$ values were converted to distances and used to construct UPGMA dendrograms. This approach considers taxonomic (OTU-based) similarities in community structure, but disregards inter-sample phylogenetic distances.

3.2.5 Statistical analysis

The effects of sample type—natural ecotypes and disturbed—on bacterial *phylogenetic* structure were assessed using P- and UniFrac tests as described above. We applied a two-way permutational multivariate analysis of variance ('PERMANOVA'; Anderson, 2001) to test the interactive effects of soil horizon and ecotype on shifts in community structure at the natural sites. To model community composition based on soil characteristics (standardized to remove effects of different measurement units), we used non-parametric multivariate multiple regression (DISTLM; McArdle and Anderson, 2001). Significant variables, selected with a step-wise forward selection procedure using an inclusion threshold of $P<0.05$, were determined with 9999 permutations of residuals under the reduced model (McArdle and Anderson, 2001). Models were built separately for the whole data set (excluding labeled samples) and for natural sites. The relationships between individual bacterial class abundances and soil attributes were further examined using simple linear regression models.

3.3 Results

3.3.1 Taxonomic distribution and diversity of 16S rRNA clone libraries

After removing low-quality 16S rRNA sequences according to the criteria outlined above, our final dataset ($n = 674$) yielded 577 OTUs-97 (i.e., those clustered around a sequence similarity threshold of 97%) and 321 OTUs-90 (Table 2). There were no distinctive patterns of bacterial diversity between the organic and mineral horizons —particularly for species-level estimates— as indicated by overlaps of 95% confidence intervals (Table 2). However, we found a clearer trend of lower diversity in active bacterial communities (BrdU-labeled

samples, ALBL and WA5L) compared to the total community (non-labeled samples, ALB and WA5) (Table 2).

Members of the classes α-*Proteobacteria* (28%), *Acidobacteria* (19%), and β-*Proteobacteria* (10%) dominated the complete sequence data set (Table 3). The relative abundances of bacterial classes varied across site type. Sequences representing the α-*Proteobacteria* were found in roughly the same proportion across all sites. However, dominant alphaproteobacterial OTU-97, shown in Table B.1 and Figures B.1 and B.2 (Appendix B), were primarily restricted to natural sites. In the disturbed sites fewer of the α-*Proteobacteria* were active—BrdU-labeled samples (ALBL and WA5L) contained a comparatively lower incidence of this class (12 and 16%, respectively)—but there was a ~2-3-fold higher proportion of *Sphingobacteria* and unclassified sequences that were active compared to the total community. The relative abundance of *Acidobacteria* was generally ≥15%, whereas the β- and Gamma γ-*Proteobacteria* were represented by ~7 and ~9% of the sequences from natural and disturbed libraries, respectively. The *Actinobacteria* tended to be abundant in organic horizons of natural sites, and the remaining class-level taxa were represented, on average, by less than 4% of the sequences (Table 3).

3.3.2 Patterns of bacterial community composition

The effects of ecotype and soil horizon on bacterial community composition were not significant ($P>0.05$ for main factors and their interaction), according to the PERMANOVAs on $\hat{J}_{abd}$ pairwise distances (as calculated for 97 and 90% sequence similarity levels). However, the environment types (ecotypes, disturbed, and labeled) were significantly clustered on the phylogenetic tree ($P<0.001$), as revealed by the P- and UniFrac tests.

Patterns of overall phylogenetic differentiation, however, appeared to be driven by the active (BrdU-labeled) communities from the disturbed sites, ALBL and WA5L, as shown by the UniFrac test (P<0.01 for both cases). Bacterial communities tended to cluster within soils with similar pH, as revealed by UPGMA clustering of the bacterial phylogenetic distances (Fig. 1a). Similar groupings were observed using Jaccard dissimilarities, though discrimination between natural and disturbed samples was clearer at 90%-level (Fig. 1c) than at 97%-level (Fig. 1b) similarities.

3.3.3 Relationships between bacterial communities and soil properties

Within natural sites, moisture content explained 32% of the variability in UniFrac-based bacterial β-diversity (DISTLM: pseudo-$F_{1,6}$ = 3.9, P<0.05). By contrast, the variance in taxonomic dissimilarities ($\hat{J}_{abd}$) was not significantly accounted for by any soil property (P>0.05). When all clone libraries were analyzed together, soil pH explained 34% of the variance in phylogenetic community structure (UniFrac distances; DISTLM: pseudo-$F_{1,8}$ = 4.1, P<0.05). pH also accounted for 14 and 18% in the variability of taxon-based dissimilarities ($\hat{J}_{abd}$) as defined by 97 and 90% cut-off levels (pseudo-$F_{1,8}$-97= 1.3, P<0.01; pseudo-$F_{1,8}$-90 = 1.8, P<0.01), which supported the qualitative groupings derived from cluster analysis.

To determine whether specific taxa were driving the observed patterns between soil properties and community composition shifts, we regressed the relative abundances of bacterial groups (Table 3) against the soil properties. The shifts in bacterial community composition with soil pH appeared to be driven by the relative abundance of *Acidobacteria*, which declined with increasing pH (Fig. 2a). Although other soil factors did not necessarily

explain the variability in overall bacterial community composition, they were linearly related to specific bacterial groups. Nitrogen concentrations positively covaried with the proportion of *Sphingobacteria* (Fig. 2b), while increasing C:N-ratios and moisture levels were associated with decreasing abundances *of Planctomycetes* and α-*Proteobacteria*, respectively (Fig. 2c and 2d). The taxonomic diversity of bacterial communities, whether observed (Shannon's index; Fig. 3a) or estimated (Chao1 index; Fig. 3b), was also positively correlated to soil pH.

3.4 Discussion

We found, as expected, greater phylogenetic and taxonomic similarity among bacterial communities in natural sites than between communities of natural and disturbed sites. However, the composition of the bacterial community sourced from a mature disturbed site (WA5) with a diverse plant community was closer to that found in the barren reclaimed site (ALB). This partial disagreement with our original expectations did not support our hypothesis that prevailing soil conditions (i.e., xeric-poor and mesic-rich) select for bacterial taxa defined by their growth strategies. Rather, and with the exception of d2 sites, which harbored a relatively high proportion of *Acidobacteria*, most of our soil samples were dominated by sequences related to the α-*Proteobacteria*. This suggested that the presence of plant cover alone may be inadequate to result in broad-scale (i.e., inter-site) bacterial community structure variations. Instead, patterns of bacterial β-diversity, whether divergence-based or OTU-based, were largely explained by soil pH.

Our findings also indicate that, although each soil harbored phylogenetically distinct bacterial lineages, there were no clear ecotype or soil-horizon effects on OTU-based

distances, irrespective of the level of taxonomic resolution. This lack of a discernable pattern in the bacterial communities was probably due to either our low number of site replicates lacking resolving power or the clone libraries not being large enough for obtaining reliable community similarity (SONS) estimates. In support of the latter possibility, among natural sites we found a close association between soil moisture (a soil characteristic that defines the position of ecotypes along the fertility gradient) and community composition—though only when we considered overall phylogenetic diversity, which is less sensitive to sample size (Lozupone and Knight, 2007). Thus, our results highlight the importance of using measures that incorporate phylogenetic divergence —as opposed to solely the fraction of shared species (e.g., Hackl et al., 2004)— to identify key features driving bacterial composition.

The importance of edaphic factors in shaping the composition of microbial communities has been recognized in a number of studies (e.g., Hallin et al., 2009; Fierer et al., 2009). In particular, our observation that soil pH was the main factor explaining the variability in bacterial community composition is in agreement with other studies that have demonstrated a close relationship between soil pH and bacterial community composition in boreal forests (Hogberg et al., 2007; Smith et al., 2008). Broad-scale surveys comparing more restricted taxonomic groups of bacteria such as betaproteobacterial ammonia-oxidizers have also found that community composition is regulated by soil pH (Stephen et al, 1998; Wakelin et al., 2009). Soil pH may act as an environmental filter, for instance, by stressing bacterial cells (McCaig et al., 1999), which results in selection of specific bacterial groups. This is demonstrated in our study by the linear decline in relative acidobacterial abundance with increasing pH values across the sampled sites. However, as soil pH can be considered as a variable that integrates a number of other abiotic and biotic soil characteristics, we cannot

determine whether pH is having a direct or indirect influence on bacterial community composition. Nevertheless, and in agreement with recent reports (Lauber et al., 2008; Jones et al., 2009), our results show that narrow-range pH values are unlikely to predict bacterial compositional shifts. Indeed, pH did not appear to significantly correlate with bacterial communities from natural sites, all of which have acidic soils.

Soil pH was also a significant predictor of overall phylotype diversity, a pattern consistent with other studies (Staddon et al., 1998; Fierer and Jackson, 2006). It is not clear, however, whether the relationship between bacterial diversity and pH is truly linear, whether diversity plateaus in soils with near-neutral pH, or whether it follows a unimodal pattern, as Fierer and Jackson (2006) suggested. Similarly, because our clone libraries most likely underestimate total bacterial diversity, we cannot predict how the absolute diversity of bacteria changes across the pH gradient. Regardless of the underlying relationship, our results demonstrate that at the scale of our study (region) pH influences soil bacteria not only through limits on the abundance of specific taxa, but also by affecting community-wide diversity levels.

Soil moisture was a major factor governing bacterial community composition in natural sites. This correlation seemed to be driven by decreases in the relative abundance of α-*Proteobacteria* with increasing moisture content. This is in contrast to results by Fierer et al. (2007) who found that moisture was only positively correlated to the *Firmicutes*. Water availability affects the osmotic status of bacterial cells and can indirectly regulate substrate availability, diffusion of gases, soil pH, and temperature. Periods of moisture limitation typical of boreal environments may affect bacterial communities through starvation, induced

osmotic stress, and resource competition, selecting for individual bacterial groups that are tolerant to moisture-limited conditions (Harris, 1981; Treves et al., 2003).

We found no evidence in favor of distinct bacterial composition in mineral and organic horizons; however, we did observe some inter-layer differences in the relative abundance of specific taxa. Within natural (d2) sites, organic horizons harbored a higher proportion of γ-*Proteobacteria* and *Actinobacteria*, whereas *Sphingobacteria* were absent from mineral horizons. In forests with the same soil type and a similar floristic composition to that of our d2 sites, Axelrood et al. (2002) found a clearer distinction between mineral and organic horizons, although they found higher actinobacterial abundance in mineral soils. Other molecular surveys have revealed inconsistent patterns. For instance, Tsai et al. (2009) reported, as we do, some compositional overlap (computed with Jaccard similarities) between organic and mineral horizons in a broad-leaved forest, while Chan et al. (2006) attributed shifts in bacterial communities to the prevalence of *Bradyrhizobium* sp. representatives in organic horizons. Spatial effects may play a substantial role in the lack of a distinct soil-horizon effect. Bengtson et al. (2007), for instance, attributed similar spatial patterns of nutrients and moisture in mineral and organic horizons to differences in topography; similarly, Agnelli et al. (2004) demonstrated the downward flow of extracellular DNA across horizons. In our study, such sources of variability may also structure bacterial communities at a scale similar to the one observed by Bengtson et al. (2007), as we indeed found in a recent DGGE-based survey at the same sites (Dimitriu, unpublished results).

Part of the variability in community composition across sites could be accounted for by pH and moisture. Nevertheless, the compositional uniqueness of disturbed sites is likely an indirect consequence of unmeasured factors. Soil acidity has been linked to a decrease in

the availability of carbon to microbial communities (Leifeld et al., 2008). In contrast to our natural sites, disturbed sites had neutral-pH soils, which may have been associated with the accumulation of C sources selectively utilized by bacteria endemic to disturbed sites. While a lack of vegetation cover in the young site may be expected to lead to different bacterial communities, the biochemical composition of the litter derived from the dominant tree species in the vegetated site may not have had a measurable impact on microbial community structure (Priha et al., 2001). In addition, a patchy distribution of organic litter may have caused a spatial aggregation of the forest soil microbiota at the site scale (Saetre and Bååth, 2000), a likely scenario given the heterogeneity of reclaimed soil profiles (Mummey et al., 2002).

Active (BrdU-labeled) bacterial communities were more similar to total communities of the corresponding unlabeled samples than to any other sample. This was especially true for phylogenetic distances and for OTUs defined at a similarity threshold of 90%. We also observed lower richness and diversity in active samples, in agreement with published studies investigating active bacterial communities in soil (Girvan et al., 2003; Felske et al., 2000). Whereas active bacterial populations may have responded to the incubation conditions in the presence of the analog rather than to environmental cues in the field, their composition more likely reflects the *in situ* conditions at the time of soil sample collection (e.g., Allison et al., 2007). In this sense, the lower abundance of active α-*Proteobacteria* is consistent with the growth strategy that has been proposed for this group, which is considered an r-selected taxon that thrives under, e.g., high concentrations of available nutrients and labile carbon (Smit et al., 2001; Fierer et al., 2007).

In conclusion, 16S rRNA-gene sequence analyses proved appropriate for detecting compositional differences in the bacterial communities inhabiting various boreal forest soils. This study represents one of the few to examine β-diversity of bacteria in boreal environments. While methodological constraints may have restricted our ability to infer true patterns at the site scale (e.g., α-diversity), compositional differences among sites appeared to be associated primarily to the variability of soil properties. In most studies, discrimination of microbial communities is based on assessing overlaps in community membership or on comparing community composition qualitatively (e.g., by computing relative abundances of taxa). Whereas such strategies may be valid for an approximation of taxonomic affiliations, unlike phylogenetic tests they are more sensitive to undersampling and thus may not detect differences in community structures (Schloss, 2008). Our results suggest that the composition of bacterial communities inhabiting natural forest soils is primarily regulated by moisture; disturbing these soils, in turn, may elicit further shifts in community composition provided a "master" variable such as pH changes concomitantly. Finally, we show that in disturbed sites a reduction in the diversity of active bacterial communities is linked not only to a decline in overall richness but also to lower evenness of dominant taxa. In disturbed sites, the phylogenetic differentiation of bacterial communities suggests an unmeasured suite of soil properties (e.g., soil organic matter quality) that locally constrain the distribution of taxa and were not superseded by plant cover effects.

Figure 3.1 UPGMA dendrograms of the pairwise estimates of bacterial community dissimilarities. (A) UniFrac distances; (B) $\hat{J}_{abd}$ distances (97% cut-off); (C) $\hat{J}_{abd}$ distances (90% cut-off). Filled squares represent nodes with bootstrap support values of ≥50%.

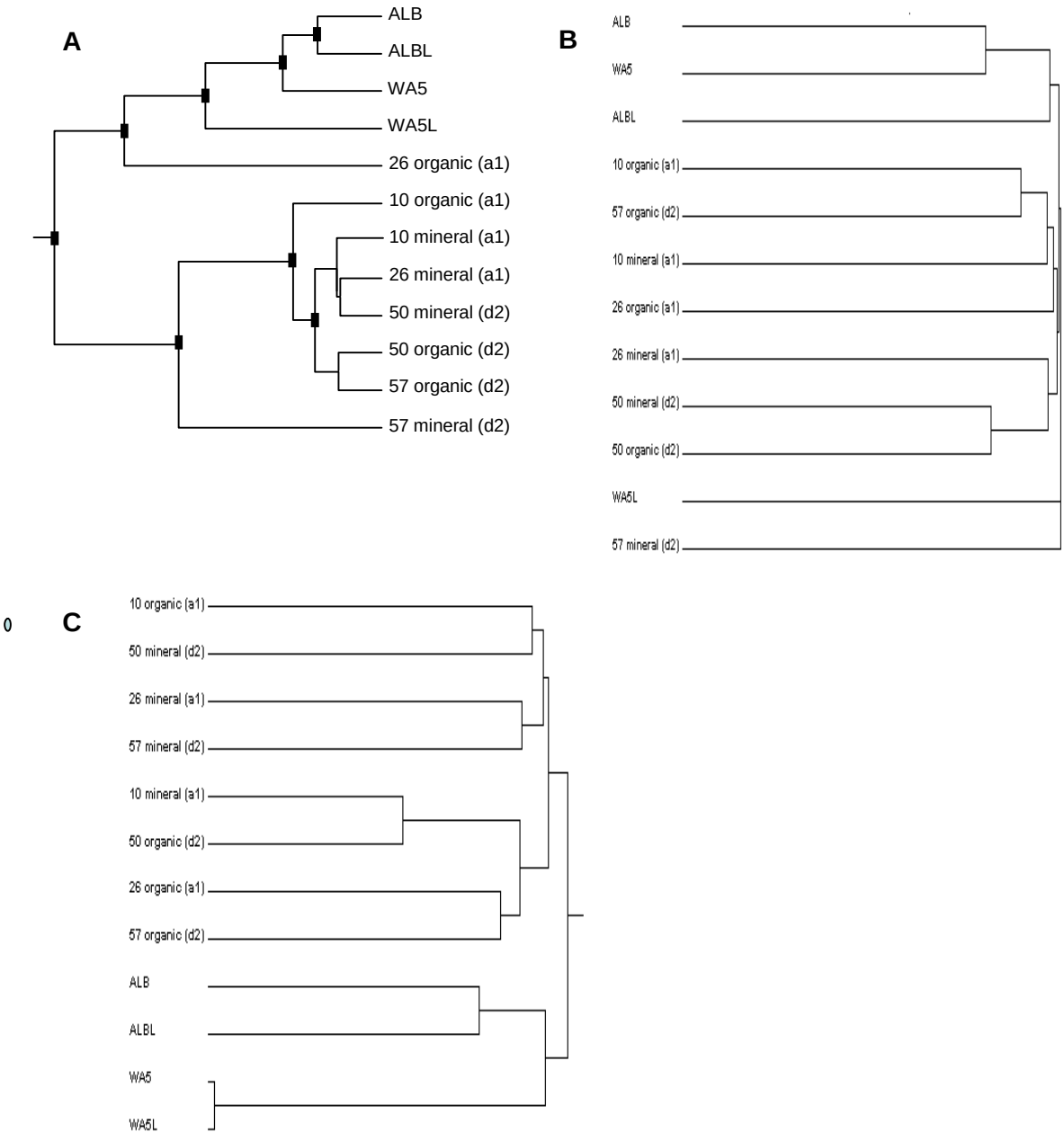

Figure 3.2 Linear regressions showing the relationship between (A) acidobacterial abundance and pH, (B) sphingobacterial abundance and total soil N, (C) planctomycetal abundance and C:N ratios, and (D) alphaproteobacterial abundance and soil moisture across the 10 sampled sites. Dotted lines are 95% confidence bands.

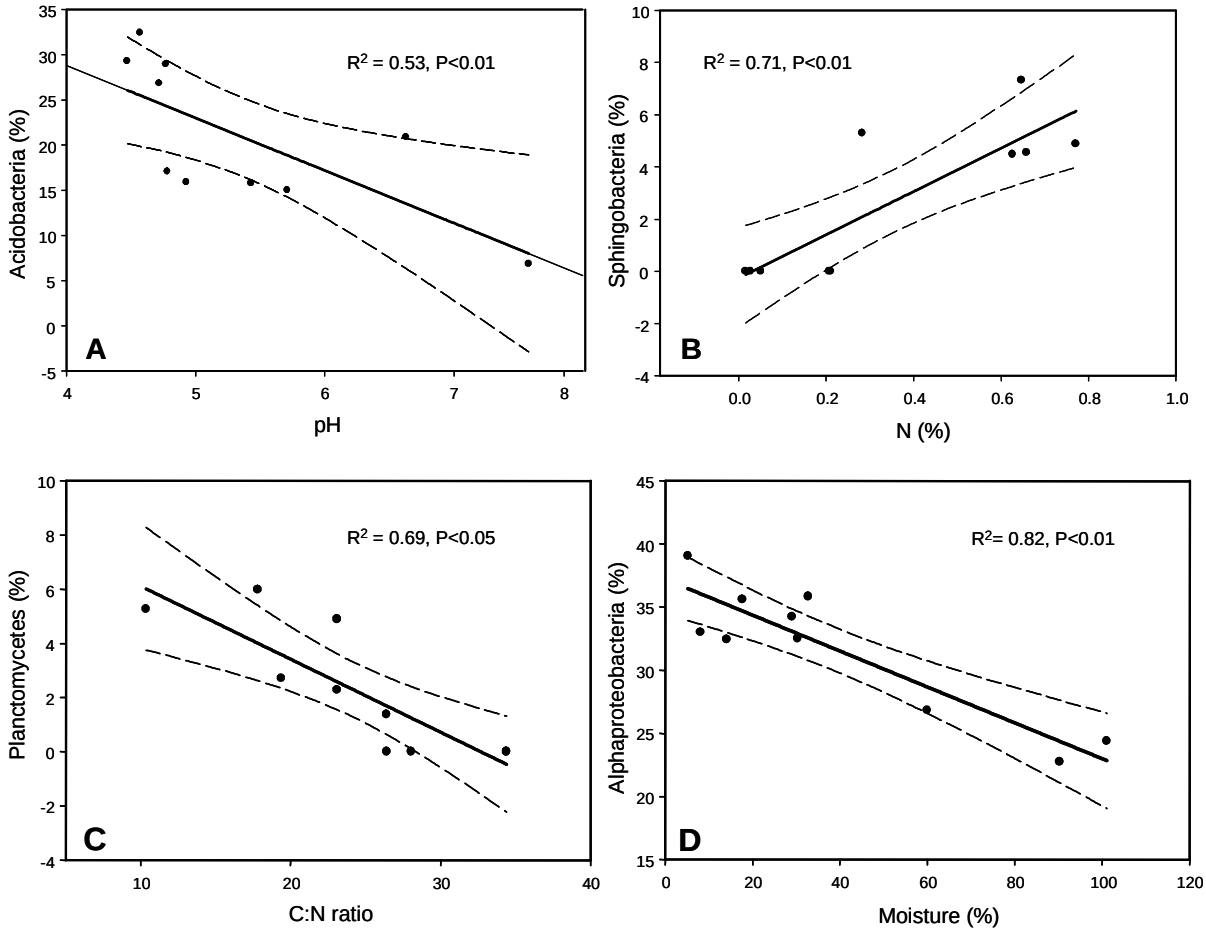

Figure 3.3 Linear regressions between pH and measures of diversity. (A) Shannon-Weaver index. Closed circles, linear relationship for OTU-90; open circles, linear relationship for OTU-97; (B) Chao1-90 index.

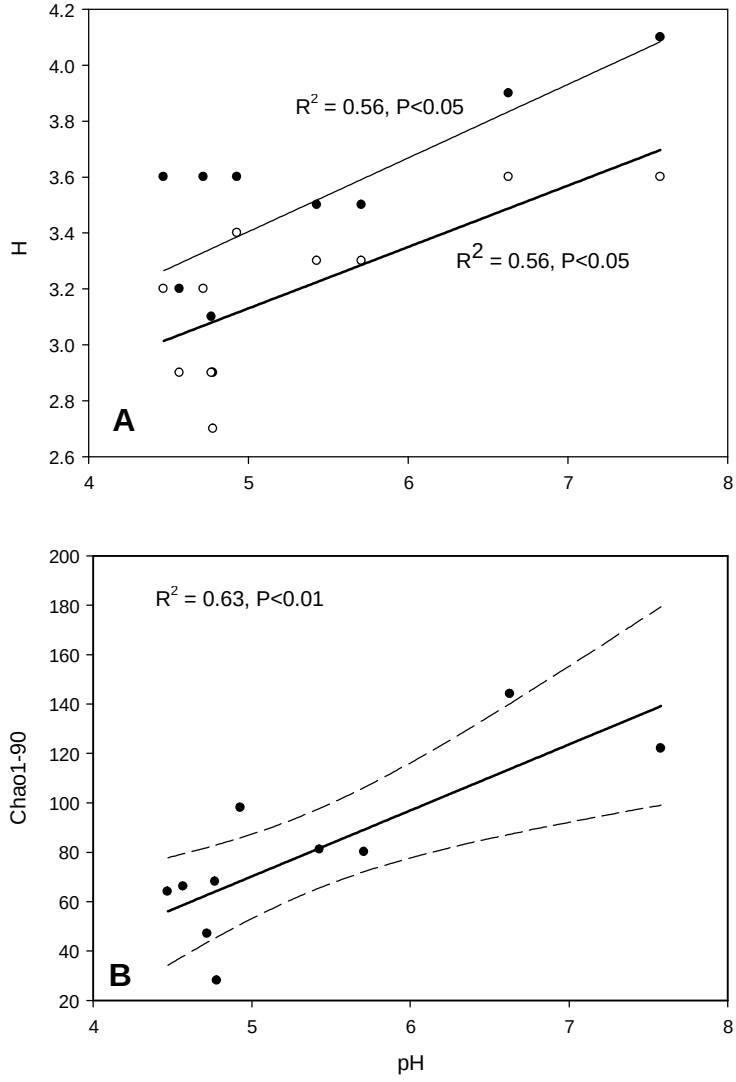

Table 3.1 Soil chemical characteristics of the sites sampled in the oil sands region

Site	pH (water)	Total soil N (%)	Total soil C (%)	C:N ratio	Moisture (%)
*a1**					
10 organic	5.2	0.21	7.2	34.4	30.4
10 mineral	5.1	0.02	0.4	25.4	5.2
26 organic	4.8	0.65	22.3	34.4	60.0
26 mineral	5.4	0.03	0.7	26.4	8.1
d2					
50 organic	4.7	0.77	21.6	28.1	101.1
50 mineral	4.6	0.05	1.0	19.4	14.1
57 organic	4.9	0.66	15.2	23.1	90.3
57 mineral	4.8	0.21	2.1	10.3	29.1
Disturbed					
ALB	7.6	0.28	7.5	26.4	17.7
WA5	6.6	0.63	12.3	19.6	32.7

**a1* and *d2* are ecotypes phases as defined by Beckingham and Archibald (1996).

Table 3.2 DOTUR-based calculation of clone library diversity parameters

Site	*N*	OTU-97[§]	OTU-90	Chao1-97	Chao1-90	*H*-97	*H*-90	*E*
a1								
10 organic	53	49	30	181 (125-350)[†]	80 (51-180)	3.5	3.3	0.95
10 mineral	51	48	24	353 (144-780)	64 (41-130)	3.6	3.2	0.97
26 organic	50	43	17	92 (44-223)	28 (23-52)	2.9	2.7	0.78
26 mineral	53	48	25	159 (80-381)	81 (48-160)	3.5	3.3	0.96
d2								
50 organic	60	45	28	142 (76-310)	47 (35-87)	3.6	3.2	0.97
50 mineral	52	48	29	408 (215-713)	66 (47-162)	3.2	2.9	0.89
57 organic	54	42	28	187 (92-361)	98 (58-200)	3.6	3.4	0.95
57 mineral	56	40	26	91 (48-221)	68 (37-180)	3.1	2.9	0.85
Disturbed								
ALB	65	56	36	229 (148-329)	122 (80-230)	4.1	3.6	0.96
ALBL*	69	45	29	143 (90-240)	96 (63-180)	3.7	3.4	0.86
WA5	61	58	37	235 (130-450)	144 (85-300)	3.9	3.6	0.93
WA5L	50	41	22	71 (43-150)	61 (38-130)	3.1	3.0	0.82
All	674	577	321	1993 (1458-2609)	657 (518-860)	5.8	5.2	0.87

Abbreviations: *N* = no. of clones; *H* = Shannon-Weaver index; *E* = *H*/ln*N* (evenness)
*'L' = *l*abeled DNA
[§]Suffixes 90 and 97 indicate 90 and 97% sequence similarity cut-off levels, respectively
[†]Parentheses next to the estimate are 95% confidence intervals

Table 3.3 Relative abundance of bacterial classes in the 10 soil samples collected in the oil sands region

Site	Alpha	Beta	Delta	Gamma	Acido	Actino	Gemma	Plancto	Verruco	Sphingo	Unclass	Other
a1												
10 organic	32.5	7.5	0	5	15	22.5	0	0	0	0	10	7.5
10 mineral	39	0	0	4.9	29.3	0	0	4.9	9.8	0	2.4	9.8
26 organic	27.8	8.3	0	5.9	17.1	0	0	0	0	8.3	13.1	19.5
26 mineral	28.9	13.2	5.3	13.2	15.8	13.2	0	0	0	0	2.6	10.5
d2												
50 organic	24.4	4.9	4.9	14.6	26.8	12.2	7.3	0	0	4.9	0	0
50 mineral	32.4	8.1	2.7	5.4	32.4	2.7	0	2.7	8.1	0	2.7	2.7
57 organic	22.7	13.6	2.3	11.4	15.9	13.6	2.3	2.3	6.8	4.5	2.3	2.3
57 mineral	34.2	10.5	0	2.6	28.9	2.6	5.3	5.3	0	0	7.9	2.6
Disturbed												
ALB	35.6	9.6	4.1	15.1	6.8	2.7	0	1.4	0	8.2	6.8	9.6
ALBL	12.2	4.1	2.7	10.8	13.5	9.5	0	0	0	23	16.2	8.1
WA5	35.8	9	3	6	20.9	4.5	3	6	0	4.5	7.5	0
WA5L	16.3	30.6	0	5.7	7.2	0	0	3.3	0	10.7	19.3	7
Total	28.1	9.9	2.1	8.2	19.1	7	1.5	2.2	2.1	5.1	8.1	6.6

Abbreviations: Acido, Acidobacteria; Actino, Actinobacteria; Alpha, Alphaproteobacteria; Beta, Betaproteobacteria; Delta, Deltaproteobacteria; Gamma, Gammaproteobacteria; Gemma, Gemmatimonadetes; Plancto, Planctomycetes; Sphingo, Sphingobacteria; Verruco, Verrucomicrobia; Unclass, unclassified. “Other” includes BRC1, WS3, Nitrospira, OP10, OP3, Firmicutes, TM7, and Flavobacteria.

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4 An evaluation of the functional significance of peat bacteria using a reciprocal transplant approach[5]

4.1 Introduction

Despite a consolidated interest in the relationship between biodiversity and function among macroorganisms (Flombaum and Sala, 2008, Loreau et al., 2002), little attention has been paid to exploring this link using soil microorganisms as model systems (Mikola and Setala, 1998; Gans et al., 2005; Nannipieri et al., 2003). While the microbial biomass mediates key functions like nutrient cycling and energy flow, it remains unclear how (or even if) changes in these functions are linked to variation in microbial community structure. To shed light on these relationships, an important set of investigations has made use of natural differences in microbial diversity/community composition occurring in pre-existing environmental gradients, such as reclamation chronosequences (Yin et al., 2000) or sites under differing management intensities (Waldrop et al., 2000). In these studies, however, the impact of microbial diversity *per se* and environmental controls on function may be confounded. Another body of work has focused on manipulating microbial diversity and measuring the associated change in function. In general, evidence stemming from the inoculation of sterile soil with serial dilutions of the same, non-sterile soil (a common way of causing reductions in diversity) suggests a high level of functional redundancy, both for phylogenetically-broad functions such as respiration (Griffiths et al., 2001, 2004), and more specialized processes such as nitrification and denitrification (Wertz et al., 2006) or the uptake of certain compounds (e.g., aminoacids) (Franklin and Mill, 2006).

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Because microbial diversity manipulations are often associated with uncontrolled compositional changes (Franklin et al., 2001; Huston, 1997), they fail in their ability to distinguish the relative influence of taxonomic identity and richness. Although both factors are important in affecting process rates (Bell et al, 2005), the effect of composition is likely moderated by environmental parameters (Reed et al., 2007; Waldrop et al., 2000). Reciprocal transplants are a valuable tool to examine the relationship between microbial community composition and function because they incorporate the relative contribution of the environment (Reed et al., 2007; Waldrop and Firestone, 2006). Typical experimental designs involve transplanting a microbial community contained in a cage (a soil core surrounded by a mesh, for instance) into different environments and assessing changes in function and/or composition (Balser et al., 2002). Field incubations —the classic set-up— provide a framework of realism, but are not flawless. For example, chemical equilibration of the substrate material with the surrounding environment is difficult to achieve (Waldrop and Firestone, 2006), and the migration of microorganisms into the transplants cannot be completely inhibited (Reed et al., 2007). In this sense, the geophysical structure of the substrate should be kept as close as possible to that of the surrounding environment (Balser et al., 2002; Waldrop and Firestone, 2006), effectively excluding it as a potential source of variation in experimental designs.

Reciprocal transplants under laboratory conditions are conceptually similar, but differ in that the transplanted community is added directly to sterile substrate (Griffiths et al., 2008, Kirchman et al., 2004; Langenheder et al., 2005; Langenheder et al., 2006). Despite its practical potential —suggested by evidence from aquatic systems (e.g., Langenheder et al., 2005; Langenheder et al., 2006)— this approach has been overlooked in soils. Recently, a

community swap between two mineral soil types showed that the ability of microorganisms to decompose barley after a heavy metal stress can be governed by the soil's physical structure through its effect on microbial community composition (Griffiths et al., 2008). This suggests the inoculum source may have a negligible impact on function and community structure. Questions remain, however, regarding the generality of these response patterns in different contexts, such as organic soils, or for more specialized functions, such as the production of lignin-degrading and nutrient-acquiring extracellular enzymes.

To test whether or not soil microbial community composition is related to function, we performed a transplant experiment by inoculating serially-diluted suspensions (10^{-1}, 10^{-3}, 10^{-5}, and 10^{-8}) sourced from two peat types, one humified and the other partly decomposed, into each other's sterile peats. Based on the hypothesized ability of serial dilutions to establish a microbial diversity gradient in the microcosms, we predicted that the peats receiving the 10^{-1} dilution would develop a more diverse bacterial community than those exposed to the 10^{-8} dilution. After incubation for 5 months to acclimate the communities, we measured short-term respiration responses and the activities of extracellular enzymes involved in the turnover of C, P, and N substrates of various levels of recalcitrance. We hypothesized that the inoculum source and the peat type would both have a significant impact on enzyme activities. In particular, we expected the inoculum source would become more important for enzymes catalyzing the degradation of recalcitrant substrates. Similarly, the peat type would mainly affect those activities related to the acquisition of labile (i.e., C, N, and P) compounds. This hypothesis was based on the expectation that, because of high local diversity, different microbial communities inoculated into a certain peat type should develop similar communities. If this is reflected in functional similarity, then the hydrolysis of labile

compounds, of broad taxonomic distribution (Allison et al, 2007a; Lynd et al., 2002), will likely follow this trend. Conversely, the degradation of recalcitrant compounds is thought to be restricted to fewer taxa (Allison et al., 2007a, b), and therefore we also hypothesized that the higher the initial serial dilution the lower the activity of enzymes that degrade such compounds. Although microfungi play an important role in peat phytomass decomposition (Thormann et al., 2002), emerging evidence suggests bacteria are also implicated in the transformation of organic matter (Pankratov et al., 2006; Kulichevskaya et al., 2006). Thus, to assess the structure and richness of microbial communities, we focused on the 16S rRNA genes of dominant bacteria as determined by PCR-DGGE. Our analysis included both total and active bacterial communities. For the latter, we applied a relatively novel method that discriminates between active and total populations after exposing soil samples to the thymidine-analog bromodeoxyuridine (BrdU) and isolating the DNA that had actively incorporated the analog (Hjort et al., 2007; Yin et al., 2000).

4.2 Materials and methods

4.2.1 Microcosm assembly

The soil samples used in this study were collected from two long-term research installations in the oil sands region of northern Alberta, Canada (for details, see Rowland et al., 2009). Two peat forms, sedge (*s*) and fibric (*f*), which are used routinely in the reclamation of forest sites after oil extraction, were selected based on characteristics that enabled us to address our main hypothesis, namely: (i) their initial bacterial community compositions were distinct, as determined by a preliminary denaturing gradient gel electrophoresis (DGGE) of 16S rRNA genes (data not shown); and (ii) their structures were markedly distinct, i.e., they differed in

decomposition state [the *s* type is characterized by highly humified sedge (*Carex* sp.), the *f* type is dominated by undecomposed moss (*Sphagnum* sp.)]. The soils' basic chemical properties were as follows: *s* type: total C: 44.2%, total N: 1.26%, moisture: 589%, pH: 3.9; *f* type: total C: 39.5%, total N: 0.88%, moisture: 601%, pH: 4.1).

The soils were either sieved to < 4 mm (sedge) or cut into ~ 2-mm pieces (fibric) and split into two parts. The first fraction was heat-sterilized by autoclaving 2 g (dry weight equivalent) of soil in 20-ml glass vials three times for 30 min, with a 24-h waiting period between each sterilization event (Lotrario et al., 1995); sterility was verified by plating a soil suspension onto nutrient agar. The second portion was used for preparing microbial inocula. Soils slurries were prepared by mixing unsterilized soil and de-mineralized water (soil:solution ratio 1:2) and grinding with a pestle and a mortar, followed by 10-fold serial dilutions. Diluted soil was inoculated into the sterile soils, to give final dilution levels of 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-8} g non-sterile soil g sterile soil $^{-1}$. We established four replicates per dilution level of the following treatments: diluted sedge + sterile sedge (designated '*s*'); diluted sedge + sterile fibric ('*sf*'); diluted fibric + sterile fibric ('*f*'); diluted fibric + sterile sedge ('*fs*'); controls included sterile, uninoculated soils. After incubation of the Parafilm-covered vials for 5 months in the dark, to allow for microbial community establishment and stabilization, we sampled all the microcosms to determine soil function and bacterial community structure and diversity of total and active populations.

4.2.2 Soil function: respiration and potential enzyme activities

Soil gross respiration (i.e., carbon mineralization) was determined by incubating 1 g of soil in 20-ml sealed vials for 24 h at ~21°C and measuring the evolution of CO_2 with an infrared gas

analyzer. According to preliminary observations, this incubation period ensured CO_2 levels reached a plateau for both soils; thus, we report maximum, potential rates (Rousk and Bååth, 2007).

We measured the potential activities of the following enzymes: β-glucosidase, involved in cellulose breakdown; *N*-acetyl-β-D-glucosaminidase, related to chitinolytic activity; acid phosphatase, which releases PO_4^{2-} from organic phosphorous sources; and phenol-oxidase, implicated in lignin depolymerization. Assays were conducted essentially as described by Stursova et al. (2006). Briefly, 0.2 ml of soil suspensions (1 g soil in 100 ml 0.1 M acetate buffer, pH 5) was dispensed into 96-well microplates. The phenol-oxidase assays were conducted in clear microplates containing 25 mM L-3,4-dihydroxyphenylalanine (L-DOPA), a substrate whose colored breakdown product is measured spectrophotometrically at 460 nm after incubation at 20ºC for 18 h. The hydrolase assays were conducted in black microplates using 200 mM of 4-methylumbelliferyl (MUB)-linked substrates. In this case, reference standards (10 mM 4-methylumbelliferone) and quench controls were added to each plate. Sixteen replicates per sample were assayed for each enzyme. After incubation for 2-5 h, fluorescence was measured using a spectrofluorometer with an excitation of 365 nm and an emission of 460 nm. Activities were expressed as nmol (hydrolases) or μmol (phenol-oxidase) g^{-1} h^{-1} of converted substrate.

To have an estimate of the joint response of enzymes to the treatments, we condensed all activity rates into a single 'catabolic evenness' index (*E*). Originally adapted by Degens et al. (2000) from the Simposn-Yule index, *E* is calculated from the soils' respiration response profiles after individual substrate additions as $E = 1/\Sigma p_i^2$, where p_i is the respiration response of each substrate as a proportion of total respiration responses summed over all substrates.

Thus, the index gives an indication of the variability in substrate use, or functional diversity. Though infrequently (Caldwell, 2005), evenness indices have been applied to enzymes by replacing respiration responses with activity rates (Tscherko et al., 2004), so that the theoretical maximum value of *E* equals the total number of enzymes being assayed (4 in this case).

4.2.3 Bacterial community structure and richness: DGGE

DNA was extracted from 0.25-g soil sub-samples with a PowerSoil Mobio DNA extraction kit (MoBio, Carlsbad, CA, USA). The bacterial community structure of total and active bacteria was characterized by PCR-DGGE of 16S rRNA genes. We used the set of primers 338F-518R, which targets the V3 region (Ovreas et al., 1997), with a GC clamp attached at the 5' end of primer 338F. Polymerase chain reactions contained (final concentrations) 1 µM primers, 250 µM of each dNTP, 1 U of *Taq* polymerase (New England Biolabs), in 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and deionized water to a final volume of 25 µl. Amplification was conducted under the following conditions: an initial denaturation at 94ºC for 5 min; 30 cycles of 94ºC for 1 min., 55ºC for 1 min, and 72ºC for 1 min; a terminal elongation at 72ºC for 7 min. Amplification products were loaded on an 8% acrylamide gel with a 35-55% denaturing gradient (100% denaturing solution is 40% formamide and 7M urea). Gels were run for 15.5 h at 60 V and 60ºC in 1× TAE buffer. After staining with SYBR Green I (Molecular Probes, Eugene, OR) for 1 h, the gels were visualized with a Typhoon 9400 variable mode imager (Amersham Biosciences, Piscataway, NJ) and their digitized images analyzed with Gel Compar II (Applied Maths, Belgium). Richness was estimated as the number of bands unambiguously detected by the software with

the default band-searching parameters; for the sake of simplicity, we regarded a 'band' as a distinct taxonomic entity to define richness. Following band matching, presence-absence matrices were constructed for inferential significance testing as described in the 'Statistical analysis' section. In addition, we used the incidence matrices to compute, for each set of dilutions, the unbiased, non-parametric estimator Chao2, as implemented in the EstimateS software package v8.0.0 (http://purl.oclc.org/estimates). This index has proved useful to obtain an estimate of the community richness based on 16S rRNA-DGGE fingerprints, as it circumvents the inherent lack of resolution of fingerprinting methods by taking into account the undetected diversity (Hughes et al., 2001; Loisel et al., 2006).

4.2.4 Assessment of active bacterial populations: BrdU labeling and immunocapture of labeled DNA

Soil samples (0.5 g) from the most and least diverse communities (dilution levels 10^{-1} and 10^{-8}) were placed in 5-ml vials and amended with 5 µl of 100 mM BrdU. After 24 h of exposure to the analog, DNA was extracted as described above. Although BrdU labeling was only carried out on a subset of the samples, this still allowed us to assess the effects on active populations of inoculum source and soil type as described below.

Immunocapture of BrdU-labeled DNA followed the method outlined in Yin et al. (2000). This yielded two DNA fractions, one containing those populations actively incorporating the nucleotide analog and one containing the total bacterial diversity. The procedure did not produce visible PCR amplification products following pre-incubations with water instead of BrdU of randomly selected soil samples, demonstrating that the BrdU was selectively incorporated into active (i.e., synthesizing) DNA.

4.2.5 Statistical analysis

For each inoculum-peat combination, the effect of dilution level on the richness of bacteria, activity of each enzyme, catabolic evenness, and respiration was tested with a one-way analysis of variance; a least significant difference test was performed to determine which means differed significantly. To assess the relative importance of the inoculum source and the soil type, we applied a two-way permutational multivariate analysis of variance ('PERMANOVA'; Anderson, 2001) as implemented in PC-ORD v. 5.0 (MjM Software, Gleneden Beach, OR). This method constructs an *F*-ratio from sums of squared distances within and between groups that is analogous to Fisher's *F*-ratio, and significance is evaluated with permutation tests (Anderson, 2001). Individual enzyme activities, catabolic evenness, respiration, and richness were tested separately. Multivariate responses (bacterial community structure and aggregated enzyme activities) were tested on Bray-Curtis transformed matrices. Finally, Mantel tests were performed to determine if there were significant correlations between active and total populations.

4.3 Results

Post-incubation bacterial numbers were comparable across dilution levels as determined by performing plate counts on nutrient agar (data not shown). Although not necessarily related to uncultivated bacterial biomass, the plate count strategy has been successfully applied as a proxy for biomass in sterile substrates inoculated with diluted soil suspensions (Wertz et al., 2006). Final numbers for each treatment were as follows [log(CFU) g^{-1} ± SEM]: *s* = 8.04 ± 0.68; *fs* = 7.95 ± 0.81; *f* = 6.36 ± 0.76; *sf* = 6.6 ± 0.9.

4.3.1 Bacterial richness and community structure

After incubation, the observed richness of bacterial DGGE fingerprints was found to exhibit mixed patterns (Fig. 1A). For the *f* treatment, richness peaked at a dilution level of 10^{-3}, while for the *s* treatment richness was significantly lower at 10^{-8} than at 10^{-1}. Though the trends in *fs* and *sf* treatments were similar to those of *f* and *s*, respectively, they were not significant. This discrepancy between samples inoculated with communities sourced from the same peat (*f* and *s*) and those inoculated with reciprocal peat suspensions (*fs* and *sf*) was partly replicated by the richness values obtained by calculating Chao2 indices (Fig. 1B). The active richness, whether observed or estimated, was always higher than total richness at 10^{-8} dilutions (Fig. 1A and 1B). According to the PERMANOVA, there was a significant inoculum source-soil type interaction effect on total richness (Table 1).

The genetic structure of the total bacterial community was significantly affected by the interaction of soil type and inoculum source (Table 1). By contrast, the experimental factors did not have a significant impact on active populations, which was reflected in the lack of an association between the active and total populations (Mantel test: $r = 0.02$, $P = 0.41$).

4.3.2 Functional parameters

Respiration fluctuated according to treatment: a peak at 10^{-3} in *f* and *fs* treatments and a linear decrease with increasing dilution in *s* and *sf* treatments (Fig. 2); the effects of treatments, however, were not significant (Table 2). Typical hydrolytic enzyme responses consisted of a progressive increase in activity with increasing dilution for *f* samples, little to no activity in *fs*

and *s* samples, and a maximum at 10^{-3} in the *sf* series (Fig. 3). By contrast, the activity of phenol-oxidase increased with increasing dilution only in *s* and *sf* microcosms (Fig. 3). A PERMANOVA of the aggregated enzyme activity response revealed soil type explained most of the variability in the data (Table 2). This trend was largely paralleled by individual enzyme activities, which were significantly affected by peat type (Table 3). The exception was peroxidase, which responded significantly to the interaction of peat type and inoculum source, but not to peat type alone (Table 3). Catabolic evenness tended to decline with increasing dilution (Fig. 4) and, like enzyme activities, was significantly affected by soil type (Table 2).

4.4 Discussion

We originally hypothesized that the inoculum source (i.e., the starting microbial community) would primarily constrain those activities presumably performed by a narrow sub-set of the microbial community (e.g., lignin depolymerization), and this would be most evident at high dilution levels where rare members of the community may be absent. Equally, the receiving peat type would become more important for activities carried out broadly across the community (e.g., nutrient acquisition). We found, as hypothesized, a stronger dependence of peroxidase activity on the inoculum source (and its interaction with the medium) and a preponderance of peat type as the main shaping factor for β-glucosidase, *N*-acetyl-β-D-glucosaminidase, and phosphatase activities, all three hydrolases produced by a variety of bacteria and fungi (Lynd et al., 2002; Thormann et al., 2004). Respiration was insensitive to both peat type and inoculum source, suggesting a high degree of functional redundancy. However, the variability in respiration responses revealed by the serial dilutions, which likely

dampened the influence of treatments, also suggests there were taxon-specific effects (Huston, 1997) mediated by the soil environment. Similar significant interactive effects of environment and microbial community composition on respiration were found in a study performed in salt marshes (Reed and Martiny, 2007). Our results therefore suggest that differences in initial microbial composition between undecomposed and humified peats are functionally significant for peroxidase activity (a narrowly-defined function), but may have little influence on broad-scale functions related to nutrient acquisition.

In general, the outcomes of our diversity manipulations (the dilutions) depended not only on the function being performed, but also on the context in which the function was measured: samples confronted with inocula sourced from the same peat—treatments *f* and *s*—exhibited richness patterns that were significantly affected by the dilutions, while samples inoculated with the reciprocal peat—treatments *sf* and *fs*—evolved patterns that were not. Although PCR-DGGE has inherent limitations to quantitatively determine the diversity of soil bacteria (Fromin et al., 2002; Loisel et al., 2006), this method has proven useful to assess broad-scale trends of bacterial richness in microcosms inoculated with soil suspensions (Griffiths et al., 2004; Wertz et al., 2006). In our experiment, the serial dilutions did not cause a consistent reduction in observed or estimated (Chao2) bacterial richness, but catabolic evenness, a measure of functional diversity, generally declined as the dilution level increased. This weak diversity response to dilution has been observed in similar studies (Griffiths et al., 2001, 2004), and was previously attributed to the wide range of groups targeted by universal bacterial primers (Wertz et al., 2006). We also found a relatively minor relationship between dilution and respiration, which again may be attributable to the taxonomic breadth underlying such function. However, re-growth of dominant

microorganisms (and their associated functions) from the highest dilutions may occur even if a large proportion of diversity is eroded: according to simulations, over 99% of the diversity may be eroded at 10^{-8} (Wertz et al., 2006).

Enzyme assays conducted under optimized conditions may include stabilized extracellular enzymes derived, for instance, from plants (Burns, 1978). However, this is unlikely in our experimental system: by heat-sterilizing the soils, we ensured that preexisting enzyme pools were denatured before inoculation (McNamara et al., 2003). Consequently, microbial colonization of the soil matrix and subsequent growth was likely the main source of new enzyme production. Growth alone, however, would not explain the patterns we observed. While in the *f* treatment hydrolytic enzyme activities increased with dilution level, other treatments had the opposite effect —a similar dual trend was observed for phenol-oxidase. Whereas a detrimental effect on abundant microbial members may be assumed following dilution, our results suggest that after inoculation certain inoculum-peat combinations promoted the dominance of relatively rare species (which may include both fungi and bacteria) with the ability to produce functioning extracellular enzymes (as suggested by the low richness and elevated phenol-oxidase activity at high dilution levels). Serial dilution may have randomly selected for subsets of the microbial community physiologically adapted to mediate the (enzymatic) breakdown of specific compounds. Once in the microcosm, the soils' environment established novel constraints under which the nascent microbial assemblages produced extracellular enzymes that modified their substrates, which in turn may have led to further changes in microbial community composition and function (Allison et al., 2007a). This soil (peat) type effect was quantitatively the most important factor shaping the enzyme activity profiles of our microcosms, and was the only

one significantly influencing catabolic evenness. These results lend support to the idea that both abundant and rare populations are potentially important for overall ecosystem functioning (cf. Szabó et al., 2007), with their relative functional relevance likely being context-dependent (e.g., Cardinale et al., 2000; Orwin et al., 2006).

Our findings indicate that the peat type in conjunction with the initial inoculum composition affected the total bacterial community structure. Saison et al. (2006) showed that the effects of compost amendment on soil microorganisms were due to the physicochemical characteristics of the compost matrix rather than a shift in microbial community composition through inoculation of compost-borne microorganisms. In a transplant experiment comparing two soils, Griffiths et al. (2008) observed that the structures of transplanted and native microbial communities converged after incubation, which they attributed to a soil habitat effect. However, the initial composition of the inocula was not taken into account. Equally, our observed enzymatic rates (high hydrolytic activity in *f* and *sf* treatments and higher phenol-oxidase activity in *s* and *fs* soils) may well correspond with a resource acquisition strategy (Allison and Vitousek, 2005) constrained by the peats' expected substrate quality (e.g., high concentrations of phenolics in the *s* peat and predominance of labile organic carbon in the *f* peat (Williams et al., 2000; Barkovski et al., 2008). Changes in microbial community structure can be driven by the nature (e.g., glucose vs. xylene; Nakatsu et al., 2005) and concentration (Griffiths et al., 1999; Langenheder and Prosser, 2008) of organic carbon compounds. Consequently, the enzymatic release of assimilable substrates might be an important agent that constrains the microbial community over limited time scales.

Unlike most studies reporting the use of BrdU, we were not concerned with identifying active bacteria proliferating in response to specified stimuli (e.g., carbon

substrates; cf. Urbach et al., 1999). Rather, our experiment sought to examine the active bacterial community's response to the environmental signals underlying incubation, some of which involve cumulative effects (e.g., stabilized microbial biomass) and some that result from shorter-term constraints (e.g., physical proximity to and utilization of labile compounds). The BrdU labeling method likely detects active members that are selected under these short-term limitations, which may explain why in our study the active richness was higher than the total richness: in the absence of an added substrate, or any resource that stimulated the enrichment of specialized taxa during incubation, the extent of competition effects may not have been captured. Moreover, the finding that the total and active community structures were dissimilar, and the lack of a response by active populations to the treatments, may imply that the taxa uptaking the BrdU are also selected by the incubation conditions in the presence of the analog. Indeed, Edlund and Jansson (2008) showed that the taxonomic affiliation and richness of active bacteria in phenanthrene-amended sediments varied depending on the time of exposure to BrdU. Together, these results question the assumption that only a small subset of the total bacterial community is active at any particular time (e.g., Artursson et al., 2005; Hjort et al., 2007). This may be true for soils under field conditions, where many microorganisms are dormant (Tate, 2000). However, the contrivances imposed by the microcosm environment mean the degree of overlap between active and total populations cannot be established *a priori*, and may fluctuate depending on the developmental stage of the community.

In conclusion, we found evidence of a general decrease in respiration rates and catabolic evenness as dilution increased, but this reduced functionality did not correspond with diversity erosions of equivalent magnitude. Furthermore, the episodic supply of

substrates may be instrumental in shaping microbial communities within a soil, reflected in our case in the inoculum effect, but when these communities are exposed to a new habitat they undergo additional compositional shifts governed by the habitat's physico-chemical properties (Ekschmitt et al., 2005), expressed through the peat-type effect.

The functional and structural (species composition) responses of microbial communities to uncontrolled diversity manipulations such as serial dilution depend not only on the immediate consequences of the manipulation (e.g., a reduction in richness due to the progressive removal of taxa), but also on the dynamics of substrate re-colonization. In turn, these characteristics will likely be limited by the taxa that are retained in the inoculum after manipulation (compositional effect) and by further context-dependent ecological constraints. Determining the total and active bacterial populations allowed us to discriminate between short- and longer-term physiological consequences of treatment effects. However, these temporal considerations should not be confused with the main goals of this study, which sought to address the functional consequences of microbial compositional changes. Further studies may benefit from the use of BrdU to track the specificity of phylogenetic scales across physiological trait value space (e.g., carbon mineralization) in response to perturbations which may influence changes in process rates (Allison and Martiny, 2008). Finally, even more promising would be to apply this labeling technique in combination with methods to estimate potential changes in functional gene abundances (e.g., real-time PCR), in particular those coding for products directly involved in nutrient turnover and geochemical cycling.

Figure 4.1 Impact of dilution on richness values calculated from DGGE fingerprints of (A) observed bacterial richness and (B) Chao2 richness. Note the different *Y*-axis scales in (A) and (B). Treatments are designated according to the following examples: *f* (1) represents sterile fibric soil inoculated with a 10^{-1} dilution of fibric soil and *fs* (3) represents sedge peat inoculated with a 10^{-3} dilution of fibric. Bars represent standard errors of the means (n = 4).Values with different letters differ significantly ($P < 0.05$).

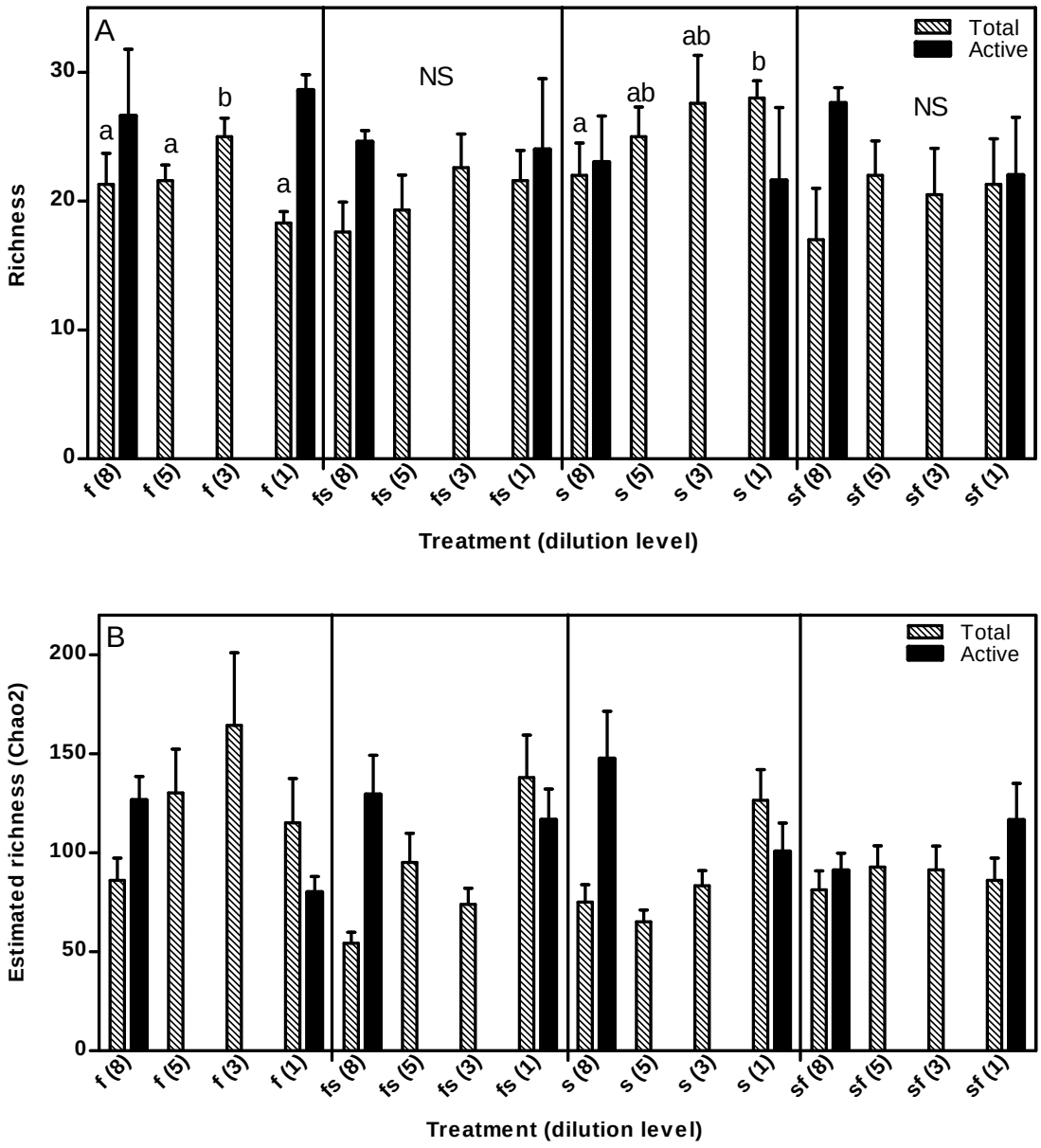

Figure 4.2 Impact of dilution on carbon mineralization rates. For details, see Fig. 4.1.

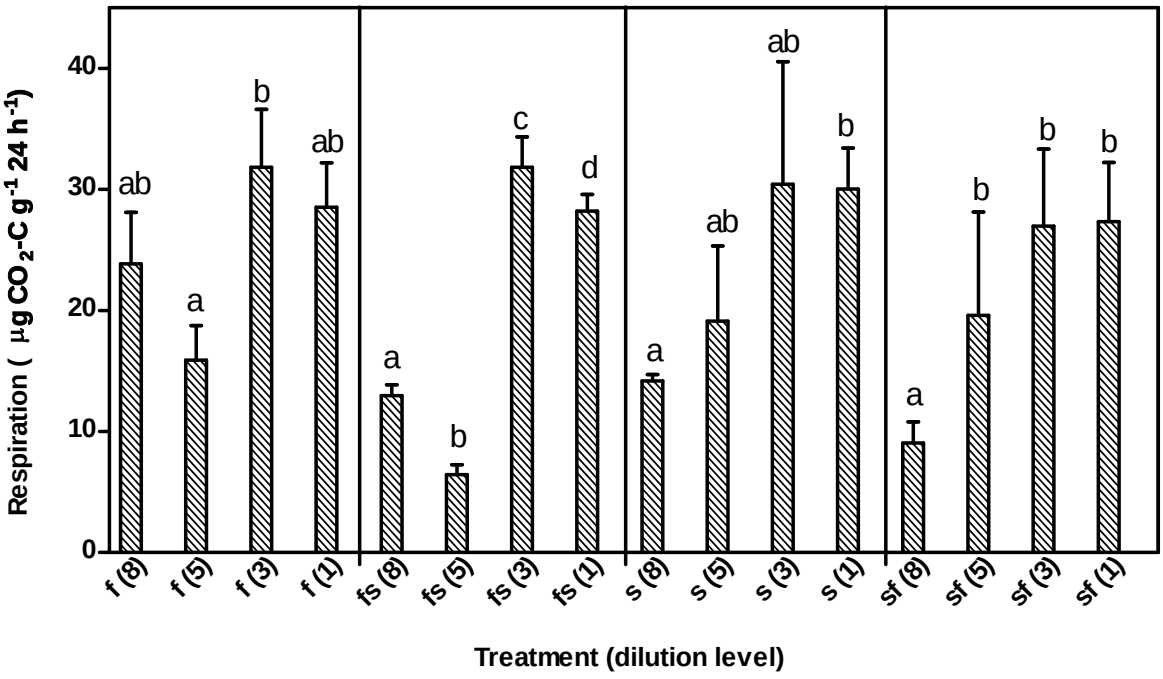

Figure 4.3 Impact of dilution on enzymatic activities. Abbreviations: Pase, phosphatase; Bgluco, β-glucosidase; NAG, N-acetyl-β-D-glucosaminidase; Pox, phenol-oxidase. For details, see Fig. 1.

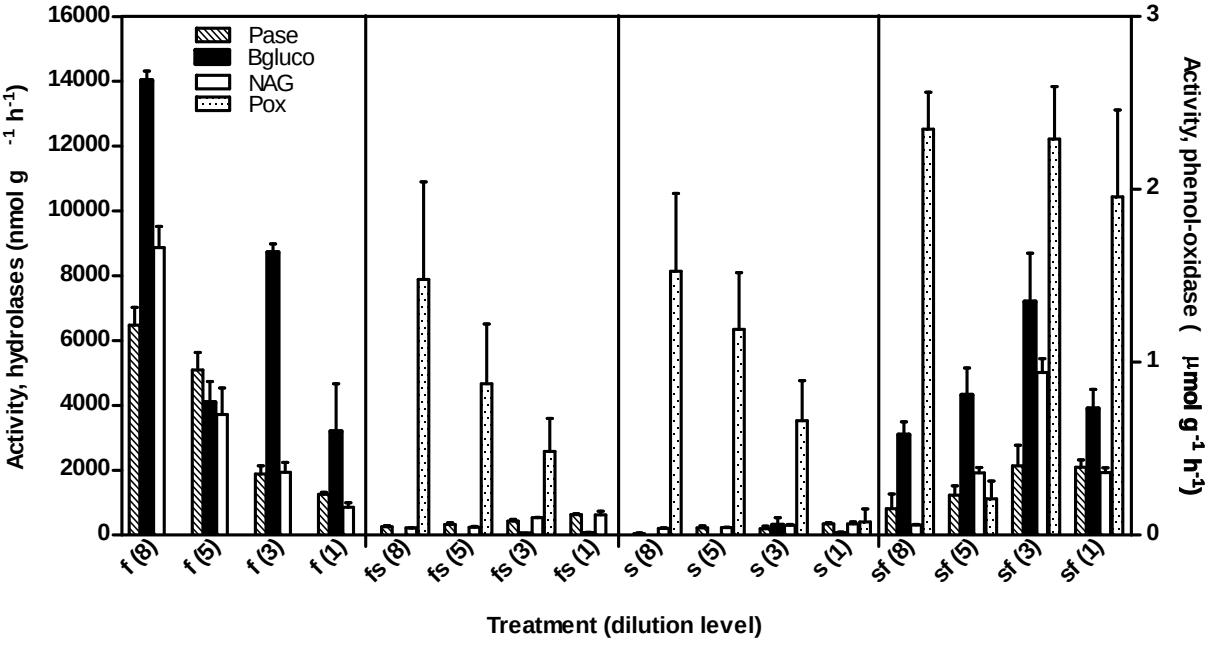

Figure 4.4 Impact of dilution on catabolic evenness values computed from enzyme activities. For details, see Fig. 1.

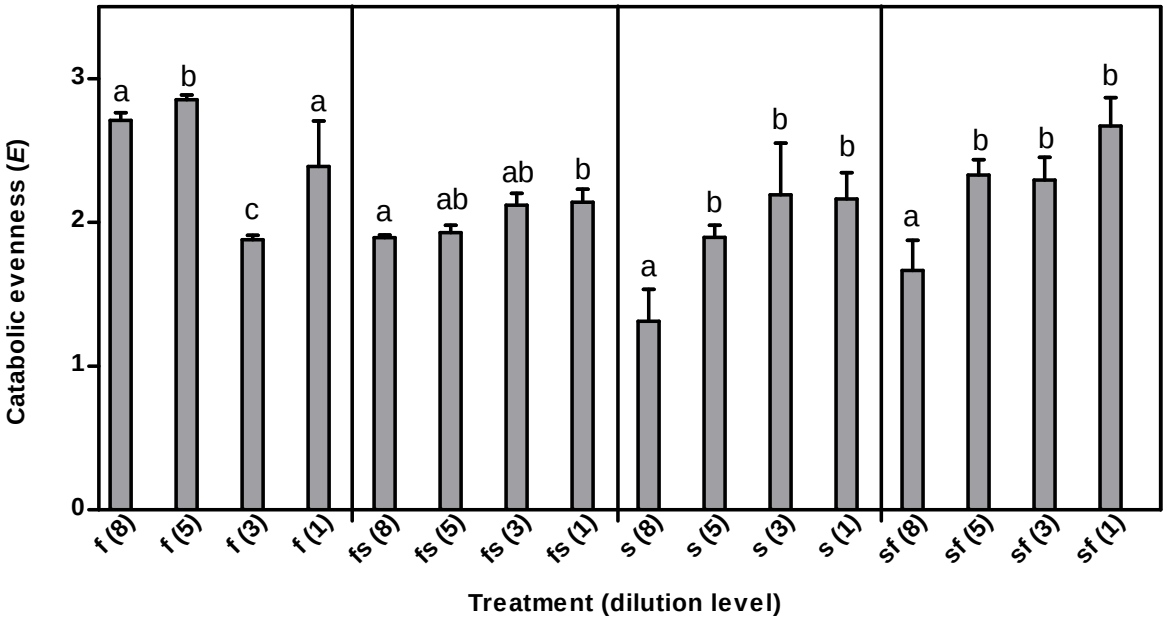

Table 4.1 Effects of peat type and inoculum source on bacterial richness and community structure

Source of variation	d.f.	Richness*			Community structure*		
		%SS	*F*	*P*-value	%SS	*F*	*P*-value
Inoculum source	1	--	--	NS	7.90	3.24	< 0.01
Peat type	1	--	--	NS	--	--	NS
Inoculum × peat type	1	8.0	4.0	< 0.01	7.70	3.17	< 0.001

--, not applicable.

*The effects of treatments on *active* members were not significant.

Table 4.2 Effects of peat type and inoculum source on functional parameters

Source of variation	d.f.	Enzyme activities*			Catabolic evenness		
		%SS	*F*	*P*-value	%SS	*F*	*P*-value
Inoculum source	1	2.51	3.75	< 0.01	--	--	NS
Peat type	1	64.7	95.50	< 0.0001	17.80	24.1	< 0.001
Inoculum × peat type	1	2.80	4.25	< 0.01	--	--	NS

*Significance was tested with a PERMANOVA using inter-sample Bray-Curtis distances calculated using all four activities.

The effects of treatments on *respiration* were not significant.

Table 4.3 Effects of peat type and inoculum source on individual enzyme activities

Source of variation	d.f.	*F-value*			
		Pase	Bgluco	NAG	Pox
Inoculum source	1	--	--	--	12.1**
Peat type	1	35*	52.5**	45.8**	--
Inoculum × peat type	1	--	--	--	13.2**

Pase, phosphatase; Bgluco, *β*-glucosidase; NAG, *N*-acetyl-*β*-D-glucosaminidase; Pox, phenol-oxidase.
*P<0.01; **P<0.001.

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5 CONCLUSIONS

Historically, the major emphases of reclamation have been the amelioration of adverse physical and chemical properties of mine spoils to ensure proper plant growth. Study foci have mirrored such tradition, but may not reflect a reasonable approach: Yarmuch (2003), for instance, observed that in the Athabasca oil sands region some physical properties (bulk density, particle size distribution) of reclaimed soils were not significantly different from those measured in natural soils. However, self-sustaining plant communities can only be established by successful rehabilitation of (micro)biological components responsible for nutrient cycling (van der Heijden et al., 2007). Following this basic tenet, the microbial constituent of the decomposer community is increasingly being acknowledged as an integral aspect of research programs that aim to understand the long-term ecological consequences of ecosystem rehabilitation (Harris, 2003; Claassens et al., 2008; Banning et al., 2008).

In this thesis, I investigated how microorganisms respond to reclamation of surface-mined boreal forest soils. My research centered on three objectives. First, I evaluated changes in bacterial and fungal community fingerprints (PLFAs,16S rRNA genes, and ITS regions) and enzyme activities in response to different prescription/age-since-reclamation combinations in post-mining boreal forest soils. To assess the effectiveness of reclamation, I included a sequence of sites representing a typical range of natural variability in the region. Using a higher-resolution approach (i.e., cloning and sequencing), I also assessed broad-scale phylogenetic patterns of bacteria in soils of natural boreal forest sites characterized by contrasting moisture and nutrient regimes, and compared them to two disturbed sites. Finally, I assembled laboratory-scale reciprocal transplants between two distinct peat types used as reclamation amendments to examine the relative influence of bacterial community

composition and the environment (i.e., peat type) on the production of extracellular enzymes and respiration rates.

5.1 Main findings

Within the time frame of the ecosystem rehabilitation chronosequence I analyzed, reclamation of surface-mined boreal forest soil profiles did not situate soil microbial attributes on a predictable recovery path; indeed, time-since-reclamation effects (which have produced measurable changes in vegetation cover, for instance; Rowland et al., 2009) were overridden by the influence of reclamation amendment (Chapters 2 and 3). I determined, in support of my hypothesis, that microbial community fingerprints (PLFA) in soil profiles containing tailings sand were different from those in natural sites at the productive end of a typical regional environmental gradient. However, it is unclear whether this implies a compositional convergence of communities in tailings-sand-based prescriptions and nutrient-poor sites or whether my survey, which provides a single snapshot of microbial properties, has excluded other potential sources of variability, such as seasonal-dependent dynamics (Bardgett et al., 2005), that may have prevented an accurate assessment of long-term reclamation effects. In general, however, when considered *en bloc* reclamation resulted in microbial communities (PLFA and DGGE) that were dissimilar to those in natural sites. I illustrate that this compositional dissimilarity was underpinned by at least two phenomena: (i) an increase in the abundance of fungal-specific markers, potentially dominated by ectomycorrhizal types, in natural sites (Chapter 2), and (ii) the presence of 16S rRNA gene variants leading to phylogenetically-unique bacterial communities in reclaimed sites (Chapter 3).

The response patterns of potential enzyme activities differed from those of microbial communities: overburden-based prescriptions affected enzyme activities most, causing a decrease in phenol-oxidase activity (mineral horizon) in comparison to productive natural sites and an increase in β-glucosidase activity (organic horizon) in relation to nutrient-poor natural sites (Chapter 2). Additionally, I identified a trend of increasing β-glucosidase, chitinase, peroxidase, and phosphatase activities along the natural fertility gradient, at least in organic horizons. These results are consistent with a resource allocation strategy in response to nutritional demands of the decomposer community, and indirectly point to the activities of key enzymes as indicators of soil resource quality (Sinsabaugh et al., 2002).

Another important aspect of my thesis attempted to establish links between environmental variables and inter-site variability in microbial community composition. The fungal-to-bacterial biomass ratio and total soil N pools each explained about one-third of the variability in microbial community composition (PLFA) in reclaimed sites and natural sites, respectively. Across all sites, however, part of the variance in community structure was also accounted for by the presence of downed woody debris. This suggested that microbial responses to reclamation were partially governed by vegetation cover development in post-mining landscapes, as additionally implied by the fact that some of the variability (~27%) in enzyme activities in reclaimed forest floors was explained by broadleaves abundance. Soil pH was also significantly associated with changes in soil microbial composition (Chapters 2 and 3). Using a higher-resolution method (sequencing of a collection of the community's 16S rRNA genes and analyzing its taxonomic and phylogenetic diversity), I established that, at least among bacterial community members, pH-mediated patterns of whole-community β-diversity were driven by the abundance of Acidobacteria, of wide distribution in terrestrial

environments (Janssen, 2006) (Chapter 3). Within natural sites, phylogenetic β-diversity patterns were largely explained by soil moisture and were driven by the abundance of alphaproteobacterial sequence types (Chapter 3). Nevertheless, my studies do not discount that other, unmeasured variables may have contributed to explaining the distribution of microbial taxa; for instance, my results indicate the possibility of substantial regional-scale spatial structuring (Chapter 2).

Although potential enzyme activities and microbial community composition (PLFA) responded differently to the reclamation treatments, I found that enzyme activities covaried with the relative abundances of several bacterial and fungal biomarkers (Chapter 2). This apparent ability of specific subsets of the microbial community to produce macromolecule-degrading enzymes was especially prevalent in reclaimed soils (where microbial biomass was generally lower), likely reflecting an adaptive strategy of microorganisms to derive growth substrates in C-limited materials.

My thesis' major second theme explored the possibility that microorganisms may control the production of enzymes. My laboratory-scale experiment showed, as I originally hypothesized, that differences in initial microbial community composition between undecomposed and humified peats were functionally significant for peroxidase activity (Chapter 4). In contrast, the activities of nutrient-acquiring enzymes (β-glucosidase, *N*-acetyl-β-D-glucosaminidase, and phosphatase) were mostly influenced by the peat type. These patterns are in agreement with a basic premise underlying my main original hypothesis: enzymes responsible for lignin depolymerization are likely produced by a narrow subset of the microbial community, while nutrient acquisition functions are more ubiquitously distributed. Following incubation of peat inoculated with dilutions of the same

or reciprocal peat, there was a decline in both peat respiration rates and functional evenness with increasing dilution, a trend that did not appear to be related to the richness of abundant bacteria detected by DGGE.

A likely (and common) interpretation of the pattern of sustained function even after diversity loss is that functional redundancy among microbial taxa is sufficient to maintain whole-community process rates—even if functionally relevant microbial groups are eliminated after a disturbance, as indicated by, in general, a trend of decreasing respiration and functional evenness in microcosms inoculated with high-dilution peat. At the same time, increases in enzyme activities at higher dilutions, which were observed both for hydrolases and peroxidase, implied that the experimental conditions may have selected for rare members of the microbial community capable of producing functioning enzyme pools.

At few cautionary notes deserve consideration. First, community-fingerprinting methods (such as DGGE) only detect a minor fraction of the diversity harbored in an environment. This limitation prevents the assessment of members present at very low abundances (i.e., <1%), so that their putative functional roles in environments characterized by extreme "right-tail" distributions cannot be established (e.g., Szabó et al., 2007). A second caveat is our inability to ascribe functions to taxonomic entities defined with fuzzy boundaries. Higher-resolution approaches such as sequencing of large sequence inventories, however, may help circumvent these problems, and would also allow a more detailed examination of which taxonomic resolutions, if any, are relevant to producing positive richness-function relationships. Finally, even if I show that bacteria may be implicated in enzyme production (more specifically peroxidase, as suggested by a significant effect of the inoculum source on both community composition and peroxidase activity), fungal

populations, which play a key role in peat decomposition (Thormann, 2006), were probably contributing to the observed enzyme activity patterns. Nevertheless, I also provide correlative evidence (Chapter 2) of a negative association between the abundance of bacterial biomarkers and peroxidase activity among reclaimed soils, which contain salvaged materials mixed with peat. This supports the notion that, under certain conditions, biomass reductions of functionally relevant microbial taxa may not necessarily correlate with declines in process rates—for instance, the activities of extracellular enzymes involved in nutrient cycling.

5.2 Future research directions

While I offer support for clear effects of reclamation on microbial community composition and function, my thesis also opens avenues for potential research directions. In the Athabasca region currently being exploited for oil, the majority of mined land was formerly peatland but is generally reclaimed to upland forests. Peat-dwelling microorganisms differ in their decomposition capabilities under short-term disturbance regimes (e.g., temperature increases; Thormann et al., 2004), and this variability in function may be governed both by temperature and litter quality effects (Thormann et al., 2003). Similarly, studying the range of microbial community variation along natural upland-to-peatland gradients is in itself of interest in the face of current reclamation practices. Whether environmental factors (disturbance, hillslope position) alone explain functional attributes or whether it is microbial community composition that affects process rates could be resolved with a reciprocal transplant experiment, either under laboratory conditions or directly in the field. Using coarse- and fine-mesh bags to allow or exclude fine root in-growth, Hannam et al. (2007) performed a field-scale reciprocal transplant to examine how microbial community composition in aspen- or

spruce-dominated forest floors responded to changes in conditions of the surrounding environment. Such a set-up could be useful to explore how a microbial community's history of exposure to reclaimed materials affects its functional performance once it is confronted to a new (e.g., "natural") environment.

For my thesis, I compared microbial properties in a reclamation chronosequence to those in a range of natural sites corresponding approximately to a xeric-poor/mesic-rich gradient. Our understanding of potential post-mining trajectories may improve by including a broader range of site qualities covering a similar spatial scale, which in turn could result in different patterns between microbial community β-diversity and soil properties. Similarly, future regional-scale surveys of reclamation effects could incorporate an analysis of microbial-plant responses to other forest disturbances (natural or human-induced) typical of the region; not only would this provide a stronger framework in which to interpret long-term compositional and functional recovery trends, but may also allow testing whether differences in community composition are functionally equivalent or dissimilar using factorial cross-inoculations among distinct site types (i.e., a combination of reciprocal transplant and "common garden"; Strickland et al., 2009).

Finally, further studies may also benefit from the use of BrdU to track the specificity of phylogenetic scales across different physiological traits (e.g., carbon mineralization, the ability to hydrolyze macromolecules) in response to perturbations which may influence changes in process rates. Applying the active DNA labeling technique in combination with methods to quantify gene pools (e.g., real-time PCR) could help us to understanding whether functional responses (for instance, changes in enzyme activities) are indeed caused by concomitant shifts in the abundance of specific subsets of the active community or result

primarily from covariation in abundance patterns of genes coding for the functional trait being investigated.

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APPENDICES

Appendix A: Description of abiotic and plant community characteristics of the 47 sites used for this study

Table A.1 Physico-chemical properties of materials used for oil sands reclamation

Name of material	Origin and description	Principle mineral component	Physico-chemical properties
Peat:mineral	Peat and clay mineral topsoils salvaged and mixed by stripping from fibrosol sites	Clay loam or clay overburden (shallow depth)	2% - 17% organic C Near-neutral pH P:M ratio by volume varies by company, between 3:2 to 3:4
Tailings sand	Cretaceous-era marine sands with occasional shale beds Ejected as waste after bitumen has been extracted	95% quartz sand, 4% silt (feldspar and mica), 1% clay (kaolinite, illite and montmorillonite)	Hydrophobic after air-drying due to residual 0.1% - 0.4% hydrocarbons; virtual absence of plant nutrients; sand grains very fine to fine-grained (95% between 50- to 250-µm); excessively drained, unstable, subject to wind and water erosion
Subsoil	Pleistocene Epoch glacial drift Shallow depth (<3m from surface), comprising the B- and C-horizons	Silt-clay shales and glacial clay-rich tills (kaolinite, illite and montmorillonite)	Non-saline pH 5.0 – 8.0; low organic C (<2%) but may be locally enriched by peat during salvage operation
Overburden	Pleistocene Epoch glacial drift over Cretaceous-era sedimentary deposits	Silt-clay shales with clay-rich tills (kaolinite, illite and montmorillonite)	Fine- or coarse textured; non-saline; slightly alkaline (pH 8.0+) *in situ*, may be acidified by oxidation of sulphites (eg. pyrite, FeS2) in the reclamation landscape; low to no organic C
Lean oil sand	Cretaceous marine sediments, with migrated bitumen	Relatively impermeable, non-cemented quartz sand with shales, silts and clays	< 6% bitumen by weight pH 5.5 – 6.0; rejected as 'ore' because clay content too high
Clearwater shales	Cretaceous-era, impermeable marine shales deposited from ancient Boreal Sea	Silt shales with swelling clays (illite, montmorillonite)	Fine-textured; saline-sodic (10-20dS/m); pH 8.0+ Sodium-saturated (SAR >20)

Table A.2 Soil properties of the 47 sites sampled for this study

Site	Ecotype/prescription	Horizon	Age (y)	pH(w)	N (%)	C (%)	Moisture content (% dry wt)
1	H	Organic	14	6.72	0.88	14.60	48
2	b3	Organic	97	6.20	0.81	21.18	72
3	B	Organic	14	6.53	1.48	26.93	106
4	d1	Organic	71	4.90	1.08	25.94	191
7	E	Organic	12	6.39	0.90	16.21	136
8	d1	Organic	53	5.53	1.04	18.85	126
10	a1	Organic	44	5.71	0.21	7.23	30
12	F	Organic	14	6.46	0.99	13.64	71
14	E	Organic	10	6.41	1.36	26.07	130
16	H	Organic	9	6.50	0.68	13.10	57
17	H	Organic	13	6.40	0.68	10.49	41
19	d2	Organic	70	5.15	1.18	34.23	172
20	d3	Organic	160	4.63	1.25	32.92	137
21	d3	Organic	84	4.78	1.10	30.20	122
23	d3	Organic	101	6.71	0.80	18.77	117
24	H	Organic	34	6.04	0.57	10.92	49
25	I	Organic	21	7.08	1.09	27.67	99
26	a1	Organic	69	4.78	0.65	22.26	60
27	a1	Organic	79	4.67	0.38	12.07	31
28	I	Organic	22	7.36	1.32	19.09	89
29	b1	Organic	46	5.05	0.44	10.79	55
30	H	Organic	34	6.69	0.18	3.49	22
32	I	Organic	21	6.95	0.73	17.02	91
34	I	Organic	24	7.22	1.13	23.56	138
36	A	Organic	11	6.66	1.24	31.61	70
37	A	Organic	24	7.18	1.15	17.43	12
38	B	Organic	19	6.67	0.91	21.23	72
39	B	Organic	9	6.45	0.38	7.55	53
40	A	Organic	14	6.59	1.76	31.00	100
42	B	Organic	11	6.44	0.99	16.38	72
43	E	Organic	22	6.57	2.06	35.09	138
46	B	Organic	10	6.60	0.83	12.23	80
49	b3	Organic	44	4.73	0.55	15.90	92
50	d2	Organic	77	4.72	0.77	21.63	101
57	d2	Organic	73	4.93	0.66	15.21	90
61	d1	Organic	56	5.46	1.10	24.44	116
62	b1	Organic	65	5.83	0.36	9.96	58
63	b1	Organic	61	5.24	0.78	22.51	75
64	b3	Organic	50	4.12	0.62	17.81	97
75	I	Organic	3	NA	NA	NA	NA
86	M	Organic	6	6.44	1.15	21.58	127
87	A	Organic	5	6.91	1.38	25.36	85
88	A	Organic	5	NA	NA	NA	NA
89	A	Organic	5	6.68	1.26	25.87	86
ALB	ALB	Organic	3	NA	NA	NA	NA
SYN	SYN	Organic	3	NA	NA	NA	NA
WA5	WA5	Organic	29	6.37	1.55	25.08	112
1	H	Mineral	14	6.88	0.25	6.56	21
2	b3	Mineral	97	4.70	0.07	1.81	29
3	B	Mineral	14	7.41	0.09	2.76	23
4	d1	Mineral	71	4.94	0.06	1.04	16

Site	Ecotype/prescription	Horizon	Age (y)	pH(w)	N (%)	C (%)	Moisture content (% dry wt)
7	E	Mineral	12	6.40	0.24	5.49	35
8	d1	Mineral	53	6.38	0.18	2.37	32
10	a1	Mineral	44	4.47	0.02	0.41	5
12	F	Mineral	14	7.42	0.05	1.58	17
14	E	Mineral	10	7.39	1.38	25.44	113
16	H	Mineral	9	5.49	0.29	8.52	22
17	H	Mineral	13	6.43	0.20	5.19	20
19	d2	Mineral	70	5.83	0.16	3.14	25
20	d3	Mineral	160	4.49	0.04	0.53	22
21	d3	Mineral	84	4.66	0.10	1.96	24
23	d3	Mineral	101	6.14	0.20	3.32	41
24	H	Mineral	34	5.36	0.27	5.61	15
25	I	Mineral	21	6.07	0.42	9.48	32
26	a1	Mineral	69	5.43	0.03	0.71	8
27	a1	Mineral	79	5.22	0.02	0.75	7
28	I	Mineral	22	8.01	0.21	6.14	21
29	b1	Mineral	46	4.51	0.05	1.46	10
30	H	Mineral	34	6.40	0.05	1.23	8
32	I	Mineral	21	7.34	0.54	12.97	49
34	I	Mineral	24	7.95	0.31	7.87	27
36	A	Mineral	11	7.44	0.16	3.66	33
37	A	Mineral	24	7.18	0.35	7.98	35
38	B	Mineral	19	8.00	0.05	1.56	16
39	B	Mineral	9	7.51	0.09	2.08	23
40	A	Mineral	14	7.23	0.41	9.57	56
42	B	Mineral	11	6.18	0.14	3.40	31
43	E	Mineral	22	5.63	0.54	12.67	34
46	B	Mineral	10	7.33	0.07	2.22	24
49	b3	Mineral	44	4.78	0.06	1.43	8
50	d2	Mineral	77	4.57	0.05	0.99	14
57	d2	Mineral	73	4.77	0.21	2.14	29
61	d1	Mineral	56	5.50	0.16	2.33	23
62	b1	Mineral	65	5.33	0.04	1.14	9
63	b1	Mineral	61	5.11	0.03	0.61	8
64	b3	Mineral	50	3.84	0.07	1.85	20
75	I	Mineral	3	7.23	0.17	4.85	28
86	M	Mineral	6	6.66	0.12	3.25	37
87	A	Mineral	5	7.23	0.63	15.64	59
88	A	Mineral	4	5.91	0.33	7.24	55
89	A	Mineral	5	7.36	0.42	11.98	50
ALB	ALB	Mineral	3	7.58	0.28	7.47	18
SYN	SYN	Mineral	3	4.94	0.40	12.85	76
WA5	WA5	Mineral	29	6.63	0.63	12.27	33

Table A.3 Cover percentage of the dominant plant functional groups in the 47 reclaimed and natural sites sampled for the study

Site[§]	Vegetation cover class (%)*									
	Bare	Moss	Lichen	Grass	Forbs	Shrub	Broadleaf	Spruce	Pine	Woody debris
1	54.8	32.4	1.6	3.3	12.8	8.7	7.0	0.0	31.0	7.0
2	32.1	28.1	1.0	11.4	26.1	15.1	63.3	49.4	0.0	20.5
3	27.9	0.8	0.0	4.2	54.9	6.3	0.0	0.0	30.5	0.0
4	20.5	6.9	0.0	4.3	38.7	44.5	32.7	3.0	0.0	7.6
7	34.9	31.1	0.1	7.2	24.5	1.4	0.0	41.0	0.0	0.1
8	46.1	11.6	1.0	4.2	36.0	52.0	39.3	0.0	0.0	13.4
10	3.0	20.0	8.9	1.7	14.7	42.5	0.0	0.0	15.6	2.0
12	44.2	1.8	0.0	1.6	54.5	0.3	37.3	19.4	0.0	0.0
14	30.3	2.2	0.0	5.0	54.7	2.2	52.6	0.0	0.0	0.0
16	33.2	65.8	2.3	2.0	24.2	14.5	2.0	16.7	41.3	2.0
17	22.0	11.0	0.0	49.0	49.4	16.8	0.0	0.0	53.8	0.0
19	27.4	47.0	3.7	0.2	30.6	5.1	35.5	56.4	0.0	16.9
20	25.9	66.5	0.1	2.8	27.8	12.3	1.0	30.3	0.0	2.6
21	22.1	43.5	0.7	1.5	20.8	17.5	0.2	19.5	0.0	13.5
23	14.1	50.5	0.8	1.9	19.4	20.8	0.2	26.3	0.0	10.6
24	63.6	30.4	0.2	16.6	0.5	9.2	0.5	63.3	50.0	0.1
25	54.6	10.4	0.4	7.1	23.5	24.4	99.7	45.6	75.0	3.2
26	44.7	2.3	34.2	1.1	2.7	13.6	0.0	0.0	5.1	2.2
27	50.2	9.9	21.6	0.0	3.0	4.3	0.2	0.0	13.9	6.3
28	22.7	74.8	1.5	1.0	29.0	3.5	38.0	41.1	0.0	10.8
29	35.7	24.5	0.4	3.4	37.7	18.3	9.8	75.0	25.8	13.5
30	42.0	0.0	1.0	38.1	2.7	5.3	0.0	0.0	57.2	0.0
32	63.3	24.5	14.8	2.1	4.4	2.8	11.5	61.0	25.0	1.6
34	27.6	75.0	1.8	1.2	15.9	6.3	11.5	41.7	0.0	2.3
36	15.1	7.3	0.0	37.1	15.3	10.7	5.0	8.0	0.0	0.0
37	22.2	3.6	0.0	14.9	48.3	2.3	0.0	44.9	0.0	0.0
38	32.4	5.2	0.0	11.8	37.7	18.8	3.9	0.0	6.0	1.2
39	23.8	3.1	0.0	20.2	57.7	4.9	3.5	2.0	0.0	0.0
40	22.9	2.2	0.0	51.8	14.1	11.0	2.4	16.5	0.0	0.0
42	11.1	10.5	0.0	7.2	51.2	7.5	13.5	0.0	3.0	0.0
43	67.7	4.0	0.0	5.3	9.2	15.6	40.0	57.4	0.0	7.2
46	24.4	8.6	0.0	53.6	36.8	5.3	3.0	3.4	0.0	0.0
49	44.0	19.6	0.5	0.5	28.9	23.3	2.7	42.6	20.5	13.9
50	26.4	38.3	0.4	0.8	22.2	8.0	2.6	48.3	0.0	11.2
57	10.6	25.8	0.3	0.3	31.7	6.3	0.2	30.1	0.0	11.8
61	25.4	1.4	0.1	3.0	28.2	42.6	3.6	34.3	0.0	4.3
62	30.1	53.8	0.7	1.7	1.7	27.7	0.7	7.1	0.0	7.7
63	21.0	54.4	3.5	2.4	8.9	23.5	0.6	34.5	0.0	4.9
75	24.7	5.7	0.0	2.5	71.4	2.5	14.7	0.0	0.0	0.0
86	10.8	2.2	0.0	30.6	55.2	9.7	2.1	0.4	0.0	0.0
87	27.0	1.6	0.0	18.3	59.6	0.0	0.0	0.5	0.0	0.0
88	24.7	17.4	0.0	6.4	36.3	1.9	0.7	0.0	0.0	0.0
89	42.3	7.6	0.0	28.6	27.6	1.0	1.0	3.0	0.0	1.0
ALB	99.7	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0
SYN	83.2	7.1	0.0	5.2	23.6	0.0	2.4	0.0	0.0	1.4

Site	Vegetation cover class (%)*									
	Bare	Moss	Lichen	Grass	Forbs	Shrub	Broadleaf	Spruce	Pine	Woody debris
WA5	59.6	1.5	1.0	3.6	2.4	52.9	85.3	0.0	0.0	9.6

* Percentage values are the mean of 10 measurements per site

§ Site 64 had been destroyed at the time plant cover percentages were estimated

Table A.4 *In situ* mean nutrient bio-availability obtained with the PRS™ probe method, in micromoles per 10 cm^2 per 95 days burial period

Site	NO3--N	NH4+-N	Ca	Mg	K	P	Fe	Mn	Cu	Zn
1	17.8	4.0	6140.0	687.4	49.7	19.5	39.9	1.6	0.9	2.1
2	10.0	8.2	2669.2	404.7	610.4	18.5	14.6	22.2	0.4	1.4
3	5.6	5.2	6152.0	520.0	86.7	4.3	13.3	1.6	0.9	1.1
4	6.4	5.0	3046.6	896.2	506.4	26.9	10.3	13.8	0.4	2.1
7	8.6	5.6	4214.4	840.2	186.4	7.2	12.8	2.2	0.6	0.9
8	6.0	4.0	3596.2	912.6	81.6	10.4	5.3	1.7	0.4	0.9
10	5.8	4.4	1200.6	261.6	336.7	4.4	11.5	11.9	0.4	1.4
12	10.4	4.0	5520.0	678.2	103.5	13.1	7.9	1.5	0.6	1.2
14	6.6	4.0	6250.0	360.3	28.5	3.2	63.7	1.2	0.4	2.2
16	11.6	9.8	4664.0	730.8	52.0	6.5	117.8	8.0	0.6	2.1
17	175.4	4.8	3908.4	1028.0	301.0	73.4	13.3	1.3	0.4	1.5
19	7.0	4.0	2380.0	435.8	1065.0	42.7	2.1	17.4	0.4	1.6
20	7.8	13.4	1817.4	324.0	772.2	21.2	2.4	24.0	0.4	1.1
21	9.2	9.2	1280.2	311.0	704.0	5.9	4.1	83.9	0.4	1.0
23	7.8	4.0	4241.4	351.7	420.7	20.7	10.5	2.1	0.4	1.0
24	7.4	22.2	3658.6	797.2	167.2	6.4	55.2	3.0	0.4	1.1
25	20.0	4.0	4054.4	834.0	189.6	4.2	18.1	1.7	0.4	1.1
26	6.8	7.2	567.4	91.8	174.9	3.0	9.7	7.3	0.4	1.2
27	6.8	4.0	612.2	109.6	158.0	3.1	9.6	11.8	0.4	1.6
28	6.4	4.0	5940.0	611.6	66.5	3.2	55.5	2.5	1.6	4.6
29	7.4	14.0	1171.8	286.3	270.4	8.3	8.5	35.6	0.4	2.8
30	239.0	7.8	3067.0	724.8	586.2	26.1	26.7	6.2	0.4	1.3
32	10.6	4.0	4422.0	1195.6	74.9	1.7	69.4	4.0	1.0	1.7
34	8.6	4.0	5350.0	731.4	66.1	2.8	33.1	2.1	1.1	2.6
36	9.6	4.6	5416.0	725.4	109.6	18.8	12.8	0.6	0.7	1.5
37	7.0	4.0	6006.0	514.2	92.4	5.3	32.0	1.1	0.5	1.9
38	97.2	4.8	4410.8	550.0	353.5	9.1	10.8	1.9	0.7	1.8
39	51.2	4.0	4922.0	720.2	60.4	15.9	32.8	1.6	1.8	3.5
40	132.8	4.0	5416.0	742.8	70.9	9.6	67.2	1.2	0.4	1.5
42	49.2	7.2	5264.0	767.8	252.4	11.4	39.6	1.7	0.8	2.2
43	119.0	4.0	4769.8	819.6	261.3	4.3	22.0	2.8	1.4	3.7
46	4.2	4.0	4900.0	700.4	133.3	9.5	25.1	2.7	0.9	1.1
49	7.4	4.4	3739.8	467.5	486.0	14.6	12.8	7.6	0.7	1.2
50	5.4	5.0	1930.6	482.5	1025.6	24.7	13.6	71.2	0.4	1.4
57	6.2	57.0	2093.0	528.8	730.6	18.4	6.8	10.3	0.4	1.7
61	5.4	4.0	3633.4	988.2	260.1	25.9	8.5	5.4	0.4	3.2
62	5.8	4.0	2239.0	595.0	176.9	16.3	8.1	3.8	0.4	1.3
63	5.8	4.8	668.2	104.7	269.7	8.2	10.3	15.5	0.4	1.0
75	100.0	4.0	3169.2	413.2	232.5	28.3	33.7	20.9	0.4	1.3
86	7.6	4.0	5758.0	616.2	38.1	24.4	8.7	0.9	0.4	0.9
87	119.8	4.0	6436.0	515.6	139.1	4.1	47.5	1.8	0.5	2.4
88	6.8	4.0	4980.0	888.6	43.9	13.4	19.6	1.6	0.4	1.3
89	47.0	4.0	6648.0	439.6	30.2	1.3	67.4	0.4	0.8	2.4
ALB	279.2	4.0	5760.0	450.2	17.3	0.9	166.1	2.0	0.6	1.0
SYN	7.2	4.0	4736.0	782.4	24.5	2.4	82.4	33.9	0.4	1.3
WA5	341.2	9.4	5672.0	957.8	194.6	2.5	54.3	4.6	1.3	4.6

Appendix B: Taxonomic distribution of dominant bacteria and phylogenetic relationships of α-Proteobacteria

Table B.1 GenBank database sequences with the highest identity match to dominant OTU-97 found in the ten study sites used for Chapter 3*

OTU	Accession no.	Identity (%)	BLAST match
OTU2	EF018575	94	Uncultured *Oxalobacteraceae* bacterium Amb_16S_843
OTU6	AY043763	100	Uncultured alpha proteobacterium NMS8.103WL
OTU16	AY673132	96	*Bradyrhizobiaceae* bacterium Ellin5405
OTU46	EU861823	98	Uncultured soil bacterium A10_bac_con
OTU50	AY043699	100	Uncultured gamma proteobacterium NOS7.145WL AY043699
OTU67	DQ829272	91	Uncultured Bacteroidetes bacterium DOK_NOFERT_clone317
OTU80	AY957916	99	Uncultured bacterium B3NR69D1
OTU81	AJ292648	96	Uncultured eubacterium WD2116
OTU101	AY043834	97	Uncultured Acidobacteria bacterium NMS8.141WL
OTU142	EU861823	95	Uncultured soil bacterium A10_bac_con
OTU145	EF551925	97	Uncultured bacterium P1fT_001
OTU152	DQ510022	97	Uncultured bacterium Toolik_Jun2005_shruborg_129
OTU155	DQ451521	96	Uncultured bacterium FAC82
OTU158	EU133920	95	Uncultured bacterium clone FFCH18791
OTU167	EU644207	97	Uncultured bacterium RT_57 EU644207
OTU169	AF432606	98	Uncultured bacterium SMW4.66WL
OTU170	FJ624937	94	Uncultured bacterium HF_C_64
OTU177	EU335157	96	Uncultured bacterium BacC-s_011
OTU197	AF407198	98	Uncultured bacterium GIF7 AF407198
OTU203	AF433173	98	*Flavobacterium xinjiangense*
OTU212	EF688362	90	Uncultured soil bacterium F3-25
OTU216	AM934729	97	Uncultured Ectothiorhodospiraceae bacterium AMNC9
OTU217	EF575563	97	*Flavobacterium resistens* BD-b365
OTU222	AB234239	98	Uncultured bacterium LS4-132
OTU332	FM206290	100	Uncultured bacterium partial 16S rRNA gene clone T7_138

* 'Dominant OTUs' are those represented by at least three sequences at the defined taxonomic cut-off level

Figure B.1 Sequence distribution of the dominant OTUs found in the ten study sites used for Chapter 3

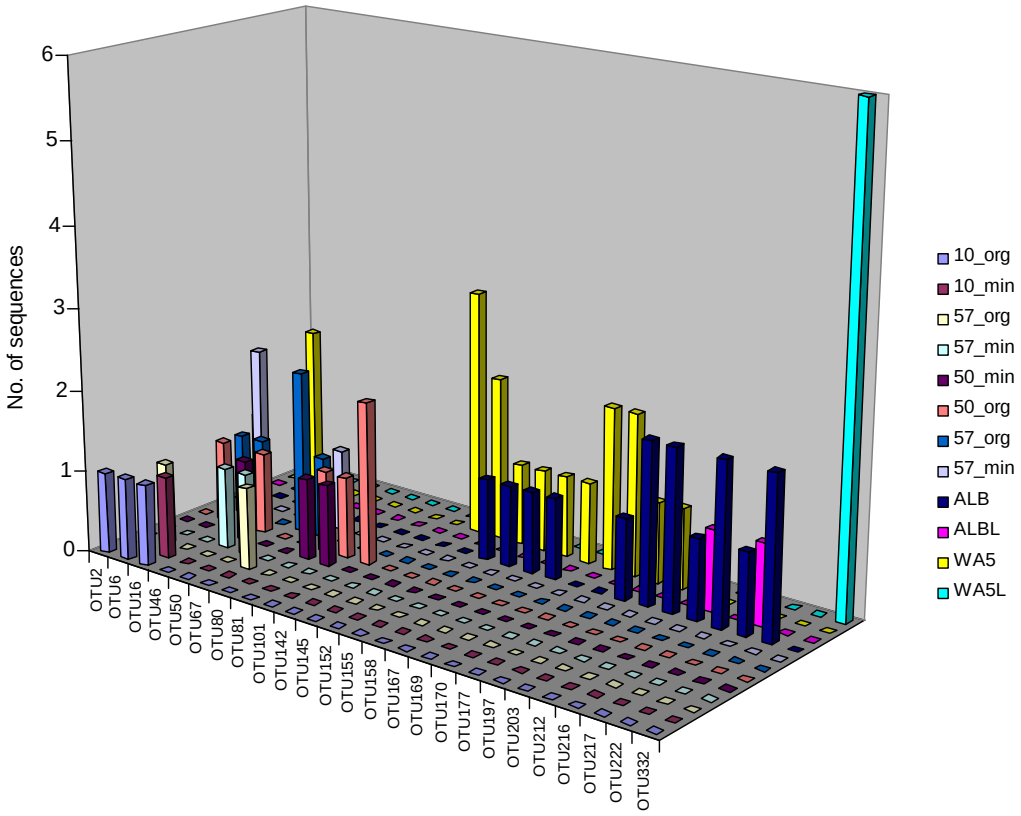
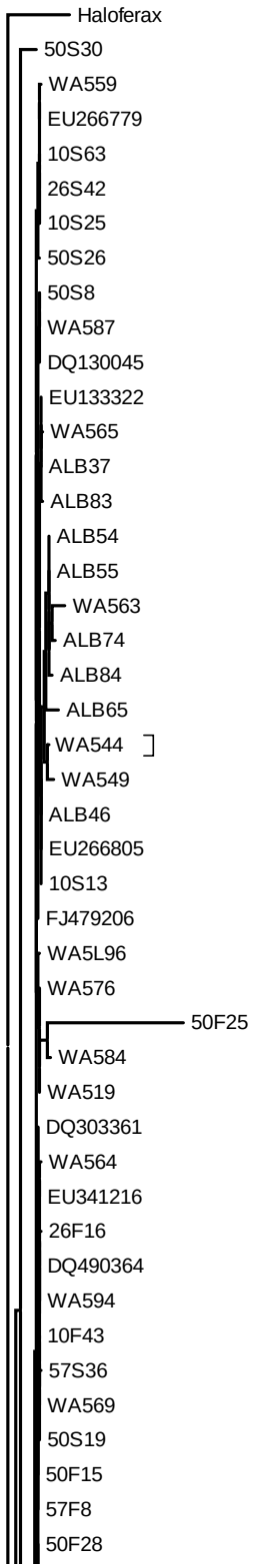
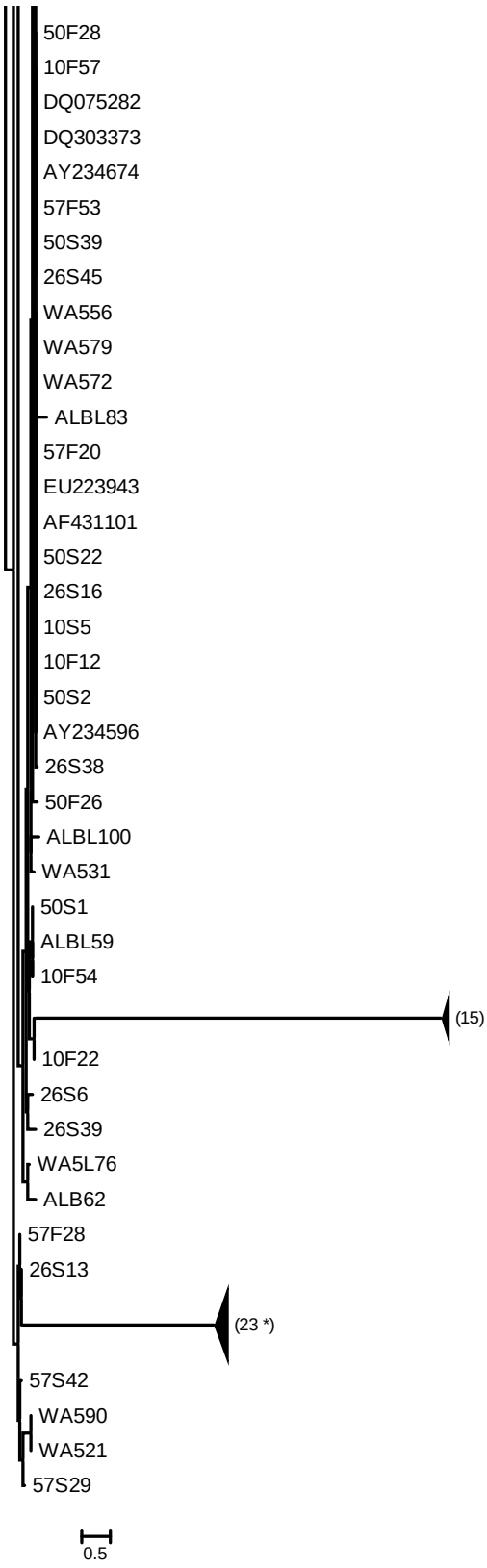


Figure B.2 Maximum-likelihood tree of the α-*Proteobacteria* identified in the 10 samples used for Chapter 3. Representative GenBank sequences, shown as accession numbers, were retrieved using the Seqmatch tool of the Ribosomal Database Project (release 10). Numbers in parentheses indicate numbers of sequences. The tree was rooted with a *Haloferax volcanii* 16S rRNA gene sequence.

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DQ075282
DQ303373
AY234674
57F53
50S39
26S45
WA556
WA579
WA572
ALBL83
57F20
EU223943
AF431101
50S22
26S16
10S5
10F12
50S2
AY234596
26S38
50F26
ALBL100
WA531
50S1
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10F22
26S6
26S39
WA5L76
ALB62
57F28
26S13
(23 *)
57S42
WA590
WA521
57S29
0.5