# NUTRIENT MANAGEMENT EFFECTS ON SOIL QUALITY, MICROBIAL COMMUNITY COMPOSITION, AND NITROGEN CYCLING IN TALL FESCUE FORAGE GRASS PRODUCTION SYSTEMS

by

KATARINA NEUFELD

BSc (Env), University of Guelph, 2011

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Soil Science)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2015

## Abstract

In the Fraser Valley of southwest BC, dairy production is an important industry but large numbers of dairy cows present challenges for manure management. Dairy manure is a valuable source of plant nutrients, yet surplus application may lead to N loss through $NO_3^-$ leaching and $N_2O$ emissions. Removing solids from whole dairy manure reduces the organic N and C contents, potentially improving crop N uptake, but reducing soil microbial activity compared to whole manure.

The objective of this study was to quantify long term effects of contrasting nutrient applications to perennial grass on soil microbial activity and community structure, and to test relationships with soil properties and rates of N transformation. Microbial community structure and activity (biomass, phospholipid fatty acid biomarkers, hydrolyzing enzyme activities) and N dynamics (net mineralization and nitrification, lysimeter leachate $NO_3^-$, $N_2O$ emissions) were measured in 2013 and 2014 on a stand of tall fescue (*Fetusca arundinacea Schreb.*) established in 2002 at Agassiz, BC, on soils receiving: whole dairy slurry manure, separated liquid fraction, $NH_4NO_3$ fertilizer, or alternating manure-fertilizer (all applied at 400 kg N/ha/yr equivalent) four times per year. In the autumn of 2013, the nitirifcation inhibitor, Nitrapyrin®, was applied to sub-plots of each treatment to assess its potential to minimize N losses from nutrient amendments.

Soil in plots receiving whole or liquid manure had higher microbial biomass than plots receiving commercial fertilizer or unamended plots, and higher activity of cellulose-degrading enzymes than plots receiving no amendment. Both microbial biomass and cellobiosidase activity (cellulose-degrading enzyme) were positively correlated with total soil C, N, and P. Fungal:bacterial ratios were higher in control and whole manure than fertilizer and liquid treatments. Emissions of $N_2O$ and concentrations of $NO_3^-$ in leachate were consistently positively correlated with abundance of bacterial biomarkers, but not total microbial biomass. N mineralization and nitrification were not correlated with any microbial group, but were positively correlated with $NO_3^-$ in leachate.

The nitrification inhibitor Nitrapyrin® had no significant impact on soil inorganic N concentrations, N mineralization or nitrification, or $N_2O$ emissions, however it increased soil microbial biomass and changed community structure and surprisingly increased $NO_3^-$ leachate.

# Preface

The work presented henceforth is the result of a collaboration between the Pacific Agri-Food Research Centre - Agassiz, and the Sustainable Agricultural Landscapes Lab, and Belowground Ecosystems Group at the University of British Columbia. Design and initiation of the current experiment in 2002 was performed by Dr. Shabtai Bittman and his research crew at the Pacific Agri-Food Research Centre.

I was responsible for all data collection, data analysis and interpretation in Chapter 2. With a team, I carried out all soil sampling, except soil elemental analysis in October of 2012. PLFA and enzyme analysis was performed by myself according to protocols developed in the Belowground Ecosystems Group Laboratory. All data analysis and interpretation is my original work.

Chapter 2 and 3 data was collected in collaboration with myself and the research team at the Pacfici Agri-Food Research Centre, including Dr. Shabtai Bittman, Anthony Friesen, and many other technicians and coop students. Pierre Groenenboom aided me with the calculation of nitrous oxide fluxes.

The experimental design for application of Nitrapyrin® was developed by myself in conjunction with Dr. Shabtai Bittman, Dr. Sean Smukler and Dr. Sue Grayston. All installation of new equipment required was supervised by me, with the help of Anthony Friesen, Frederic Bounaix, and many other technicians and coop students. All data analysis and interpretation for Chapters 3 and 4 is my original work.

# Table of Contents

**ABSTRACT** ..... **II**
**PREFACE** ..... **III**
**LIST OF TABLES** ..... **VII**
**LIST OF FIGURES** ..... **VIII**
**LIST OF ABBREVIATIONS** ..... **XI**
**ACKNOWLEDGEMENTS** ..... **XII**
**1. GENERAL INTRODUCTION** ..... **1**
1.1. Agricultural Management Strategies to Enhance Crop Yield ..... 2
1.1.1. Impacts of Manure and Fertilizer on Nitrogen Losses ..... 4
1.1.2. Impacts of Manure and Fertilizer on Soil Microbial Communities ..... 5
1.2. Soil Quality and Biological Indicators ..... 7
1.3 Nitrogen Cycling ..... 9
1.3.1. Nitrification ..... 10
1.3. Nitrification inhibitors ..... 13
1.4. Research Objectives and Hypotheses ..... 14
1.4.1. Research Objectives ..... 14
1.4.2. Research Questions ..... 15
1.4.3. Hypotheses ..... 15
**2. SOIL MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY IN TALL FESCUE FORAGE PRODUCTION AFTER LONG-TERM APPLICATION OF WHOLE AND SEPARATED DAIRY SLURRY AND AMMONIUM NITRATE FERTILIZER TREATMENTS IN AGASSIZ, BRITISH COLUMBIA** ..... **18**
2.1. Introduction ..... 18
2.2. Materials and Methods ..... 21
2.2.1. Study Site ..... 21
2.2.2. Soil Sampling ..... 23
2.2.3. Soil Physical and Chemical Analysis ..... 23
2.2.4. Microbial Biomass Carbon and Nitrogen ..... 24
2.2.5. Microbial Community Structure - Phospholipid Fatty Acid Analysis ..... 25
2.2.6. Enzyme Analysis ..... 27
2.2.7. Statistical Analysis ..... 28
2.3. Results ..... 29
2.3.1. Soil Physical and Chemical Properties ..... 29

2.3.2. Microbial Biomass Carbon and Nitrogen ..... 30
2.3.3. Phospholipid Fatty Acids ..... 33
2.3.4. Enzyme activities ..... 36
2.3.5. Relationships Between Microbial Community Structure and Activity and Soil Properties ..... 39
2.4. Discussion ..... 42
2.5. Conclusions ..... 48
**3. NITROGEN DYNAMICS AND RELATIONSHIPS WITH SOIL MICROBIAL COMMUNITIES ON TALL FESCUE IN AGASSIZ, BRITISH COLUMBIA AFTER LONG-TERM CONTRASTING NUTRIENT MANAGEMENT TREATMENTS ..... 49**
3.1. Introduction ..... 49
3.2. Materials and Methods ..... 52
3.2.1. Study Site, Soil Physical and Chemical Analysis, and Biological Measurements ..... 52
3.2.2. Nitrogen Pools and Transformations ..... 53
3.2.3. Nitrogen Losses ..... 55
3.2.4. Statistical Analysis ..... 56
3.3. Results ..... 57
3.3.1. Soil Nitrogen Pools and Transformations ..... 57
3.3.2. Nitrogen Losses ..... 62
3.3.3. Relationships Between Microbial Community Structure and Nitrogen Transformations ..... 66
3.4. Discussion ..... 71
3.5. Conclusions ..... 77
**4. POTENTIAL OF NITRAPYRIN® TO REDUCE SOIL N LOSSES AND EFFECTS ON MICROBIAL COMMUNITY STRUCTURE ..... 79**
4.1. Introduction ..... 79
4.2. Materials and Methods ..... 80
4.2.1. Study Site ..... 80
4.2.2. Application of Nitrapyrin® ..... 81
4.2.3. Soil Measurements ..... 82
4.2.4. Soil Nitrogen Pools and Transformations ..... 82
4.2.5. Soil Nitrogen Losses ..... 83
4.2.6. Microbial Community Structure ..... 83
4.2.7. Statistical Analysis ..... 83

4.3. Results....84
4.3.1. Soil Nitrogen Pools....84
4.3.2. Soil Nitrogen Transformations....85
4.3.3. Nitrogen Losses....87
4.3.4. Microbial Community Structure....90
4.4. Discussion....93
4.5. Conclusions....95
**5. GENERAL SUMMARY AND CONCLUSIONS....97**
5.1. Research Conclusions....97
5.1.1. Effects of Nutrient Amendments on Microbial Community Structure and Activity....97
5.1.2. Effects of Nutrient Amendments on Nitrogen Cycling and links to Microbial Communities....98
5.1.3. Potential of the Nitrification Inhibitor, Nitrapyrin, to Prevent N Losses after Nutrient Amendment....99
5.2. Strengths and Contribution to the Field of Study....99
5.3. Limitations and Directions for Future Research....100
5.4. Implications of this Study for the Dairy and Agriculture Industry in the Fraser Valley....102
**REFERENCES....103**
**APPENDIX....116**

# List of Tables

Table 1 Approximate nutrient content of ammonium nitrate fertilizer, whole slurry manure, and liquid fraction slurry manure. Values are averages of total annual applications from 2002 – 2014. Values in brackets represent one standard error. Adapted from Bittman et al. (2011). ..... 22

Table 2 Soil physical and chemical properties from 0-15 cm depth after long-term application of *fertilizer*, *manure*, *combination* and *liquid* treatments. Values represent sample means (n=4), with standard error in brackets. Letters represent treatments that are not significantly different ($p<0.05$). ..... 29

Table 3 Microbial biomass, community structure (PLFA) and activity (enzymes) (n=20) in 2013 and 2014 after long-term fertilizer, whole manure (manure), whole manure/fertilizer combination (combination), liquid fraction (liquid) treatments, and no amendment (control). Significant results highlighted in bold. ..... 32

Table 4 Soil nitrogen pools and transformations in 2013 and 2014 after long-term application of fertilizer, combination, liquid fraction, and no amendment; n=20 for all variables. ANOVAs on soil $NO_3^-/NH_4^+$ at individual dates (due to treatment*date interaction), as well as averaged winter resin strip extraction (due to non-normality of residuals in the original model), were run with only treatment as a fixed effect, therefore Date and Treatment*Date effects are not applicable (N/A). Values in bold represent significance at $p<0.05$. ..... 60

Table 5 Potential N losses ($N_2O$ emissions, $NO_3^-$ leachate concentrations in lysimeters) over 2 years from control, fertilizer, combination, and liquid fraction. ANOVAs on averaged seasonal $N_2O$ and $NO_3^-$ leachate (run due to non-normality in residuals of original models) were run with only treatment as a fixed effect, therefore Date and Treatment*Date effects are not applicable (N/A); n=20. Values in bold represent significance at $p<0.05$. ..... 63

# List of Figures

Figure 1 The Nitrogen Cycle. From Hofman & Van Cleemput (2004). ..... 10

Figure 2 Microbial biomass carbon (A) and nitrogen (B) for control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error.Treatments with the same letter are not significantly different (p<0.05). ..... 31

Figure 3 Microbial Biomass Carbon:Nitrogen ratio for control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different (p<0.05). ..... 33

Figure 4 PLFA concentrations in soils from control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) plots. Bars represent sample means across two years (n=20), and error bars represent ± one standard error. Treatments with the same letter are not significantly different (p<0.05). ..... 34

Figure 5 Fungal:bacterial PLFA ratio over 1 year in soils from control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) treated plots. Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different (p<0.05). ..... 36

Figure 6 Potential activity of (A) β-glucosidase and (B) cellobioside over 1 year in soils from control plots, and those receiving fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different (p<0.05). ..... 38

Figure 7 Spearman's correlation matrices between microbial community structure and soil properties. Values represent positive (blue) or negative (red) correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, (C) early summer 2014, and (D) late summer 2014. Variables included were MBC, MBN, C:N ratios (cn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gneg), actinobacteria (actin), bacteria (bact), fungi (fungi), and fungi:bacterial ratios (fb). Values with stars represent significant correlations (p<0.05). ..... 40

Figure 8 Spearman's correlation matrices between microbial activity and soil properties. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, and (C) early summer 2014. Variables included are the activity of b-glucosidase (bgluc), cellobioside (cello), n-acetyl-glucosaminidase (nag), phosphatase (phos), sulfatase (sulf) (all units of nmol activity/h/g soil), and nitrogen mineralization (nmin) and nitrification (nitr) (units of mg N/g soil/day). Values with stars represent significant correlations (p<0.05). ..... 41

Figure 10 Concentrations of soil nitrate (A) and ammonium (B) (mg N/kg soil) obtained from soil extracts in 2013 and 2014. Bars represent treatment means (n=4) ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$). ..... 59

Figure 11 Net nitrification rates (mg $NO_3$-N/g soil/21 days) in soils in control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) plots over 1 year. Treatments with the same letter are not significantly different ($p<0.05$). ..... 61

Figure 12 Nitrate collected on resin strips (µg $NO_3^-$N/$cm^3$/day) measured over the winter of 2013. Lines represent mean (n=1-4) of treatments ± one standard error. ..... 62

Figure 13 Nitrous Oxide emissions (g $N_2O$-N/ha/day) measured in (A) summer 2013 and (B) winter 2013. Lines represent mean (n=4) of treatments, and error bars are ± one standard error. ..... 64

Figure 14 Nitrate (ppm) retrieved from lysimeter water measured over one year. In the summer of 2013, fertilizer and combination were not measured. Lines represent mean (n=1-4 due to samples missing on some dates) of treatments, and error bars are ± one standard error. The gap between July and October is due to dry soils and no leaching would have occurred. Leaching proceeded after that dry period..... 66

Figure 15 2013 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, and (B) late summer 2013. Variables included were microbial biomass carbon (mbc), microbial biomass nitrogen (mbn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin), bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil organic soluble N (nsol), and soil total N (N) in in mg N/kg soil, and $N_2O$ emissions (n2o) in g $N_2O$-N/ha/day. Values with stars reresent significant correlations ($p<0.05$). ..... 68

Figure 16 Fall/winter 2013/2014 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R). Variables included were total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin), bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), and soil total N (N) in in mg N/kg soil, $NO_3^-$ in leachate (no3leach) in mg N/L, $N_2O$ emissions (n2o) in g $N_2O$-N/ha/day, and $NO_3^-$ taken up by resin strips (resin) in ug/$cm^2$/day . Values with stars rerpesent significant correlations ($p<0.05$). ..... 69

Figure 17 2014 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R) for (A) early summer 2014, and (B) late summer 2014. Variables included were microbial biomass carbon (mbc), microbial biomass nitrogen (mbn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin),

bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil soluble organic N (nsol), and soil total N (N) in in mg N/kg soil, and $NO_3^-$ in leachate (no3leach) in mg N/L. Values with stars reresent significant correlations ($p<0.05$). ..... 70

Figure 18 Spearman's correlation matrices between microbial nitrogen fluxes and soil N pools. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, (C) fall/winter 2013/14, (D) early sumer 2014, and (E) late summer 2014. Variables included were net N mineralization (nmin) and nitrification (nitr) in mg N/g soil/day. Nitrogen variables included soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil soluble organic N (nsol), and soil total N (N) in in mg N/kg soil, and $NO_3^-$ in leachate (no3leach) in mg N/L. Values with stars repesent significant correlations ($p<0.05$). ..... 71

Figure 19 Soil $NH_4^-$ and $NO_3^-$ concentrations 17 days following manure/fertilizer application without (noNI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Bars represent means (n=4) ± one standard error. ..... 85

Figure 20 Net rates of nitrification 17 days following manure/fertilizer application without (no NI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Bars represent means (n=4) ± one standard error ..... 86

Figure 21 Nitrate released onto resin strips in the winter of 2013, following whole slurry dairy manure application only, without (no NI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Lines represent means (n=4) ± one standard error. ..... 87

Figure 22 $N_2O$ emissions for October 2013 – February 2014 following eleven years of fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4). ..... 88

Figure 23 $NO_3^-$ concentrations in suction cup lysimeters for October 2013 – August 2014, following eleven years of fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4) ± one standard error. ..... 89

Figure 24 PLFA concentrations in soils in October 2013 following long-term fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4) ± one standard error. Asterisks represent significant differences between NI and no NI. ..... 91

Figure 25 Results of a principal components analysis (PCA) between molar % of PLFA biomarker groups. n=40. Points (with adjacent numbers) represent individual samples and are colour-coded by treatment. Labelled arrows represent the association of each microbial variable with each Principal Component (Dim 1 or Dim 2). ..... 92

Figure 26 Percentage increase in total PLFA concentration in soils after Nitrapyrin® addition. Bars represent mean (n=4) ± one standard error. ..... 92

# List of Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| C | Carbon |
| CEC | Cation Exchange Capacity |
| DMPP | 3,4-dimethylpyrazole phosphate |
| K | Potassium |
| MBC | Microbial Biomass Carbon |
| MBN | Microbial Biomass Nitrogen |
| MUB | 4-methylumbelliferone |
| MWD | Mean Weight Diameter |
| N | Nitrogen |
| NAGase | β-1, 4-N-acetylglucosaminidase |
| $N_2$ | Nitrogen gas |
| $NH_3$ | Ammonia |
| $NH_4^+$ | Ammonium |
| $NO_3^-$ | Nitrate |
| P | Phosphorus |
| PCA | Principal Components Analysis |
| PLFA | Phospholipid Fatty Acid |
| S | Sulfur |
| SOM | Soil Organic Matter |
| SWC | Soil Water Content |

# Acknowledgements

I would like to start by acknowledging my co-supervisors, Dr. Sean Smukler and Dr. Sue Grayston, for allowing me the opportunity to carry out this work. Without their balance, support, and input I would not be where I am today. Particular thanks to Dr. Smukler for always keeping me on task, and Dr. Sue Grayston for helping me to unravel the strange world of belowground Ecology. I would like to thank committee members Dr. Shabtai Bittman and Dr. Maja Krzic for their thoughtful and constructive feedback.

This research was supported by a graduate fellowship provided by the Natural Sciences and Engineering Research Council of Canada, and a Research Affiliate position at the Department of Agriculture and Agri-Food Canada; without these sources, I could not have completed this work.

In addition, I'd like to thank the laboratory and research crew at the Agri-Food Research Station in Agassiz, BC. Particular thanks go to Dr. Shabtai Bittman, who suggested this project and along with Derek Hunt initiated and maintained the long research project, enabling me to carry out this work. Dean Babuin, Anthony Friesen, Frederic Bounaix, Xiao Wu and Connie Pietrafesa showed me the ropes and gave me vital insight into methods. Thanks also to the wonderful coop students who helped me with my data collection and provided support during long hours in the field.

My lab mates in the Sustainable Agricultural Landscapes Lab and the Belowground Ecology Lab were integral to my success; without them I would not have anyone with which to discuss the ins and outs of research, to bounce ideas off of, or to share office space with.

Finally, I'd like to thank my friends and family who stuck by me while I undertook this degree, to listen to me ramble about soil microbes (even if they didn't know what I was talking about), as fresh pairs of eyes on my thesis, and support when I needed it most. Thanks goes to my many cheerleaders who completed graduate degrees in the years before me, and who assured me that I also could get it done. And most especially to my parents, Dave and Sharon Neufeld, for their unwavering support and willingness to stand by me, no matter how far from home I may be.

# 1. General Introduction

Agricultural management practices have the potential to degrade, or improve soil quality; and to contaminate the environment. In North America today, one of the most important challenges for agriculture is the mismanagement and over-application of fertilizers and manure, leading to nutrient leaching, runoff, and increased emissions of ammonia, nitrous oxide ($N_2O$) and other greenhouse gas emissions from the soil (Smil, 1999). Soil properties (e.g. soil texture and cation exchange capacity) and microbial activity have important effects on the retention of water and nutrients in the soil, and the emission of greenhouse gases (Ginting et al., 2003; Gómez-Rey et al., 2012; Schloter et al., 2003). Reducing the environmental impacts of agriculture therefore requires an understanding of the processes and microbial communities which govern soil nutrient retention and cycling. Furthermore, it is important to understand how agricultural management strategies in turn may impact both the processes and the microbial communities.

The type of manure or fertilizer used in agricultural management has the potential to impact the soil microbial community, as well as the magnitude of N loss from the system. Manure, which is high in organic matter and essential plant nutrients, may benefit the soil microbial community and help to build soil quality (Ginting et al. 2003). Fertilizer, on the other hand, has been shown in some studies to negatively impact microbial populations (Sun et al., 2004; Truu et al., 2008). Another option for manure application is separated liquid dairy slurry, which has the solid fraction including phosphorus (P) and organic matter separated out, and therefore contains a higher proportion of nitrogen (N) in the soluble form. The different quantities of nutrients in this

organic liquid manure fraction compared to whole manure may favour some microbial species over others.

In this study I characterized soil microbial community structure (through phospholipid fatty acid (PLFA)) and activity (hydrolytic enzyme activities), as well as N dynamics (net N mineralization, net nitrification, $NO_3^-$ leaching, $N_2O$ emissions) and soil chemical and physical properties in tall fescue forage grass soils over two years after receiving long-term amendments of fertilizer, whole manure, separated liquid manure or a combination of fertilizer and manure. My objective was to assess how these nutrient management strategies impact soil microbial communities, N loss to the system, and how indicators of microbial community structure and activity can be used to better understand and predict N losses.

### 1.1. Agricultural Management Strategies to Enhance Crop Yield

The Fraser Valley of southwest British Columbia (BC) generates 62% of BC's gross farm receipts, on only 1.6% of the province's farmland (Fraser Valley Regional District, 2011). In this area, the agricultural industry is highly dependent on additions of fertilizers or slurry manure to supply the essential nutrients for optimum crop production. The region has a humid, maritime climate; mild winters bring only a few freezing events per year, with the longest frost-free periods in Canada. The length of the growing season is approximately 230 days, and summers are generally drier with sunny conditions prevailing. The average precipitation the area is 1720 mm, with most falling as rain in the winter.

Dairy production is an important industry for the Fraser Valley, but large numbers of dairy cows present challenges for the management of manure. High feed and forage costs make growing forage on-farm an attractive option, and provide a potential use for the manure generated from the dairy operation (Sheppard et al., 2011). Using manure can substantially offset nutrient input costs and can build soil organic matter (SOM) which has numerous reported benefits including building nutrient stocks. Manure, however, is often not the most efficient method of nutrient application, since about 50% of manure N is in the organic form, which has been shown to be less available to plants than inorganic forms of N such as $NO_3^-$ in the short term (Webb et al., 2010). In addition, the relative quantities of nutrients in manure may not be appropriate for crop demand. In the Fraser Valley, dairy manure usually contains more P per unit N than is required for crop growth; when manure is applied to meet crop N demands, P may build up in the soil (Bittman et al., 2011). Therefore, farmers may supplement with inorganic fertilizer, or use altered forms of manure to improve crop response.

One potential strategy to improve crop uptake of manure N is to separate the liquid from the solid fraction in the manure; the liquid fraction can then be applied to crops while the solid fraction can be stored and transported more easily, and used for annual crops with higher P requirements. Because P is much less soluble than N, P remains in the solid fraction (as well as a larger fraction of the organic matter, including organic N). Buildup of P in the soil can lead to P leaching (Gasser et al., 2012). Because inorganic N is more available to crops, increasing the fraction of inorganic N through removal of the solids may improve crop response per unit N in the applied manure. Bittman et al. (2011) found that forage grass yield and N uptake was improved with the liquid fraction

over the whole fraction of manure, compared at similar total (and mineral) N rates. As there is less organic matter and organic N in the separated liquid fraction, and significantly less P, I may expect differences in the composition and activity of the microbial community, as well as magnitudes of N loss, in response to separated manure slurry.

### 1.1.1. Impacts of Manure and Fertilizer on Nitrogen Losses

There are several important pathways of loss of soil N to the environment; volatilization of ammonium ($NH_4^+$), $NO_3^-$ leaching into groundwater and aquatic ecosystems, and $N_2O$ or $N_2$ emissions; all of which may be impacted by manure, liquid fraction manure, and fertilizer applications. $NH_3$ volatilization can happen right from the soil surface, before manure (or fertilizer) has infiltrated the soil. This can be reduced with injection or by applying manure to the surface in bands below the crop canopy (Bittman et al., 2007). $NO_3^-$ leaching is concern particularly given the large amount of precipitation in the humid, maritime climate of the Fraser Valley. As $NO_3^-$ is highly soluble, any excess N in the soil is potentially vulnerable to leaching, making it a serious environmental concern, particularly for the water quality of shallow aquifers found in the region (Kowalenko, 2000). Studies have shown that dairy manure applications increase $NO_3^-$ leaching relative to fertilizer applications, due to the mineralization of organic N from manure. For example, $NO_3^-$ leaching was higher with dairy manure over fertilizer applications under corn after three years of management on a loam soil (Stoddard et al., 2005); after six years of maize-alfalfa rotation, also on a loam (Basso and Ritchie, 2005); and a rotation of barley-ryegrass-sugar beet on a loamy sand (Thomsen et al., 1993). However, this was not always the case; Di et al. (1999) found in New Zealand, with a warm temperate

climate and annual rainfall of 1295 mm, under pasture that $NO_3^-$ leaching was greater under fertilizer than manure applications, likely due to increased microbial N immobilization after manure applications. Microbial populations may have an impact on the outcomes of inorganic and manure N application.

$N_2O$ emissions may also be impacted by manure and fertilizer applications. Multiple studies have shown higher $N_2O$ emissions after manure application than after fertilizer application in corn on loams and sandy loams (Chantigny et al., 2007; Paul and Zebarth, 1997a) and on grass on a gravelly loam (Philippe Rochette et al., 2008). However, in clay soils, the opposite effect was found (fertilizer higher than manure) (Chantigny et al., 2010). Jin et al. (2010) found, on a perennial grassland in China, that manure and fertilizer did not have significantly different annual emissions, despite an almost double total manure N application rate; the emission factor ($N_2O$-N emitted/N applied) was much higher in fertilizer plots. Controls on $N_2O$ production include soil $NO_3^-$ concentration, water and $O_2$ content of the soil, as well as the amount of available C for microbial communities, and the microbial community structure; therefore different soils, under different conditions and with different microbial communities, may respond differently to amendments (Xue et al., 2013).

### 1.1.2. Impacts of Manure and Fertilizer on Soil Microbial Communities

Manure and fertilizer have the potential to influence the soil microbial community in different ways. Bittman et al. (2005) reported higher bacterial populations after manure applications to forage grass production and lower bacterial populations after fertilizer applications, but fungi were depleted after both forms of amendment, leading to no difference in total microbial biomass compared with unamended controls after six years

of application. Truu et al. (2008) also found increased microbial biomass after manure addition, but decreased microbial biomass with fertilizer addition, to agricultural crops in a range of soils in Estonia. Truu et al. (2008) also found that a variety of microbial activity indicators (nitrification, N mineralization, alkaline and acid phosphatase activity, and respiration) increased with increasing N and C availability in the same study. Sun et al. (2004) used Denaturing Gradient Gel Electrophoresis *(*DGGE), a type of DNA fingerprinting analysis, to determine that a silt loam in Oklahoma, planted to winter wheat and treated for 100 years with manure, had a greater bacterial diversity than for the same period with inorganic fertilizer. At the same study site, Parham et al. (2002) examined soil P dynamics in manure vs. fertilizer-treated soils, and found that there was more phosphatase activity per unit of microbial biomass in the manure treatment relative to the fertilizer treatment. As there was no clear increase in total microbial biomass in the manure plots it appeared that P was more mobile. Lower P application rates in nutrient amendments, relative to C and N inputs, may favour fungi over bacteria in soils (Liu et al., 2013). Cruz et al. (2008) found on tallgrass prairie in Saskatchewan, that phospholipid fatty acid (PLFA) biomarkers for arbuscular mycorrhizal fungi increased in soils which had been amended with N, but no P (in comparison with soils amended with both nutrients). Beauregard et al. (2010), in the same study site, determined that changes in P availability changed the bacterial and fungal community without affecting diversity, as measured by denaturing gradient gel electrophoresis (DGGE) and PLFA analysis. Therefore, I might expect that reduction in C and P content through removal of the solid fraction of manure may impact microbial community structure and functioning relative to applications of whole manure. However, to date, no

studies have examined the impact of manure liquid fraction-only application on the microbial community.

Although there is an increasing recognition of the role of soil in mediating environmental processes such as nutrient cycling, fluxes of greenhouse gases, and the leaching of nutrients into aquatic ecosystems, I still do not have a firm understanding of how soil properties and microbial communities influence these processes. This is a problem, in particular due to the use of microbial measurements in soil quality assessments, in which the 'value' of microbial communities to soil processes is evaluated.

### 1.2. Soil Quality and Biological Indicators

The concept of soil quality has been used to attempt to integrate soil properties into a representative description of "the fitness of a specific kind of soil, to function within its capacity and within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Arshad and Martin, 2002). Attempts to assess soil quality involve using a variety of measureable physical, chemical and biological indicators to draw conclusions about larger-scale processes in the soil. Some well-established indicators include soil aggregate stability, pH, CEC, and nutrient content (Carter et al., 1997). Biological indicators of soil quality tend to be much more difficult to measure and interpret. Soil microbial communities, which perform a variety of functions including breaking down organic matter and regulating the flow of nutrients to and from the plants, are essential to the functioning of soil; however, measuring and interpreting them in the context of soil quality and soil function is still in development (Brussaard et al. 2007).

Ritz et al. (2009) evaluated indicators based on their relevance to multiple soil functions and ease of deployment. They pointed out that while microbial biomass is easy to measure, it represents only a ‘black box’ of microbial activity; the overall biomass may not represent the potential of the soil to carry out specialized functions.

Ritz et al. (2009) identified several other techniques, which may be more appropriate to reflect actual processes in the soil. PLFA profiling extracts the fatty acids from the cell membranes of soil micro-organisms, and as they are rapidly broken down in soil they reflect the viable (i.e., living and active) biomass of the microbial community. PLFAs can be identified, based on their length, number of double bonds, and any associated functional groups. Different functional groups of organisms (for example, fungi, bacteria, Gram-negative and Gram-positive bacteria, actinomycetes) can be identified using characteristic PLFAs. This provides a useful indicator for both total biomass and community structure, enabling a broad resolution analysis of changes in microbial community (Frostegård et al., 2011). Beauregard et al. (2010); Bohme et al. (2005); Sánchez-Moreno et al. (2007); and Zhang et al. (2012) found that different agricultural management strategies (from tillage, to fertilizers and manure, to non-production farm habitat structure) resulted in changes in the PLFA profile of the soil, but what this means for soil function is unclear. Schloter et al. (2003a,b) found that microbial activity rates (measured by soil N cycling activity rates such as nitrification and denitrification) changed due to farm management, while the microbial community structure (as measured by PLFA) did not. Therefore, this indicator of microbial community structure requires more investigation to determine if microbial groups are representative of specific processes, such as N cycling, in the soil.

Alternatively, biological soil quality indicators can reflect the activity of organisms in the soil. Ritz et al. (2009) suggest multiple enzyme analysis using a microplate fluorimetric assay. This allows the investigation of multiple soil functions, especially pertaining to organic matter decomposition, at one time (i.e., the activity of C, N, and P cycling enzymes). The activity of extracellular enzymes has been directly connected to rates of mineralization and nutrient cycling in the soil, and change quickly with land use, making them good potential indicators of soil function, specifically nutrient cycling, and soil quality (Sinsabaugh et al., 2008).

Direct links to process rates would better establish the interpretation of these 'indicators.' Direct links, for example, could enable the use of PLFA and enzyme analysis to predict N cycling dynamics in the soil (Bastida et al., 2008).

### 1.3 Nitrogen Cycling

Nitrogen is often the limiting nutrient in agricultural systems, but N pollution through improper application (surplus application, or improper location and timing of application) causes many environmental problems worldwide; thus, the N cycle is one of the most intensively studied nutrient cycles. The N cycle is intimately linked with soil microbial processes; however, there are still many gaps in our knowledge of the controls on rates of N transformations in the soil, and our ability to predict the N retention capacity of a soil using current soil quality indicators is lacking.

Biological N fixation is often one of the main sources of biological N in soil; in terrestrial ecosystems, it is primarily provided by bacteria and actinobacteria in symbioses with plants (legumes amid others) (Vitousek et al., 2002). N mineralization is the release of

ammonium ($NH_4^+$) from organic N and is carried out by a wide variety of organisms (Weiske et al., 2001; see Figure 4). $NH_4^+$ is one of the primary forms of nitrogen in some soils. It is available for plant and microbial uptake (although less so than $NO_3^-$), and can be toxic to plants at high concentrations.

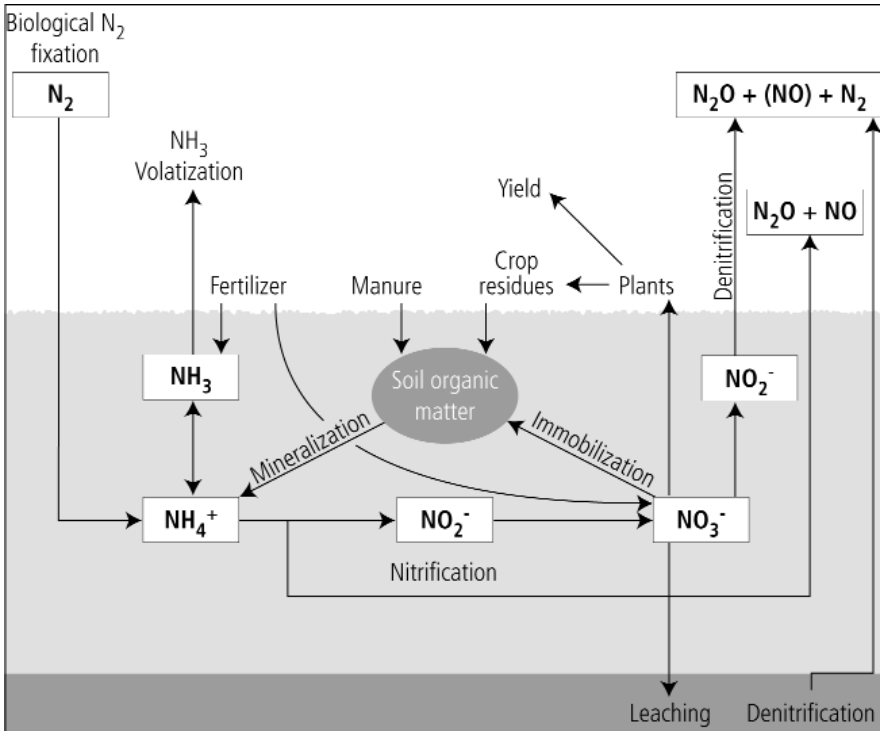


**Figure 1** The Nitrogen Cycle. From Hofman & Van Cleemput (2004).

### 1.3.1. Nitrification

Following mineralization, the next step in the N cycle, nitrification, is both extremely important to nutrient availability for plants, and the potential cause of environmental problems such as $NO_3^-$ leaching and the emission of $N_2O$. Nitrification involves the conversion of $NH_4^+$ into $NO_3^-$ and takes place over two steps, each of which are performed by specific groups of soil bacteria and archaea. $NO_3^-$ is preferentially used by plants over $NH_4^+$, however it is also much more mobile in the soil and is vulnerable to

denitrification. Complete denitrification results in the release of gaseous nitrogen ($N_2$) back into the atmosphere. If denitrification is incomplete, (e.g., in low pH soils, which inhibit the final enzyme in denitrification), $N_2O$ may be released into the atmosphere, where it has consequences for climate change (Weiske et al., 2001).

Most studies of nitrification focus on a relatively limited group of Gram-negative ammonia-oxidizing bacteria, such as *Nitrosomonas* spp (Slangen and Kerkhoff, 1984). These are organisms which perform the first step of nitrification (the conversion of $NH_4^+$ into nitrite ($NO_2^-$)) using the enzyme ammonia monooxygenase. New, culture-independent DNA techniques have revealed that ammonia-oxidizing archaea are also potentially important in this first step. Archaea are a distinct group of single-celled organisms that, while visually very similar to bacteria, have a unique genome (more similar to eukarya/multicellular organisms than bacteria) (Gattinger et al., 2003). Archaea do not use fatty acids to build their cell membranes, but rather polar ether lipids (phospholipid ether lipids, or PLEL), which cannot be detected in PLFA analysis (Gattinger et al., 2003). Molecular techniques can identify both bacteria and archaea by targeting functional genes, for example *amoA*, which codes for ammonia monooxygenase; however, these techniques cannot capture the total microbial biomass or other populations (e.g., decomposers) in the soil (Kleineidam et al., 2011). Kleinedam et al. (2011) found, using this technique, that the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) was only effective at inhibiting bacterial, but not archaeal nitrification. Therefore, it may be important to test the impact of treatments separately on bacterial and archaeal nitrifiers. The second step in the process of

nitrification is nitrite oxidation (the conversion of $NO_2^-$ into $NO_3^-$), carried out primarily by *Nitrobacter* spp. (Slangen and Kerkhoff, 1984).

As nitrification is carried out by a relatively limited community of bacteria and archaea in the soil, it may be difficult to link to indicators of microbial community diversity and structure (e.g. PLFA) or activity (e.g. enzyme analysis). For example, total microbial biomass declined after tillage on a field of grass in Oregon, USA, while rates of net nitrification increased (Nelson et al., 2006). This is in contrast with the popular 'more diversity = more function' interpretation of biological properties (Andrews et al., 2004). Phospholipid Fatty Acid Analysis is more specific, including microbial groups such as Gram negative bacterial populations; as nitrifiers are gram negative bacteria, this specific microbial group may be a better indicator of the soil function of nitrification.

Denitrification

Denitrification is the conversion of $NO_3^-$ (produced by nitrification, or added as fertilizer) to $N_2O$ and finally to $N_2$. This process is usually carried out under low-oxygen conditions, when heterotrophic microbial groups use $NO_3^-$ as an alternate electron acceptor (Zumft, 1997). Denitrification requires both available $NO_3^-$ and adequate C to be oxidized by denitrifying organisms. Thus, different amounts of available C (through manure application) may have an impact on denitrification rates (Paul and Beauchamp, 1989).

There is a very broad range of prokaryotes and eukaryotes involved in denitrification, comprising up to 5% of the total soil microbial community; while some organisms can reduce $NO_3^-$ to $N_2$, others produce a mix of $N_2O$ and $N_2$, and others, (such as fungi)

produce only $N_2O$ (Philippot et al., 2007). Many studies focus only on denitrifying bacteria (Miller et al., 2009; Paul and Zebarth, 1997b); however, several studies have shown that fungal denitrification may contribute significantly to $N_2O$ production in annual production systems (Herold et al., 2012) and upland pasture used for overwintering cattle (Jirout et al., 2013). Molecular techniques have allowed for targeted investigations of microbial denitrification, however, these genes are, as yet, limited to bacteria (Philippot and Hallin, 2005). Therefore, PLFA may still be useful for determining relative importance of different microbial groups to $N_2O$ production and denitrification rates.

### 1.3. Nitrification inhibitors

One potential solution to preventing soluble $NO_3^-$ from being leached from agricultural ecosystems into groundwater or converted into gaseous $N_2O$ is preventing nitrification. Nitrification inhibitors comprise a wide variety of products designed to reduce the activity of ammonia mono-oxygenase and therefore slow the conversion of ammonium ($NH_4^+$) to $NO_3^-$ (Grant and Wu, 2008). As $NH_4^+$ is less soluble than $NO_3^+$, and therefore less mobile in the soil, it is less likely to be leached into the groundwater (Kowalenko, 2000). Therefore, nitrification inhibitors have the potential to inhibit $NO_3^-$ formation, decrease leaching and $N_2O$ emissions, and increase crop yield due to increased retention of available N in the soil. The search for specific nitrification inhibitors started in the late 1950s (reviewed by Slangen and Kerkhoff, 1984). Many nitrification inhibitors have been developed; three of note include nitrapyrin (2-chloro-6(trichloromethyl)-pyridine), DMPP, and dicyandiamide (DCD) (Zerulla et al., 2001).

Nitrapyrin is the most widely used nitrification inhibitor in the United States. There are several reasons for environmental concern with the use of this chemical, including plant

phytotoxicity and persistence in the soil (Maftoun et al., 1982; Sander and Barker, 1978). In addition, the enzyme that nitrapyrin inhibits (ammonia monooxygenase) is very similar to methane monooxygenase, which oxidizes methane ($CH_4$). Several studies have reported a suppression of $CH_4$ oxidation with nitrapyrin, leading to increased emissions of $CH_4$, another potent GHG, from soil.(Bronson and Mosier, 1994; Topp, 1993). Nitrapyrin is bactericidal (i.e. it kills the bacteria, as opposed to merely reducing their activity) (Trenkel, 1997). While it is targeted towards *Nitrosomonas* bacteria, Roberts et al. (2003) found that 3.1 mg/kg of nitrapyrin decreased N mineralization in soil. Few studies have investigated nitrification inhibitor effects on overall microbial community structure and activity. If nitrapyrin does negatively affect the general microbial population, a more diverse and abundant microbial community may be better able to respond to this chemical and show more resilience in retaining microbial community function following its application (Griffiths and Philippot, 2013). Thus, long-term manure additions, if they do increase the microbial population and diversity through addition of labile C, may improve the communitiy's ability to retain soil quality (function) following nitrapyrin addition (Gregory et al., 2009).

## 1.4. Research Objectives and Hypotheses

### 1.4.1. Research Objectives

My research objectives were to (1) identify how specific long-term nutrient management strategies affect soil microbial community structure (PLFA) and function (hydrolytic enzyme activities), (2) identify how these long-term nutrient management strategies affect soil N pools (organic N, microbial N, soil $NH_4^+$ and $NO_3^-$ concentrations), transformations (N mineralization and nitrification, capture onto resin strips) and

potential losses ($NO_3^-$ in leachate, $N_2O$ emissions), and (3) to investigate relationships between microbial community structure and N cycling (using Spearman correlations).

### 1.4.2. Research Questions

Therefore, this research addressed four research questions.

1. How do contrasting nutrient management strategies (fertilizer and manure application techniques) affect microbial community structure and function on a long-term study site?
2. How do these nutrient management strategies (fertilizer and manure application techniques) affect rates of soil N transformations, the N content of different soil pools (organic N, microbial biomass N, inorganic N), $N_2O$ emissions and potential $NO_3^-$ leaching from the system?
3. Do biological indicators of soil quality, microbial community structure and function, reflect pools, transformations and losses of N in the soil?
4. Does application of the nitrification inhibitor Nitrapyrin have non-target effects on soil microbial community structure and function? Does nitrapyrin inhibit nitrification in the contrasting nutrient treatments?
    a. Does Nitrapyrin affect N transformation in the soil?
    b. Does Nitrapyrin affect microbial structure and function?
    c. Are there interactions between Nitrapyrin and the nutrient management strategies?

### 1.4.3. Hypotheses

Following these questions, I developed four main hypotheses:

**H1**. Soils will have higher microbial biomass, fungal:bacterial ratios, activity of enzymes involved in C, N, S and P cycling after long-term application of dairy slurry, liquid fraction dairy slurry, and a combination of manure and fertilizer compared to commercial fertilizer applications or the control (i.e. no nutrient additions).

Microbial biomass and activity will be slightly lower after application of the separated liquid fraction than of whole manure due to decreased C and increased inorganic N inputs, with the exception of fungal biomass and phosphatase activity, which will be higher due to limited P availability.

**H2.** The content of soil organic pools (total soil N, microbial biomass N), and rates of transformation (net N mineralization and nitrification) will be higher, but rates of N loss ($N_2O$ emissions and $NO_3^-$ leaching) will be lower, after applications of dairy slurry, liquid fraction dairy slurry, and the combination over fertilizer treatments and the control.

**H3.** Specific microbial groups will be correlated with potential rates of N transformation and N loss. For example, total biomass will be correlated with mineralization, gram negative bacteria will be correlated with nitrification rates, and total soil bacteira will be correlated with $N_2O$ emissions.

**H4a.** Nitrapyrin will impact nitrification and so reduce $NO_3^-$ in the soil, which will reduce $N_2O$ emissions, and $NO_3^-$ leaching from the soil.

**H4b.** Nitrapyrin will reduce bacterial abundance, but not fungal abundance in the soil. Due to decreased microbial abundance, Nitrapyrin will also decrease rates of C and N mineralization in the soil.

**H4c.** If there is an impact of nitrapyrin, microbial community structure and rates of enzyme activity will have different magnitudes of response in the dairy slurry, separated

dairy slurry and fertilizer treatments, due to increased resilience of the microbial community following manure application.

This research provides insights into how long-term manure and fertilizer application may impact the soil, soil communities and associated environmental outcomes. It answers questions about the impacts of nutrient management on microbial communities and N cycling after consistent, long-term management. It also provides insights regarding whether identification of broad resolution microbial groups is useful to predicting N dynamics in the soil after long-term applications.

# 2. Soil Microbial Community Structure and Activity in Tall Fescue Forage Production After Long-Term Application of Whole and Separated Dairy Slurry and Ammonium Nitrate Fertilizer Treatments in Agassiz, British Columbia

## 2.1. Introduction

Optimizing the application of dairy manure onto forage production fields to maximize yield while minimizing environmental impact is an important, but challenging task for dairy farmers (Webb et al., 2010). The balance of nutrients present in manure is often not the most efficient for crop growth. For example, there is often more phosphorus (P) per unit nitrogen (N) in whole dairy manure than is required for crop growth, which may lead to the overloading of P in the soil, even at the optimal level of N application (Bittman et al., 2011). To avoid excessive P, farmers may add mineral N, or remove some P from the manure by solid-liquid separation. Manure altered to separate the liquid from the solid fraction is one of the more prominent methods used in hopes of improving crop response. Because P is much less soluble than N, P remains in the solid manure fraction, which also includes a larger fraction of the organic matter and organic N. Organic N has been shown to be less available to crops than inorganic N, especially $NO_3^-$ (Webb et al., 2010). Therefore, increasing the fraction of inorganic N through removal of the solids may improve crop response per unit N in the applied manure. Bittman et al. (2011) found that grass yield and N uptake was improved with the application of only the liquid fraction over the whole fraction of manure, compared at similar total (and inorganic) N rates. As there is less organic matter and organic N in the separated liquid fraction, and significantly less P, I may expect differences in the

composition and activity of the microbial community in response to separated manure slurry.

There is increasing evidence that land use practices, particularly the application of manures and fertilizers, have the potential to influence the soil microbial community in different ways. Using manure instead of fertilizer may result in higher total soil microbial biomass (Truu et al., 2008), bacterial:fungal ratios (Bittman et al., 2005), and microbial diversity (Sun et al., 2004). This is likely due to the application of labile carbon (C) sources in the manure (Paul and Beauchamp, 1989). The ratio of available nutrients being applied may also have an impact on community structure (Börjesson et al., 2012); thus, the differing organic C, inorganic N, and P content of whole and liquid fraction dairy manure may impact microbial communities differently. Cruz et al. (2008) found that phospholipid fatty acid (PLFA) biomarkers for arbuscular mycorrhizal fungi increased in soils on tallgrass prairie in Saskatchewan which had been amended with N, but no P (in comparison with soils amended with both), suggesting that lower P application rates may prevent inhibition of mycorrhizal fungal populations.

Evidence suggests it is not only mycorrhizae, but a variety of other members of the soil microbial community which mediate the storage and availability of nutrients, such as N and P, in the soil. Microbes may immobilize nutrients in their biomass, or mineralize nutrients from organic matter, creating more bioavailable forms for plants to use. An important component of microbial mineralization of nutrients is the activity of extracellular enzymes, which are formed by fungi and bacteria (in varying quantities) and released from the cells to mineralize nutrients such as P, N, and sulfur (S) (Bandick and Dick, 1999). The activity of extracellular enzymes, which has been directly

connected to rates of mineralization and nutrient cycling in the soil (Sinsabaugh et al., 2008), has also been shown to change quickly with land use (Bandick and Dick, 1999), and has thus been suggested as a good indicator of changing soil quality. Agricultural management strategies have been shown to impact the activity of hydrolyzing enzymes, such as winter fallow/cover crops (Bandick and Dick, 1999), manure/fertilizer (Bohme et al., 2005), tillage (Štursová and Baldrian, 2010), and organic management practices (García-Ruiz et al., 2009), however, the direction of change is not always clear in the short term. In some cases, increased biomass production leads to higher enzyme activity (García-Ruiz et al., 2009), while in others, high applications of inorganic nutrients appear to decrease activity (Trasar-Cepeda et al., 2008). Thus, before they can be properly used as an indicator of soil quality, further research and investigation is required to understand the activity of extracellular enzymes in more contexts and with more data on the microbial community present and environmental conditions. In addition, research is required on long-term trials, including soil and environmental data, in order to determine the controls on these enzymes.

The objective of this study was to evaluate soil microbial community structure and activity in long-term forage grass plots following application of synthetic N fertilizer, whole slurry manure, and liquid fraction dairy manure, and to investigate the relationships between microbial communities and soil chemical and physical properties. My hypothesis was that soils would show higher microbial biomass, fungal:bacterial ratios, and activity of enzymes involved in C,N,S and P cycling after long-term application of dairy slurry or liquid fraction dairy slurry as well as a combination of manure and commercial fertilizer, compared to a fertilizer treatment alone and a control

(no nutrient additions). In addition, after application of the separated liquid fraction and a combination of whole manure and fertilizer, I expect that the soil microbial biomass and activitywill be slightly lower than following whole manure application (but still higher than fertilizer and the control), due to decreased C and increased inorganic N inputs. I expected the exception to this to be fungal biomass (due to increased mycorrhizal populations foraging for P) and phosphatase activity, to be higher in liquid than whole manure due to the lower P concentration in the liquid fraction vs. whole manure. I determined soil properties (pH; total soil C, N, P, and K; bulk density, and mean weight diameter of soil aggregates) to evaluate factors influencing microbial populations and enzyme activity in these soils.

## 2.2. Materials and Methods

### 2.2.1. Study Site

This study was conducted at the Pacific Agri-Food Research Centre in Agassiz, in the Fraser Valley of British Columbia (49$^{o}$ 10’ N, 125$^{o}$ 15’ W). The region has a humid maritime climate, with an annual average precipitation of 1720 mm. The study site was located on a silty to sandy loam of the Monroe series and classified as an Eluviated Eutric Brunisol (Paul and Zebarth, 1997a). Prior to initiation of the study, the soil contained about 6% organic matter, with and had moderate drainage (Bittman et al., 2005). The study was carried out on 3m by 45m plots that are part of an agronomic trial planted with forage grass tall fescue (*F. Arundinacea*) in 2002. The total trial area covers 588m$^{2}$.

Since establishment, nutrient treatments have been applied to this site continuously (4x/yr) with the exception of 2005 (replicate 1&4) and 2006 (replicate 2&3) when plots

were renovated and no nutrients were added. The plots are fertilized once in the spring before harvest, and then three more times over the course of the season (immediately following the first three harvests). Each treatment is replicated four times in a randomized complete block design. The treatments were: unfertilized control (*control*); a whole dairy slurry manure fraction (*manure*) applied at a target rate of 50 kg/ha of total $NH_4^+$-N per application (200 kg/ha annually); a separated liquid dairy slurry fraction (*liquid*) applied at a target rate of 75 kg $NH_4^+$-N/ha per application (300 kg/ha annually); an ammonium nitrate fertilizer treatment (*fertilizer*) applied at 100 kg $NH_4^+$-N/ha (400 kg/ha annually); and a combination whole manure and fertilizer (*combination*) applied at a rate of 50 kg $NH_4^+$-N/ha each manure and fertilizer (400 kg/ha annually). These treatments have been chosen to represent an equivalent application rate of total N (organic and inorganic) of 400 kg total N/ha annually (as manure generally contains approximately 50% $NH_4^+$-N and 50% organic N). Manure is applied by surface-banding using a sleigh-foot or drag-shoe slurry applicator designed to have little downward force to avoid soil penetration (Bittman et al., 2007). P, potatssium (K), and sulfur (S) are applied to the fertilizer plots at levels as recommended by a soil test. The forage grass in each plot has been harvested 4 times per year since establishment.

**Table 1** Approximate nutrient content of ammonium nitrate fertilizer, whole slurry manure, and liquid fraction slurry manure. Values are averages of total annual applications from 2002 – 2014. Values in brackets represent one standard error. Adapted from Bittman et al. (2011).

| | Carbon (kg/ha) | Total N (kg/ha) | Total Mineral N (kg/ha) | Organic N (kg/ha) | Total P (kg/ha) |
|---|---|---|---|---|---|
| Ammonium Nitrate Fertilizer | 0 | 400 | 400 | 0 | 0 |
| Whole Slurry Manure | 2928 (145) | 412 (2) | 216 (1) | 196 | 72 (1) |
| Liquid Fraction Slurry Manure | 1318 (173) | 444 (4) | 300 (2) | 144 | 44 (1) |

### 2.2.2. Soil Sampling

Soil was sampled three times over the course of the growing season in 2013 in June, August, and in October; and twice in 2014, in June, and late July. Samples were collected approximately 20 days after each fertilizer/manure application (and 32-36 days after harvest). Approximately 250 g of soil (4-6 cores) was taken from each plot at a depth of 0-15 cm using a 2.5 cm diameter probe. The cores were taken at random intervals across the plot, and composited in the field. A 100-150 g subsample was immediately taken to test for in situ N mineralization and nitrification. The rest of the soil was stored at 4$^{o}$C until preparation, then sieved to < 2 mm. A 10 g sub-sample of soil was frozen for enzyme analysis (analyzed on June and August 2013 and June 2014), another 10 g was freeze-dried for phospholipid fatty acid analysis (all dates), and another sub-sample was air-dried for total carbon (C) analysis (June 2013). The remainder was kept at 4$^{o}$C for general soil chemical analysis, carried out within a week of sampling. Microbial biomass C and N were measured in June and August 2013 and June and July 2014).

### 2.2.3. Soil Physical and Chemical Analysis

pH was measured at each sampling date (except October 2013 and June 2014) in distilled water with a suspension ratio of 1:4. Soil organic matter (SOM) content was determined for the June 2013 sample by dry combustion in a LECO CNS machine (Leco Corporation, St. Joseph, MI) (Kowalenko, 2001). Total Soil Element analysis measured total soil P (mg/kg soil) and K (mg/kg soil) on samples takin in October 2012. Soil Water Content (SWC) was measured at each sampling date by oven-drying a

known weight of field-moist soil at 105$^{o}$C until it reached a stable weight (approximately 48 h).

Bulk density was also determined on undisturbed samples taken in August 2014. Bulk density samples were collected at a depth of 0-15 cm (vegetation removed) using a core with a 2.5 cm diameter and 15 cm height. Cores were oven-dried at 105$^{o}$C until they reached a stable weight (approximately 48 h) (Blake, 1986). Aggregate stability was determined using a wet-sieving method adapted from Kemper & Rosenau (1986). Each core was gently broken apart, and soil from the centre of the core was sieved through two sieves, 6 mm and 2 mm. The 2 – 6 mm fraction was kept. A 2-5 g subsample was dried at 105$^{o}$C to determine moisture content. A 15g subsample was placed onto a nest of sieves of pore size 2 mm, 1 mm, and 0.25 mm, and moistened in a humidifier for 45 minutes to 1 hour to prevent slaking. The sieve nests were immersed and agitated for 10 min. Each fraction was dried at 105$^{o}$C for 24 hours before being weighed. Subsequently, the samples were ground and re-sieved to remove coarse fragments, and weighed again. The mean weight diameter (MWD) was calculated by summing the product of the mean diameter of each size fraction and the proportion of total weight attributed to each size fraction. Mean weight diameter is taken as a measure of the stability of aggregates in the sample.

### 2.2.4. Microbial Biomass Carbon and Nitrogen

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform fumigation-extraction technique (Vance et al., 1987) in June and August 2013, and June and July 2014. One 25 g sub-sample of soil from each plot at each sampling date was fumigated with chloroform ($CHCl_3$) for 24 hours, then extracted with

0.5 *M* potassium sulfate ($K_2SO_4$). Another 25 g sub-sample of soil was extracted directly with 0.5 *M* $K_2SO_4$. The extracted organic C was measured for dissolved organic C on a Shimadzu TOC-V Organic Carbon and Total Nitrogen Analyzer (Shimadzu Scientific Inc., Columbia, MD). Organic N was measured using a persulfate digestion (Kowalenko and Babuin, 2003). A solution of potassium persulfate, boric acid, and sodium hydroxide was mixed with the extracted sample and microwaved at 120$^{o}$C for 10 minutes in a microwave digester. The resulting digested sample was measured for $NO_3^-$-N on a flow injection analyzer. The difference in organic C and total N in the extracts was divided by a factor of 0.35 for C and 0.5 for N to calculate the amount of biomass mobilized by the chloroform (i.e., microbial biomass C and N) (Voroney et al., 2008).

### 2.2.5. Microbial Community Structure - Phospholipid Fatty Acid Analysis

To assess microbial community structure, I measured the phospholipid fatty acid profiles of microbial communities in the soil at all sampling dates. Phospholipid fatty acids were extracted in the Belowground Ecosystem Group laboratories at the University of British Columbia in Vancouver, following the procedure outlined by Frostegård et al. (1991). 1.5 g of soil were placed in Teflon tubes, and extracted with a 1:2:0.8 v/v/v mixture of chloroform, methanol and citrate buffer. The resulting solution was vortexed, shaken for one hour, and centrifuged for 30 min at 3000 rpm. The supernatant was transferred to a new Teflon tube. This process was repeated once (without the shaking). The collected supernatant was split into two phases by adding 3.1 mL of citrate buffer and 3.1 mL of chloroform, and left overnight. Following this separation, the top phase was aspirated, and the bottom phase (containing the lipids) was dried down under a stream of $N_2$. This lipid extract was then re-dissolved in a small

amount of chloroform, and eluted through a silica solid phase extraction cartridge (Agilent Technologies). Neutral lipids and waxes, glycolipids, and phospholipids were extracted with chloroform, acetone, and methanol, respectively. The phospholipids were collected in Teflon tubes, and 200 µL of an internal standard methyl nonadecanoate (C19:0) was added for calibration of the PLFA quantities. The lipids were converted into their fatty acid methyl ester forms through transesterification with methanolic potassium hydroxide. The samples were then dried under a stream of $N_2$ and stored at -20$^{o}$C until analysis.

The extracted fatty acids were measured with an Agilent 6890N gas chromatograph with an Agilent 5973N mass selective detector. The peaks were identified using a standard qualitative bacterial acid methyl ester mix (BAME) (Sigma Aldrich, Canada) that ranged from C11 to C20, and by referring to the template in Brockett et al. (2012).

Broad resolution microbial groups were identified using characteristic fatty acid markers as described by Brockett et al. (2012). i15:0, a15:0, i17:0, a17:0 were used to identify Gram-positive bacteria, 16:1ω7, cy17:0, cy19:0 were used to identify Gram-negative, and 15:0 and 17:0 were used as general bacterial biomarkers. 10Me18:0 was used to identify actinobacteria. The biomarkers 18:2ω6,9 and 18:1ω9c were used as fungal biomarkers. The Shannon Index (SI) was calculated for each sample using each microbial group as a 'species', to investigate changes in microbial community structure (Q. C. Zhang et al., 2012). I used the equation:

$$SI = -\sum_{i=1}^{N} Pi(lnPi)$$

Where Pi is the molar % PLFA belonging to the $i^{th}$ microbial group (mol of microbial group/mol total PLFA). I did not use individual fatty acids as 'species', as they are not good indicators of actual microbial species composition (Frostegård et al., 2011), but compared distributions of microbial groups in order to determine different broad resolution community structure between treatments.

### 2.2.6. Enzyme Analysis

The activity of cellobiosidase, β-glucosidase, chitinase, phosphatase and sulphatase were measured fluorimetrically, using methylumbelliferyl substrates, in 96 well microplates as described by Sinsabaugh et al. (2003) in June and August 2013 and June 2014. 0.1 g of soil (ground in a pestle and mortar from frozen samples) was suspended in 50 mL of 50 mM sodium acetate buffer and shaken on high for 1 hour. Subsequently, another 50 mL of buffer was added. 200 µL aliquots of the soil suspension were added to 96-well black microplates (Costar microplate, Corning Lie Sciences, Acton, MA) along with 50 µL of the 200 µL substrate solution (4-MUB-β-D-cellobioside, 4-MUB- β –D-glucoside, 4-MUB-N-acetyl-β-glucosaminide, 4-MUB-phosphate, 4-MUB-sulfate) and incubated for 7 (cellobiosidase), 3 (β-glucosidase, N-acetyl- β-glucosaminidase, sulfatase), or 2 (phosphatase) hours, after which the reaction was stopped with NaOH. Each soil sample was replicated in 16 wells. Each plate also contained 8 replicates of a positive control (200 µL buffer and 50 µL of 10 µM 4-methylumbelliferone standard) and 8 replicates of a negative control (200 µL buffer and 50 µL of the appropriate substrate). On a separate plate, 8 replicates of a soil background fluorescence (200 µL soil solution + 50 µL buffer) and soil quench (200 µL

soil solution + 50 µL of 10 µM 4-methylumbelliferone standard) were run for each sample.

Blank wells (acetate buffer + sample), negative controls (substrate + acetate buffer), and quench standards (4-methylumberlliferone standard + sample) were included for each sample and enzyme. There were 8 replicate wells for each blank, negative control, and quench and 16 for each sample. Fluorescence was measured using a CytofluorTMII microplate fluorimeter with 365 nm excitation and 450 nm emission filters. Units (after calculations correcting for controls, quenching and substrate background) are expressed in nmol/h/g soil.

### 2.2.7. Statistical Analysis

Differences in microbial community structure and activity were analyzed using a linear mixed effects model, R package lmerTest, with treatment and date as fixed effects and block as a random effect. An ANOVA was used to determine significant effects and where differences were found ($p<0.05$), separations were investigated using Tukey's test, R package multcomp. Data was tested for normality using the Shapiro-Wilk test, and where not normal was log-transformed to meet normality assumptions. Mixed models were tested for normality of residuals using functions plot and qqnorm in R. Pearson correlation coefficients were calculated between non-transformed variables using R package Hmisc to allow for analysis of statistical significance. All analyses were carried out in R (R Foundation for Statistical Computing, 2014).

## 2.3. Results

### 2.3.1. Soil Physical and Chemical Properties

There were significant differences among treatments in all soil chemical and physical properties measured, except for the MWD of soil aggregates and soil phosphorus (Table 2). Total soil C was 19% higher in the plots receiving the *manure* treatment, 24% in the plots receiving the *combination* treatment and 15% in the plots receiving the *liquid* treatment, compared to the unamended *control* ($p<0.05$) (Table 2). Soil C in the plots receiving the *fertilizer* treatment was not significantly different than *control.* Similarly, total soil N was 14% higher in the *manure*, 19% in the *combination* and 18% in the *liquid* treated plots than the *control* (*fertilizer*, again, was not significantly different from *control*). Soil pH was lower in the *fertilize*r plots and higher in the *manure* and *liquid* plots (as compared to the *control* (Table 2). Surprisingly, potassium was higher (55%) than the *control* only in the plots receiving the *liquid* treatment (Table 2). While *manure* treated plots had the highest, and *fertilizer* the lowest, average MWD, there were no significant differences between treatments.

**Table 2** Soil physical and chemical properties from 0-15 cm depth after long-term application of *fertilizer*, *manure*, *combination* and *liquid* treatments. Values represent sample means (n=4), with standard error in brackets. Letters represent treatments that are not significantly different ($p<0.05$).

| Treatment | MWD (mm) (May 2014) | Bulk Density (g/cm3) (August 2014) | pH (5 dates 2013-2014) | SWC (g/g) (5 dates 2013-2014) | Total Carbon (mg C/kg soil) (June 2013) | Total Nitrogen (mg N/kg soil) (June 2013) | Total Phosphorus (mg P/kg soil) (October 2012) | Total Potassium (mg K/kg soil) (October 2012) |
|---|---|---|---|---|---|---|---|---|
| Control | 2.9 (0.2) | 0.93 (0.02) | 6.3 (0.0) b | 0.39 (0.01) | 30.9 (1.5) a | 2.4 (0.1) a | 926 (71) | 729 (65) a |
| Fertilizer | 2.7 (0.4) | 0.98 (0.01) | 6.0 (0.1) a | 0.35 (0.01) | 32.7 (1.2) ab | 2.5 (0.1) ab | 788 (167) | 703 (176) a |
| Manure | 3.0 (0.3) | 0.88 (0.03) | 6.6 (0.0) c | 0.37 (0.01) | 36.8 (1.6) c | 2.8 (0.0) bc | 1089 (74) | 895 (118) ab |
| Combination | 3.0 (0.1) | 0.91 (0.02) | 6.3 (0.1) b | 0.39 (0.01) | 38.3 (0.8) c | 2.9 (0.1) c | 967 (57) | 895 (118) a |
| Liquid | 2.7 (0.2) | 0.97 (0.05) | 6.6 (0.0) c | 0.37 (0.01) | 35.6 (1.6) bc | 2.8 (0.1) bc | 970 (58) | 1133 (135) b |

### 2.3.2. Microbial Biomass Carbon and Nitrogen

Amendments significantly changed C and N pools in microbial biomass. Soil MBC and MBN were significantly different for both treatment and date, with no interaction between the terms ($p<0.05$) (Figure 2). At every sampling date, *manure*, *liquid*, and *combination* treatments all had higher MBC (32%, 19%, and 22% higher than *control*, respectively) than the *control* and *fertilizer* treatments. MBN was also significantly higher in the *manure*, the *combination* and the *liquid* treatment (48%, 50%, and 36% higher than *control*, respectively). Both MBC and MBN were lower in 2014 than 2013 (though 2014 MBN was only significantly different than August 2013) (Table 3).

The microbial biomass C:N ratio was significant for both treatment and date, and there was an interaction between the terms (Figure 3). Subsequent analysis, by date, showed the *fertilizer* and *combination* treatments had lower soil microbial biomass C:N ratios than the *control, manure*, and *liquid* treatments in August of 2013. In contrast, in June and July 2014, *combination, manure*, and *liquid* treated plots all had lower soil microbial biomass C:N than *control* plots.

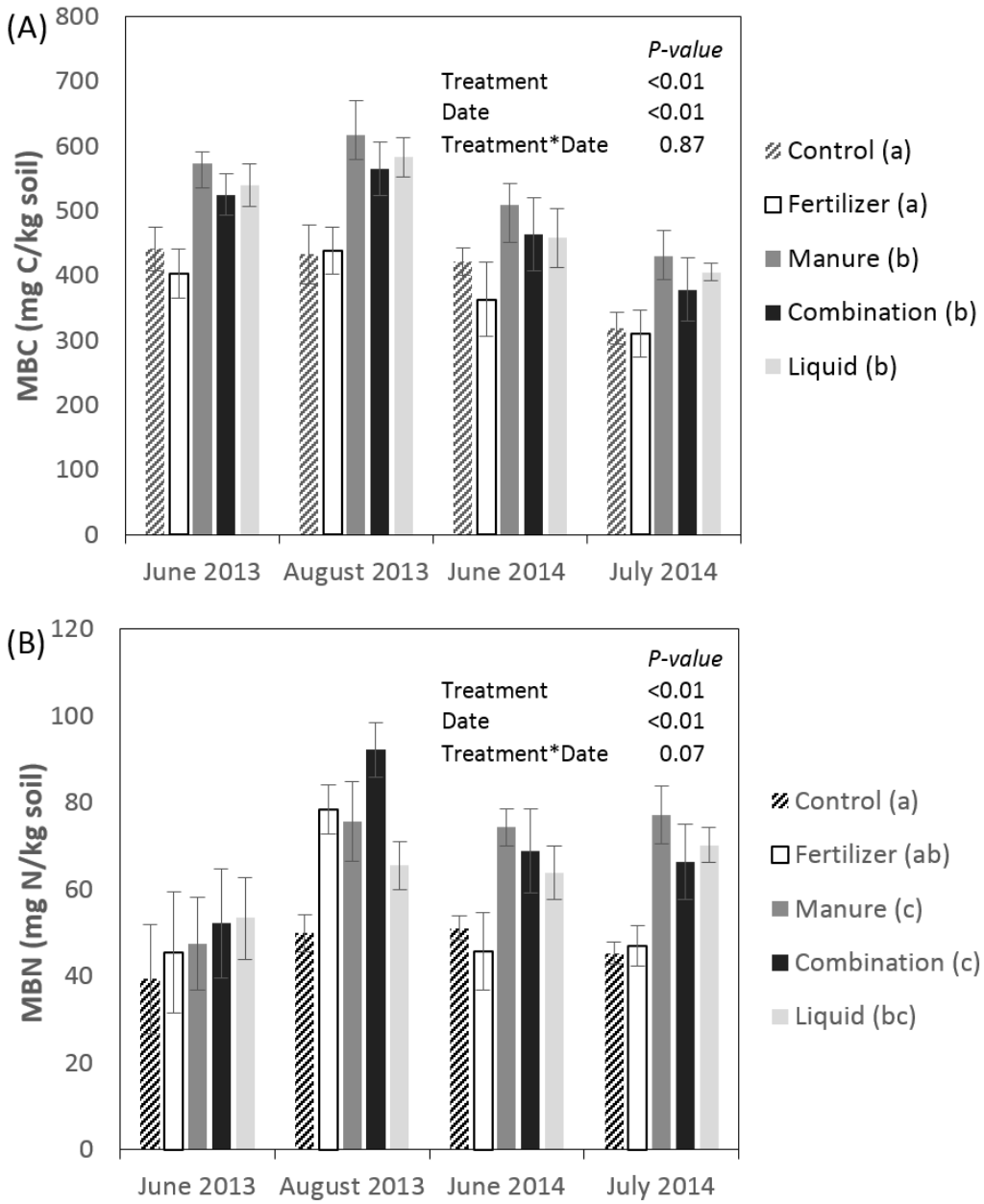


**Figure 2** Microbial biomass carbon (A) and nitrogen (B) for control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error.Treatments with the same letter are not significantly different ($p<0.05$).

**Table 3** Microbial biomass, community structure (PLFA) and activity (enzymes) (n=20) in 2013 and 2014 after long-term fertilizer, whole manure (manure), whole manure/fertilizer combination (combination), liquid fraction (liquid) treatments, and no amendment (control). Significant results highlighted in bold.

| Variable | Treatment | | Date | | Treatment*Date | |
|---|---|---|---|---|---|---|
| | F | p-value | F | p-value | F | p-value |
| Microbial Biomass Carbon | **21.36** | **<0.01** | **30.78** | **<0.01** | 0.55 | 0.87 |
| Microbial Biomass Nitrogen | **9.37** | **<0.01** | **11.94** | **<0.01** | 1.78 | 0.07 |
| C:N | **4.9** | **<0.01** | **27.08** | **<0.01** | **3.06** | **<0.01** |
| Total PLFA | **11.59** | **<0.01** | **53.43** | **<0.01** | 0.62 | 0.86 |
| Total Bacteria | **12.45** | **<0.01** | **43.22** | **<0.01** | 0.57 | 0.9 |
| Gram Positive Bacteria | **12.91** | **<0.01** | **16.77** | **<0.01** | 0.97 | 0.49 |
| Gram Negative Bacteria | **8.26** | **<0.01** | **76.17** | **<0.01** | 0.44 | 0.96 |
| Actinomycetes | **5.9** | **<0.01** | **26.4** | **<0.01** | 1.05 | 0.41 |
| Fungi | **6.98** | **<0.01** | **32.57** | **<0.01** | 0.8 | 0.68 |
| Fungal:Bacterial Ratio | **6.4** | **<0.01** | **33.89** | **<0.01** | 1.2 | 0.29 |
| β-glucosidase | **3.52** | **0.02** | **18.87** | **<0.01** | 0.77 | 0.63 |
| cellobioside | **5.14** | **<0.01** | **21.4** | **<0.01** | 0.41 | 0.91 |
| NAGase | 0.49 | 0.74 | 2.77 | 0.07 | 0.48 | 0.86 |
| sulfatase | 0.71 | 0.59 | **39.53** | **<0.01** | 0.51 | 0.83 |
| phosphatase | 0.53 | 0.71 | **56.43** | **<0.01** | 0.67 | 0.71 |

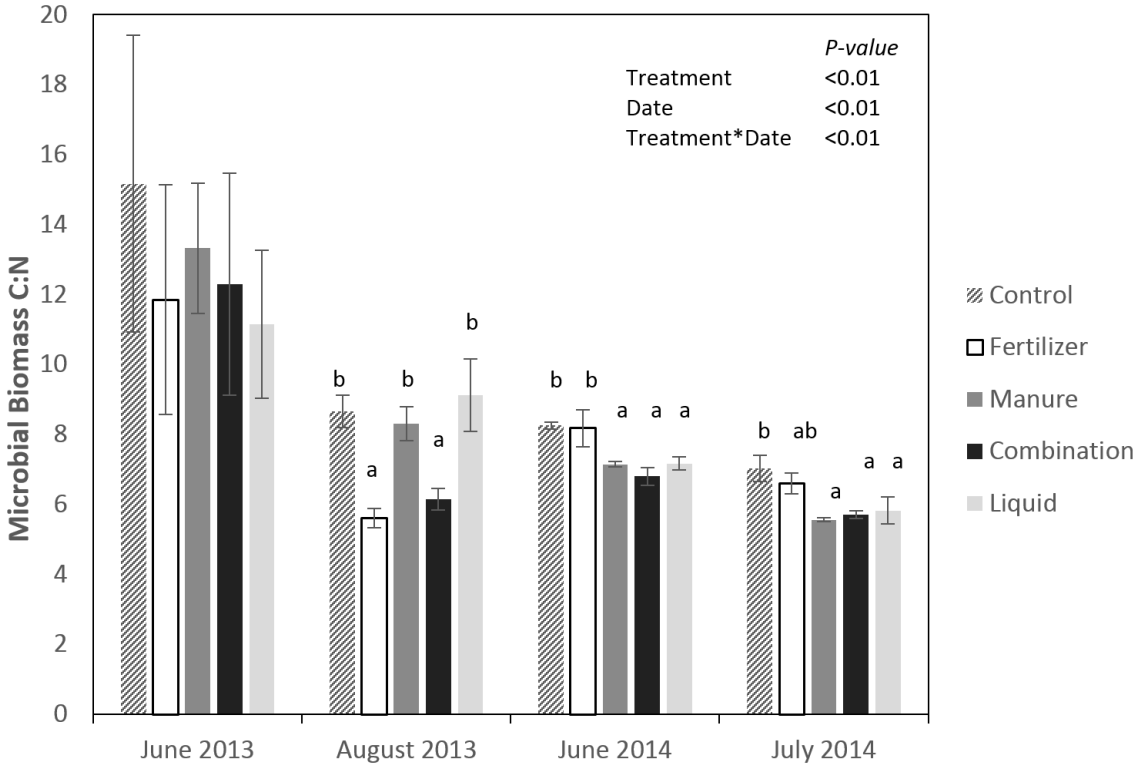


**Figure 3** Microbial Biomass Carbon:Nitrogen ratio for control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$).

### 2.3.3. Phospholipid Fatty Acids

Total PLFAs followed the same pattern as MBC; total concentrations were increased in plots receiving *manure, liquid*, and the *combination*. The relationship between MBC and total PLFAs, as the latter is often used as a proxy for total microbial biomass, had an $R^2$ of 0.35 ($p<0.05$) (see Appendix, Figure 26). Total PLFA concentrations ranged from 47 to 152 nmol PLFA/g soil.

For total PLFAs, both treatment and date had a significant effect, with no interaction (Figure 4). Similar to MBC, total PLFAs were lower in June 2014 than July and August 2013 (data not shown, Table 3). Likewise, soil from *control* and *fertilizer* treated plots had significantly lower total PLFA (94.4 and 92.9 nmol/g soil, respectively) than soil from

*manure*, *liquid*, and *combination* plots (117.9, 111.4, and 110.9 nmol/g soil, respectively) (Figure 4). Bacterial biomarkers comprised the majority (~50%) of total PLFA in all soil samples and followed a similar pattern of significance to total PLFA. Within bacteria, Gram-positive and Gram-negative bacteria also followed the same pattern, with soils receiving organic amendments (*manure*, *liquid*, *combination*) containing significantly higher concentrations of the PLFAs characteristic of these bacterial classes.

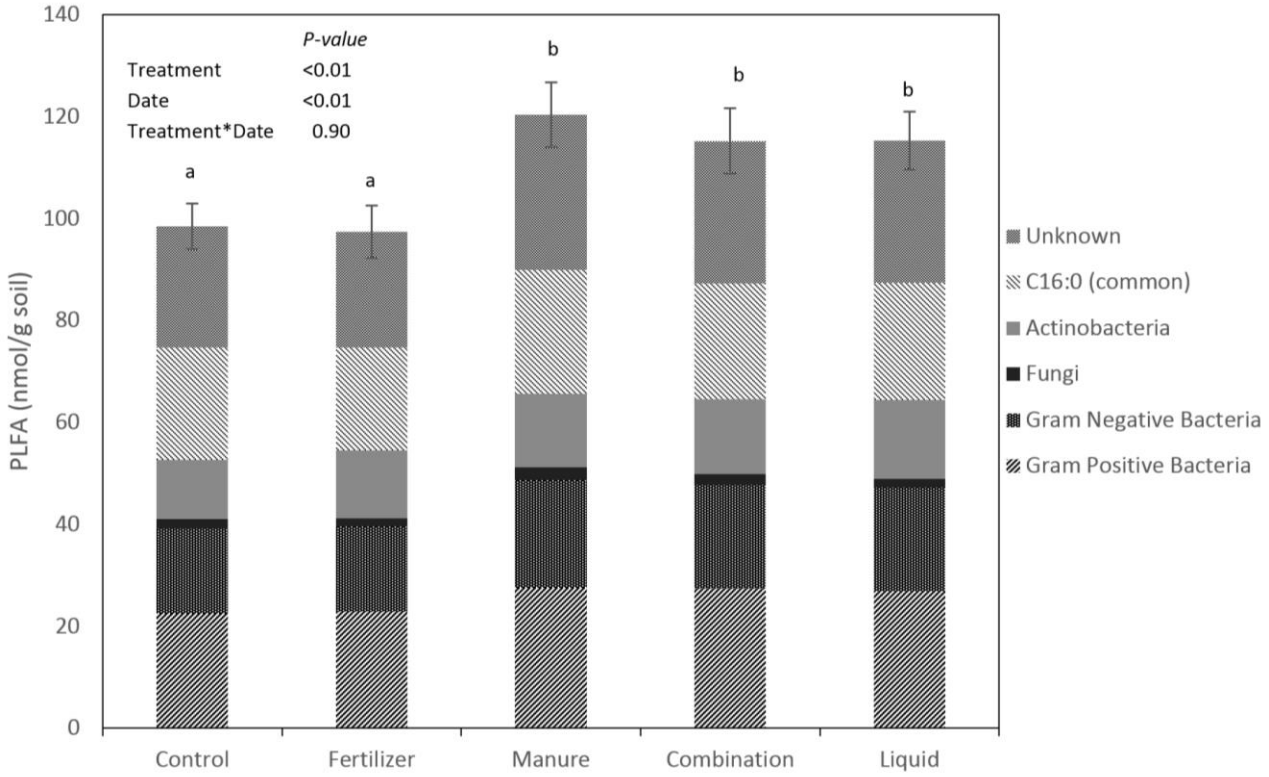


**Figure 4** PLFA concentrations in soils from control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) plots. Bars represent sample means across two years (n=20), and error bars represent ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$).

Treatment and date were significant (no interaction) for the fungal biomarker 18:2ω6,9, with a clear pattern of decreasing concentrations of fungal PLFA over the season in 2013 (Table 3, Figure 5). This pattern did not continue in 2014. Soils from *manure*

treated plots had a significantly higher (40%) abundance of the fungal PLFA (2.54 ± 0.31 nmol/g soil) than control (1.81 ± 0.26 nmol/g soil), and all other treatments, except the *combination* (1.97 ± 0.23 nmol/g soil) ($p<0.05$).

Treatment and date were significant for actinobacteria (a group of Gram-positive bacteria), again with no significant interaction. The *liquid* treatment had the highest concentrations of the PLFA characteristic of these bacteria (17.1 nmol/g soil). *Liquid*, *manure* and *combination* all contained significantly higher actinomycete PLFA concentrations than *control* (11.4 nmol/g soil for control).

The ratio of fungal:bacterial PLFA was also significant for both treatment and date with no interaction between the terms (Figure 5). The ratio decreased over the season in 2013 (mirroring the decrease in fungal PLFA biomarkers). Soils from plots receiving *liquid* had significantly lower fungal:bacterial PLFA than control, and soils from *manure* treated plots had significantly higher fungal:bacterial PLFA than plots receiving *liquid* or *fertilizer*. There were no significant differences in the Shannon Index (SI) between treatments or dates, though the control had a consistently lower SI than the other treatments.

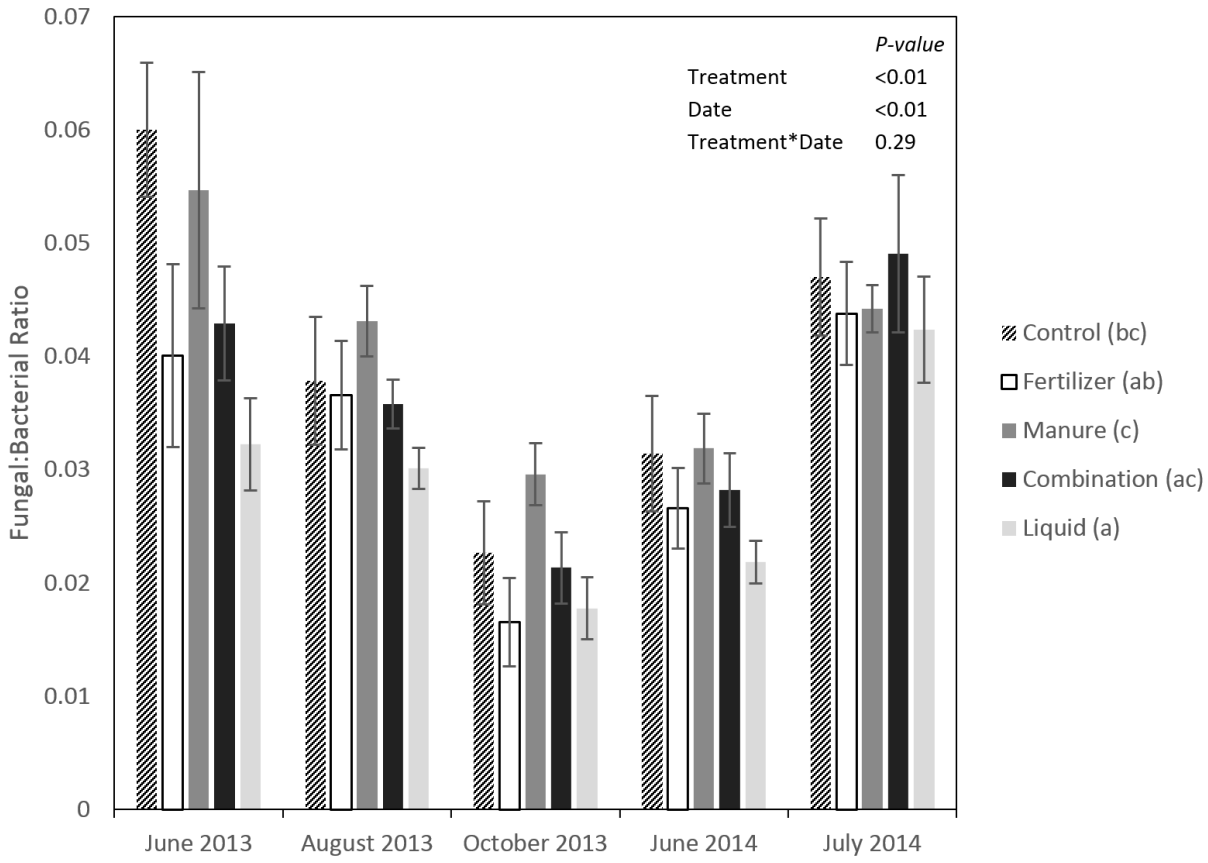


**Figure 5** Fungal:bacterial PLFA ratio over 1 year in soils from control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) treated plots. Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$).

### 2.3.4. Enzyme activities

The potential activity of both cellulose-degrading enzymes was significantly affected by treatment and date ($p<0.05$). There was no interaction between the terms. Potential cellulolytic activity was lower in June 2014 as compared to both dates in 2013 (Figure 6). The activity of cellobiosidase (the first step in cellulose decomposition, breaking down more complex molecules) was significantly lower in soils from the *control* plots than the *manure, liquid* and *combination* treatment plots; soil from plots receiving *fertilizer* had an intermediate level of potential cellobiosidase activity, but this was not significantly different from any other treatment. β-glucosidase activity (the final step in

cellulose breakdown) was significantly lower in soils from the *manure, liquid* and *combination* treatment plots, with *fertilizer* having an intermediate, but not significantly different, level of activity. There did not appear to be any impact on β-glucosidase or cellobioside activity of type (or amount) of C applied between the *manure, liquid* and *combination* plots.

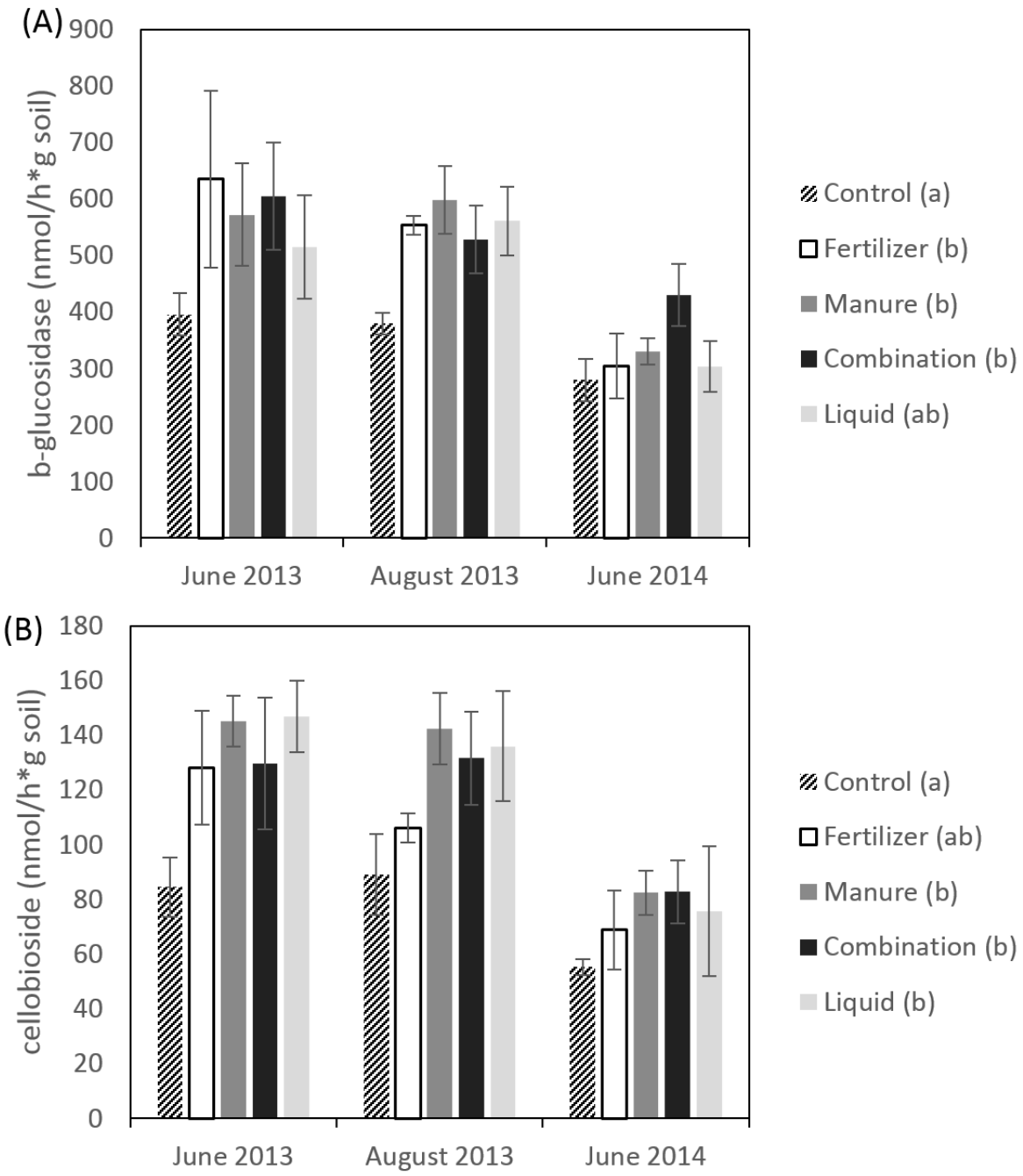


**Figure 6** Potential activity of (A) β-glucosidase and (B) cellobioside over 1 year in soils from control plots, and those receiving fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid). Bars represent sample means (n=4), and error bars represent ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$).

The potential activities of N-acetyl-β-glucosaminidase, sulfatase, and phosphatase were not significantly different between soils from any treatment (data not shown). The

variability in N-acetyl-β-glucosaminidase activity was very high (standard errors of 15-60% of the mean) across all samples.

#### 2.3.5. Relationships Between Microbial Community Structure and Activity and Soil Properties

Significant correlations between measured variables varied throughout the season. Across all dates, MBC was significantly positively correlated with soil N; it was also significantly positively correlated with soil C (significant in 2013) and P (significant in 2013 and late summer 2014). Total PLFA biomass was similarly consistently positively correlated with soil pH (significant in 2013), soil C (significant in late summer 2013 and early summer 2014), soil N (significant in 2013), and soil P (significant in 2013 and late summer 2014). MBN was consistently negatively correlated with the mean weight diameter, although this was only significant in the early summer of 2013. Gram-positive bacteria were negatively correlated with pH in the late summer of 2013, but positively correlated with pH in the late summer of 2014. Gram-negative bacteria were consistently positively correlated with soil K, however this was only significant in 2013. Actinobacteria were consistently negatively correlated with MWD, soil water content (SWC), and soil K, although these were only significant in the early summer of 2013. Total bacteria were similarly negatively correlated with MWD, SWC, and K in the early summer of 2013, although these values shifted at later dates. Fungi and the fungal:bacterial ratio showed similar patterns; negative correlations with bulk density (only significant for fungal:bacterial ratios in the early summer of 2013) and soil N.

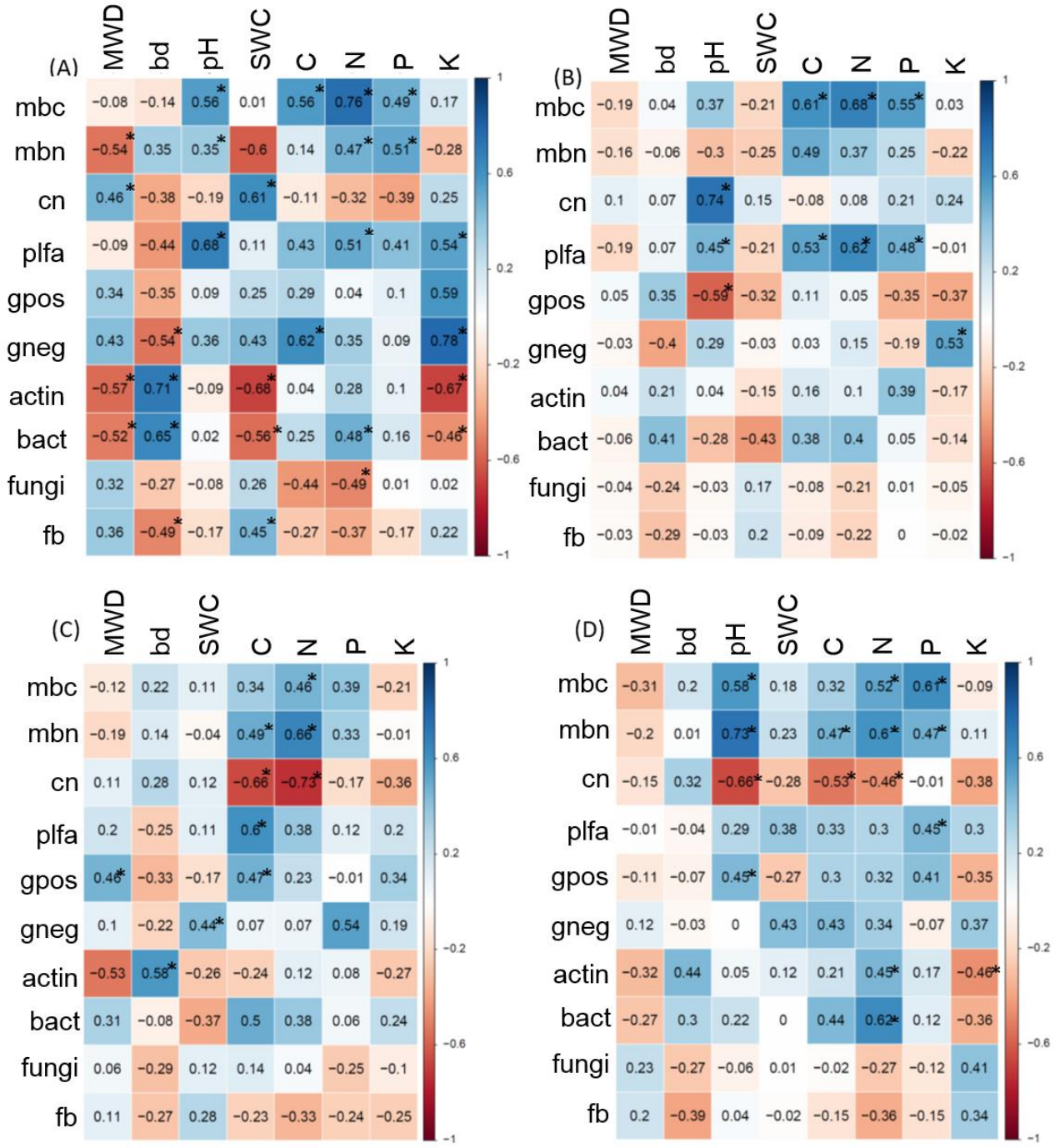


**Figure 7** Spearman's correlation matrices between microbial community structure and soil properties. Values represent positive (blue) or negative (red) correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, (C) early summer 2014, and (D) late summer 2014. Variables included were MBC, MBN, C:N ratios (cn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gneg), actinobacteria (actin), bacteria (bact), fungi (fungi), and fungi:bacterial ratios (fb). Values with stars represent significant correlations ($p<0.05$).

Microbial activity, however, was not as strongly correlated with soil properties. Cellobiosidase activity was consistently positively correlated with soil C, N, and P, but this was only significant in the early summer of 2013 (soil C and N) and the late summer of 2013 (soil P). In 2013, sulfatase activity was positively correlated with MWD and SWC. Surprisingly, phosphatase activity was neither positively nor negatively correlated with soil P, suggesting that other factors are controlling phosphatase activity (and phosphorus availability) in the soil. The activity of NAGase, which participates in the mineralization of N, was significantly positively correlated with SWC and soil C only in the early summer of 2013.

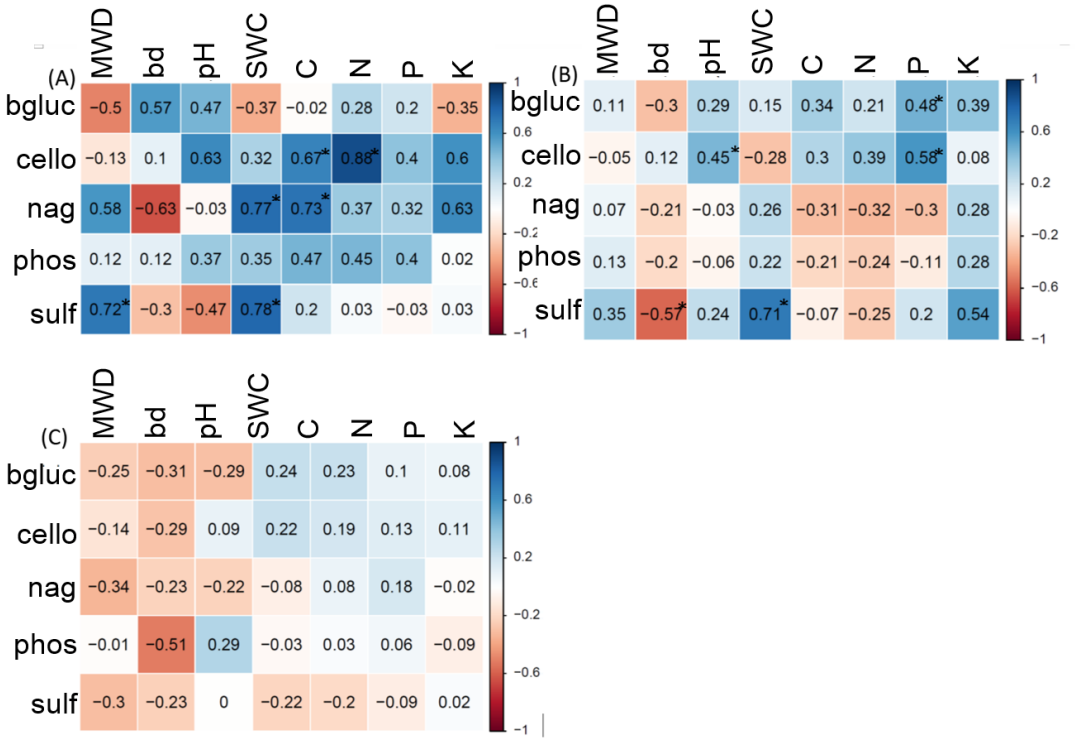


**Figure 8** Spearman's correlation matrices between microbial activity and soil properties. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, and (C) early summer 2014. Variables included are the activity of b-glucosidase (bgluc), cellobioside (cello), n-acetyl-glucosaminidase (nag), phosphatase (phos), sulfatase (sulf) (all units of nmol activity/h/g soil), and nitrogen mineralization (nmin) and nitrification (nitr) (units of mg N/g soil/day). Values with stars represent significant correlations ($p<0.05$).

## 2.4. Discussion

My hypothesis was that soils would show higher microbial biomass, fungal:bacterial ratios, and activity of enzymes involved in nutrient cycling after long-term dairy slurry, liquid fraction dairy slurry, or a combination of whole manure and fertilizer treatments compared to a fertilizer treatment or control . I expected that the microbial biomass and activity would be lower after application of separated liquid fraction manure than whole manure, with a higher fungal biomass and phosphatase activity after application of the liquid fraction.

In agreement with this hypothesis, all treatments that included manure application (*manure, liquid* and *combination*) significantly increased the biomass and cellulose-degrading activity of microbial populations. This is likely due to increased organic matter inputs, as total microbial biomass (MBC and total PLFAs) were positively correlated with soil organic matter (total soil C, N and P), at several dates during the current study. Ten years of annual applications of 2928 kg C/ha/year from the whole manure, and 1318 kg C/ha/year from the liquid fraction would amount to 29,000 and 13,000 kg C/ha added in total. In comparison, in 2013 there was approximately 323,664 kg/ha (3.67%, *manure*) and 345,514 kg/ha (3.56%, *liquid*) of C stored in the top 15 cm of the mineral soil at my sites. While I do not have measurements over time across the eleven years of management, initial SOM content was approximately 6% (Forge et al., 2005). According to the convention that SOM contains 58% C, this would have been a soil C content of 3.48%, which therefore decreased in the top 15 cm in control and fertilizer treatments (3.08% and 3.27%, respectively), but has increased marginally in all three treatments receiving organic matter (3.67% in whole manure, 3.56% in liquid fraction, and 3.83% in

the combination). Other studies have shown increased microbial biomass with increased C content in the soil (Börjesson et al., 2012; Fließbach et al., 2007; Gu et al., 2009; Parham et al., 2002). Despite differing contents of organic matter in the whole and liquid fractions, there were no differences in total microbial biomass between whole and liquid dairy slurry, contrary to my expectations that there would be slightly less microbial biomass and activity after liquid dairy slurry due to lower C applications. Therefore, it is possible that C was not limiting in this system.

MBC and MBN values reported here were comparable to those found in other studies, particularly in pasture and grassland sites. Soil from an upland pasture in the UK was shown to have microbial biomass values between 200 and 700 mg/kg soil (as compared to my soils, with a range of 300-600 mg/kg soil) (Griffiths et al., 2004). MBC values of 100-200 mg C/kg soil (~65% less than manure values in my study) were found on long-term winter wheat plots amended with cattle manure in Oklahoma, USA (Parham et al., 2002). In Switzerland, a 20-year organic and conventional farming study found MBC of 267 mg C/kg soil for organic and 218 mg C/kg soil in a conventional system, with MBN values of 40.7 and 32.8 mg N/kg soil, respectively (Fließbach et al., 2007). C:N ratios were 6.63 and 6.84 in the study by Fließbach et al. (2007).

Total PLFA content was significantly higher in *manure*, *liquid*, and *combination* treatments, with no significant difference among them. The values in the current study were higher than those previously reported in annual cropping systems, but were comparable with total PLFA content in grassland systems. Soil under corn, rice paddies, cotton-wheat rotation and alfalfa ranged from 9-89 nmol/g soil (on average 56% less than in my study) in a variety of soil types, climates, tillage, and nutrient

management strategies (Beauregard et al., 2010; Börjesson et al., 2012; Peacock et al., 2001; Watts et al., 2010; Zhang et al., 2012). Steenwerth et al. (2002) found that PLFA concentrations were 52-162% higher in perennial grassland soils than annual agricultural soils. Grayston et al. (2004) described a range of fungal PLFA (1.5-5.5 nmol/g soil) and bacterial PLFA (40 – 60 nmol/g soil) concentrations in a range of grasslands that are comparable with my results. In addition, similar to the current study, fungal PLFA were negatively affected by inorganic fertilizers (Grayston et al., 2004).

In the current study in 2014, total microbial biomass was less than in 2013. This could have been due to precipitation differences. Winter 2012/2013 precipitation was 1495 mm from October to May, and 310 mm from May to September 2013; 2014 values were lower, with 1234 mm falling between October 2013 and May 2014, and 206 mm from May to September 2014. This did not translate into differences in soil moisture on the sample dates, but differences between years in moisture could have impacted microbial biomass. Brockett et al. (2012) determined that soil moisture was the major factor affecting soil microbial community structure and enzyme activities in a range of forest ecosystems, and therefore could be playing a significant role in my systems. While MBC and SWC were not correlated at each sampling date, it is possible that seasonal differences could play a role.

In this study, soil fungi were negatively impacted by addition of commercial fertilizer and liquid fraction, but not whole manure. This refutes my hypothesis that lower P in the liquid fraction dairy slurry would stimulate fungal biomass. The *liquid* treatment increased overall microbial biomass (where fertilizer did not), but appeared to similarly negatively impact the soil fungal community (in contrast with manure). This is in contrast

to Bittman et al. (2005), who reported higher bacterial and lower fungal populations after whole manure applications to grassland and lower bacterial and fungal populations after commerical fertilization, leading to no overall difference in total microbial biomass compared with a control after six years of manure application. Bossio et al. (1998) found, consistent with my study, that organic inputs (vetch winter cover crop, manure, and seaweed fish powder) did increase fungal biomass after seven years in tomato fields. Thus, it seems clear that manure and organic matter inputs do not always affect the microbial community in the same way. Several studies have shown that inorganic N applied to the soil negatively impacts fungal biomass (Bittman et al., 2005; Q. C. Zhang et al., 2012), and the increased inorganic N fraction in the liquid fraction slurry manure may have contributed to this depression in fungal biomass in this treatment.

Microbial biomass was consistently positively correlated with total soil C, N, and P; this suggests that MBC and SOM are coupled in this study. The soil microbial community aids in the production of stable SOM by consuming easily-decomposed plant residues and exudates and creating humus (Condron et al., 2010). In a long-term study such as this one, the microbial biomass and the amount of soil C is likely in equilibrium, and thus they were strongly correlated. Microbial biomass nitrogen, bacterial biomarkers, and actinobacterial biomarkers were negatively correlated with the MWD of soil aggregates; this is slightly surprising, as others have found decreases in biomass with decreasing size of soil aggregates (e.g., in rice paddy fields in China (Chen et al., 2015)). However, it is possible that higher proportions of microaggregates associated with lower MWD provided an environment in which bacteria could thrive. Fungi were negatively associated with bulk density. Nadian et al., (1998) found that compaction in a pot

experiment decreased vesicular-arbuscular mycorrhizal fungi colonization of clover plants; thus, fungi were negatively impacted by soil compaction.Unlike biomass, microbial community as indicated by enzyme activity was not strongly impacted by nutrient management regime, or by soil properties. Enzyme activities found in this study were similar to those published in Brockett (2008) in the mineral layer of forest soils (<500 nmol/h/g soil). There are a wide range of enzyme activity rates reported in the literature, even in similar ecosystems. The current samples were taken 20 days after nutrient application. In contrast, samples taken from a 40-year-old grassland in southwest England 14 days after application of dairy slurry manure had β-glucosidase activities of 41.2 nmol/min/g soil (2,472 nmol/h/g soil; 70% higher than the control in my study), cellobiohydrolase activities of 7.2 nmol/min/g soil (432 nmol/h/g soil; 80% higher than my control), N-acetyl-β-glucosaminidase activities of 21 nmol/min/g soil (1,260 nmol/h/g soil, 105% higher than my control) and phosphatase activities of 133 nmol/min/g soil (7,980 nmol/h/g soil, 82% higher than my control) (Bol et al., 2003; Toyota and Kuninaga, 2006). Alster et al. (2013) also found much higher values than in my study for the activity of all enzymes sampled from litter bags in a California grassland (for example, 32,000 nmol/h/g dry litter of β-glucosidase, 21 times higher than the control in my study); higher values are to be expected in pure organic matter compared to mineral soil. In contrast, no-till corn and tallgrass praire had 88-93% lower activities of β-glucosidase and cellobioside than my study (153 and 31 nmol/h/g dry soil, respectively) in a loam soil in Iowa (collected in May) (Bach & Hofmockel, 2014). Thus, activity rates of enzymes in soil are highly variable and likely cannot be compared directly between ecosystems as a measure of ecosystem functioning. Similarly, there

were no strong correlations between microbial activity and any soil characteristics that I measured. The activity of cellobiosidase was positively correlated with soil C, N, and P, but the more labile β-glucosidase was not, suggesting that it is not controlled by total soil C (which includes non-labile forms of C). Similarly, the activity of phosphatase was not connected to soil P, or any of the other soil properties I measured, therefore it must be controlled by other factors at this site.

The results of my enzyme analyses were somewhat unexpected, even taking into account their variability, by the fact that inorganic fertilizer treatments increased the activity of C cycling enzymes without increasing microbial biomass. Many previous studies have shown correlations between enzyme activity and microbial biomass (Bohme et al., 2005; Peacock et al., 2001; Sinsabaugh et al., 2008; Tian et al., 2010). Instead, I found that enzyme activity was increased in the fertilizer treated plots, despite no increase in soil or microbial C. Extracellular soil enzyme activity may not be tied directly to microbial biomass, as the enzymes can bind to clay particles in the soil and remain active even after microbes have turned over; however, with the low clay content in our soil and the long-term management strategies, microbial biomass and enzyme activity have likely reached an equilibrium (Nannipieri et al., 2012; Stott et al., 2010). Rhizodeposition of labile C compounds (e.g., glucose and cellulose), could stimulate the production of C cyclying enzymes following fertilizer application as well as manure application, if they both stimulate grass growth (Burns et al., 2013). Alternatively, I did find small differences in the microbial community structure after fertilizer and manure application, specifically fungal:bacterial ratios. If C cycling enzymes are preferentially produced by bacteria, or a subset of bacterial species, their activity may not reflect the

overall microbial biomass. In fact, Brockett et al. (2012) found that β-glucosidase and cellobiosidase were correlated with Gram-positive bacteria and total bacteria in forest soils.

## 2.5. Conclusions

Forage plots receiving amendments of whole dairy manure (*manure),* liquid fraction dairy manure (*liquid)*, and a combination of whole manure and fertilizer (*combination*) had increased soil microbial biomass over two years of sampling compared to commercial fertilizer *(fertilizer*) alone, or no amendment (*control*). Fungal:bacterial ratios were lower following fertilizer and liquid slurry than with manure, a combination of manure and fertilizer, or no amendment. Enzymes involved in C cycling were increased following all treatments (except the *control*), but no other enzymes (those degrading compounds containing N, S or P) were impacted by nutrient amendments in this study. While there were some changes to the microbial community structure, in particular a negative effect of commercial fertilizer on fungal communities, seasonal differences were in many cases larger than differences among treatments.

# 3. Nitrogen Dynamics and Relationships with Soil Microbial Communities on Tall Fescue in Agassiz, British Columbia After Long-Term Contrasting Nutrient Management Treatments

## 3.1. Introduction

Manure is a valuable source of plant nutrients, but the complex composition of nutrients within manure means that some nutrients will be more available than others. For example, N in manure is typically about 50% organic and 50% inorganic (mostly ammoniacal N ($NH_4^+$)). Therefore, to get equivalent yield responses to inorganic fertilizer (for example, ammonium nitrate ($NH_4NO_3$)), manure must be applied at a higher total N rate (Bittman et al., 2007). This could lead to a buildup of organic N in the soil, overapplication of other nutrients (for example, phosphorus (P) in dairy manure tends to exceed crop requirements), or mineralization of organic N at inopportune times (e.g., non-growing season). This could also be a benefit; increased soil organic N may be mineralized and provide a slower, more resilient pool of fertilization in the long term. Due to the challenge of providing adequate mineral N from manure when the plant needs it, farmers in the Fraser Valley of British Columbia often supplement manure applications with mineral fertilizer, despite having an overabundance of organic N sources available in the region.

Manure application to soil, with additional nutrients, affects the soil microbial community (see Chapter 2). After manure application, there is more microbial N as well as total N in the soil (Chapter 2), which may impact microbial activity.

Potential changes in organic N pools, through addition of organic matter (application of carbon (C) through manure), and quantities of inorganic N, may influence N loss

pathways. $NO_3^-$ leaching into aquatic ecosystems and $N_2O$ or $N_2$ emissions are both concerns, especially with high annual precipitation in the Fraser Valley. $NO_3^-$ is highly soluble, and therefore any $NO_3^-$ not taken up by crops, in particular during the non-growing season is vulnerable to leaching. Studies have shown that dairy manure applications often increase $NO_3^-$ leaching more than fertilizer applications. Nitrate leaching has been shown to be higher in dairy manure than fertilizer applications in annual crops due to the mineralization of organic N from manure (Basso and Ritchie, 2005; Stoddard et al., 2005); though Di et al. (1999) found in New Zealand under perennial grassland that $NO_3^-$ leaching was greater after fertilizer applications than manure applications (applied at 400 kg/ha annual total N, the same as my study), likely due to increased microbial N immobilization after manure applications.

Nitrous oxide emissions through denitrification is another pathway of N loss that has been traditionally investigated through environmental controls such as temperature, moisture and oxygen ($O_2$) availability. Chantigny et al. (2010) found contrasting responses of $N_2O$ emissions to different sources of N in different types of soils; in clay soils, $N_2O$ emissions were higher after mineral fertilizer application than manure application, but in loam soils, the opposite was true, which may have been due to differences in moisture or $O_2$ availability, but also may have been due to higher organic matter content, which provides substrates which allow for higher microbial activity, even without organic manure applications. In a corn trial in Agassiz, BC, Paul & Zebarth (1997b) also found increased $N_2O$ emissions from manure compared with fertilizer plots. However, on grassland fields on volcanic soils in Japan (with a humid contenintal climate), the opposite was true (Jin et al., 2010). Increasingly it is being recognized that

there are multiple pathways of $N_2O$ production by different organisms, all of which are controlled by different environmental factors. Baggs (2011) concluded that may organisms not previously thought to denitrify may be capable of it under certain circumstances. For example, ammonia-oxidizing bacteria may be able to release $N_2O$ through both nitrification (ammonia oxidation) and denitrification, especially in conditions of high $NH_4^+$. Fungi, as well as bacteria, may contribute to $N_2O$ emissions at low soil pH (Baggs, 2011; Herold et al., 2012). Recent advances in molecular techniques and selective inhibition studies have elucidated new microbial groups which may be able to denitrify, or produce $N_2O$ through other pathways. Fungi (Jirout et al., 2013), actinomycetes (Shoun et al., 1998), and archaea (Schauss et al., 2009) may all contribute to $N_2O$ production, although the conditions controlling each group are not yet fully understood. Fungi may also be able to denitrify using a process known as co-denitrification, which combines an N atom from another source (e.g., amino acids or even $NH_4^+$) with $NO_3^-$ to produce $N_2O$ (Baggs, 2011). Fungi have been shown to denitrify in forest and grassland soils, suggesting that they may have a more important impact on $N_2O$ production than previously thought (Baggs, 2011). They have not been shown to produce the enzyme reducing $N_2O$ to $N_2$, which means that all fungal denitrification results in $N_2O$ production. Most studies on denitrifier populations have focused on non-agricultural forests or meadows (Tian et al., 2013), annual crops ( Miller et al., 2009) or bare soil in incubations (Herold et al., 2012; Jirout et al., 2013; Shoun et al., 1998). The extent to which different microbial groups contribute to $N_2O$ emissions in fertilized and manured soils under perennial grass is currently unknown.

In this study I attempted to relate soil microbial populations to N pools, fluxes and losses ($N_2O$ emissions and $NO_3^-$ leaching) in soils growing forage grass that has had long-term applications of various nutrient management options: un-fertilized; $NH_4NO_3$ fertilizer; whole slurry manure; a combination of fertilizer and manure, the liquid fraction of manure only; and a control (no nutrient additions). I quantified important soil N pools, as well as N losses to the environment, and explored relationships between these pools/losses and microbial community structure and activity. My hypotheses were (1.) The size of organic soil N pools (total soil N, microbial biomass N), and rates of transformation (net N mineralization and nitrification, $NO_3^-$ released onto resin strips) will be higher after whole and liquid fraction dairy slurry than after fertilizer applications and a combination of fertilizer and manure; though all will be higher than in a control, (2.) Rates of $N_2O$ emissions and $NO_3^-$ leaching will be lower after applications of whole and liquid fraction dairy slurry than applications of fertilizer and a combination of fertilizer and manure, but all will be higher than in a control, and (3.) Microbial community structure and activity, and specific microbial groups, will be correlated with rates of N transformation and loss.

## 3.2. Materials and Methods

### 3.2.1. Study Site, Soil Physical and Chemical Analysis, and Biological Measurements

This study was carried out on a stand of tall fescue (*Fetusca arundinacea*) established in 2002 in Agassiz, BC. Five treatments were included in this study, replicated four times in a randomized complete block design: unfertilized control (*control*); whole dairy slurry manure (*manure*); separated liquid dairy slurry (*liquid*); $NH_4NO_3$ fertilizer

(*fertilizer*); and a combination whole manure and fertilizer (*combination*) (see Chapter 2 for details). Soil samples collected from 0-15 cm depth in June, August and October 2013, and June and July 2014, were analysed for pH, total soil C and N, bulk density, mean weight diameter (MWD) of stable aggregates, phospholipid fatty acid (PLFA) content, and enzyme activity, as described in Chapter 2. PLFA concentrations will be used in correlations with N dynamics from this chapter.

### 3.2.2. Nitrogen Pools and Transformations

*Soil Nitrogen Pools*

Total soil N values were determined by dry ash combustion in a LECO CNS200 C and N analyzer (Thiessen and Moir, 1993) on samples collected in June 2013. Inorganic N ($NH_4^+$, $NO_3^-$) was determined from a subset of the soil samples collected at each date (as described in Chapter 2) by extracting 10 g of field-moist samples with 50 mL of 2 *M* KCl. Samples were shaken for 1 h and passed through 11 cm diameter Whatman No. 42 filter paper. The collected extracts were analyzed for $NH_4^+$-N and $NO_3^-$-N on an FIAStar 500 Flow Injection Analyzer (FOSS Analytical, Denmark).

Soil microbial N (as well as C) was determined with the fumigation-extraction procedure (Vance et al., 1987). Initial N extracted with 0.5 *M* potassium sulfate ($K_2SO_4$) was identified as salt-extractable N or soluble organic N.

*Soil Nitrogen Transformations*

Net rates of mineralization and nitrification were determined using the buried-bag technique (Hart et al., 1994). At each sampling date, a 100-150 g composite sub-sample of field-moist soil from each plot was placed into a polyethylene bag and buried

for a period of 21 days (except for the October date, which due to lower temperatures was buried for 153 days) at a depth of 15 cm at a random location at the edge of the trial area. To extract inorganic N, 10 g of soil pre- and post-incubation was placed in a 100 mL Erlenmeyer flask, 50 mL 2 *M* KCl added and the suspension shaken for 1 h, and passed through a filter (Whatman No. 42). The samples were analyzed for nitrate ($NO_3^-$) and ammonium ($NH_4^+$) on an FIAStar 500 Flow Injection Analyzer (FOSS Analytical, Denmark). Net mineralization rates (mineralization – immobilization) were taken as the difference between inorganic N ($NO_3^-$ and $NH_4^+$) at the beginning and end of the incubation. Net nitrification rates were taken as the difference in nitrate ($NO_3^-$) at the beginning and end of the incubation, assuming no denitrification.

Finally, anion resin strips (Binkley and Matson, 1983) were buried in the soil for 2-week periods as an indication of potential cumulative anion availability for root uptake, initiated in all treatments in October 2013 and reported from October 2013 – February 2014. Anion resin strips were cut into strips with surface area of approximately 130 $cm^2$ and buried deep enough to ensure good soil contact (approximately 5 cm) in the soil for two-week periods, where they adsorbed $NO_3^-$ as it was released into the soil and accumulated adsorbed $NO_3^-$ over the sampling period. Following removal from the soil, $NO_3^-$ was extracted from the resin strips with 2 *M* KCl and analyzed for $NO_3^-$ on an FIAStar 500 Flow Injection Analyzer (FOSS Analytical, Denmark). Values are reported as $NO_3^-$-N/$cm^2$/day and dates are reported as the midpoint in the 2-week sampling period.

### 3.2.3. Nitrogen Losses

*Greenhouse Gas Emissions*

In 2013 and the early winter/spring of 2014, greenhouse gas fluxes were measured using static chambers according to methods outlined in Bhandral et al. (2008). Square (60x60cm) collars were inserted approximately 3 cm into the soil immediately after each manure application (leaving a collar height of approximately 8-10 cm). Chamber heights were measured at least one week after collar insertion to allow for settling. To measure fluxes, lids of the same dimension and 2.7cm high were placed into channels on the top of each collar. These channels were filled with water to form an airtight seal. To minimize any pressure build-up inside the chamber, the chambers were vented and the vents had a small piece of tubing to prevent ambient air from mixing with the chamber air. Ten mL air samples were pulled through a septum in the chamber lid with a syringe every 10 minutes for half an hour and injected into pre-evacuated glass vials for storage until analysis. At the time of sampling, reference standards were injected into glass vials, taken to the field, and analyzed with the samples to account for any storage/transportation issues. In addition, three ambient samples were taken from the field at the time of sampling. The samples were analyzed for $N_2O$, carbon dioxide ($CO_2$), and methane gas ($CH_4$) on a gas chromatograph. During the growing season in 2013, plots were sampled for GHG emissions once/day for three days following each soil sampling event. In the fall/winter of 2013-2014, plots were measured bi-weekly.

*Nitrogen Leaching*

Estimates of dissolved N that may be leached from the field were measured using porous ceramic suction cup lysimeters. Lysimeters were installed to a depth of 45cm in each plot, and vacuumed and pumped bi-weekly from May 2013-August 2014. In the summer of 2013, lysimeters were only present in control, manure and liquid plots; lysimeters were installed in the combination and fertilizer plots in August/September of 2013, and were not sampled until October. Samples were immediately frozen and kept at -20$^{o}$C until analysis for $NH_4^+$ and $NO_3^-$ (see above). Where N levels were higher than calibration standards (<10 mg N/L), samples were diluted by a factor of 5-10 and rerun. Levels of N are reported as mg $NO_3^-$N/L or mg $NH_4^+$-N/L.

### 3.2.4. Statistical Analysis

All analyses were carried out using R statistical software (R Foundation for Statistical Computing, 2014). Differences in total soil N, MBN, and soil $NO_3^-$ and $NH_4^+$ were analyzed using a linear mixed effects model, R package lmerTest with treatment and date as fixed effects and repetition as a random effect. An ANOVA was used to determine significant effects and where differences were found, separations were investigated using Tukey's test. Data was tested for normality using the Shapiro-Wilk test, and where not normal was log-transformed. Mixed models were tested for normality of residuals using function qqnorm in R. This did not always meet normality requirements (nitrous oxide and N leaching data were not normal). In these cases, seasonal averages were taken. Values were averaged for each treatment for the periods between manure applications; the early summer (May – July), late summer (July – October) of 2013 and 2014 and over the fall/winter (October – May) of 2013. As

the sampling intensity for $N_2O$ and $NO_3^-$ leaching was designed to capture peak activity, it was disproportionate annually, but consistent across treatments. Therefore, averages are not representative of annual rates of losses, but they can be compared across treatments to evaluate relative fluxes.

Spearman correlation coefficients were calculated between non-transformed variables using R package Hmisc for analysis of statistical significance.

## 3.3. Results

### 3.3.1. Soil Nitrogen Pools and Transformations

*Soil Nitrogen Pools*

Plots receiving manure (*manure*, *liquid*, and *combination*) contained more total soil N than *control* plots. Total soil N was 17% higher in the *manure* and *liquid* treatment and 21% in the *combination* treatment than the *control* ($p<0.05$). The *combination* was also significantly higher (16%) than the *fertilizer* plots ($p<0.01$) (Table 4). As presented in Chapter 2, microbial biomass N (MBN) was also significantly higher in the *manure, combination* and *liquid* treatment (48%, 50%, and 36% higher than *control*, respectively) (Table 4).

Soil $NO_3^-$ and $NH_4^+$ concentrations were significantly affected by both treatment and date, with interaction ($p<0.05$) (Figure 9). Soil $NH_4^+$ values were not consistent and ranged from 0.60 – 17.53 mg $NH_4^+$N/kg soil (most values falling from 1-5 mg/kg soil). In 2013, and particularly in August of 2013, soil that had *fertilizer* additions had the highest $NH_4^+$ concentrations (Figure 9). Soil $NO_3^-$ concentration was significantly higher in the *combination* and *fertilizer* treatments than all other treatments, across all 5 dates.

*Manure* and *liquid* treatments had significantly higher $NO_3^-$ concentration than *control* ($p<0.05$). In June and July of 2013, as well as June 2014, the *liquid* treatment had significantly higher $NO_3^-$ concentration than *manure* plots ($p<0.05$). Values of $NO_3^-$ ranged from 0-49mg $NO_3^-$-N/kg soil. Soil contained 7 times higher $NO_3^-$ concentrations than $NH_4^+$ after *fertilizer* addition, 10 times higher after *combination,* and 3 times higher after *liquid*. Soil receiving *manure* had, on average, approximately equal concentrations of $NO_3^-$ and $NH_4^+$; in the *control* plots, $NO_3^-$ concentration was 14x lower than $NH_4^+$ concentration.

*Soil Nitrogen Transformations*

There were no significant differences in net N mineralization rates among treatments (see Appendix Figure 27). For net nitrification rates, only date of sampling had a significant effect ($p <0.05$), with nitrification (and mineralization) values being lowest in October of 2013 (Figure 10). Apparent negative net nitrification in plots receiving fertilizer and manure/fertilizer combination treatments in October of 2013 were likely due to very high initial concentrations of nitrate in these samples, resulting in overall uptake of nitrate by microorganisms (or, potentially, loss through denitrification).

In the winter of 2013, $NO_3^-$ adsorbed onto resin strips was much higher in the *fertilizer* and *combination* treatments, however again the ANOVA had a treatment interaction with the date of sampling (Figure 11). When averaged across winter 2013, $NO_3^-$ availability in soil was highest following applications of *fertilizer* and the *combination*, followed by *manure* and *liquid*, and the lowest $NO_3^-$ availability was in the *control* plots ($p<0.05$).

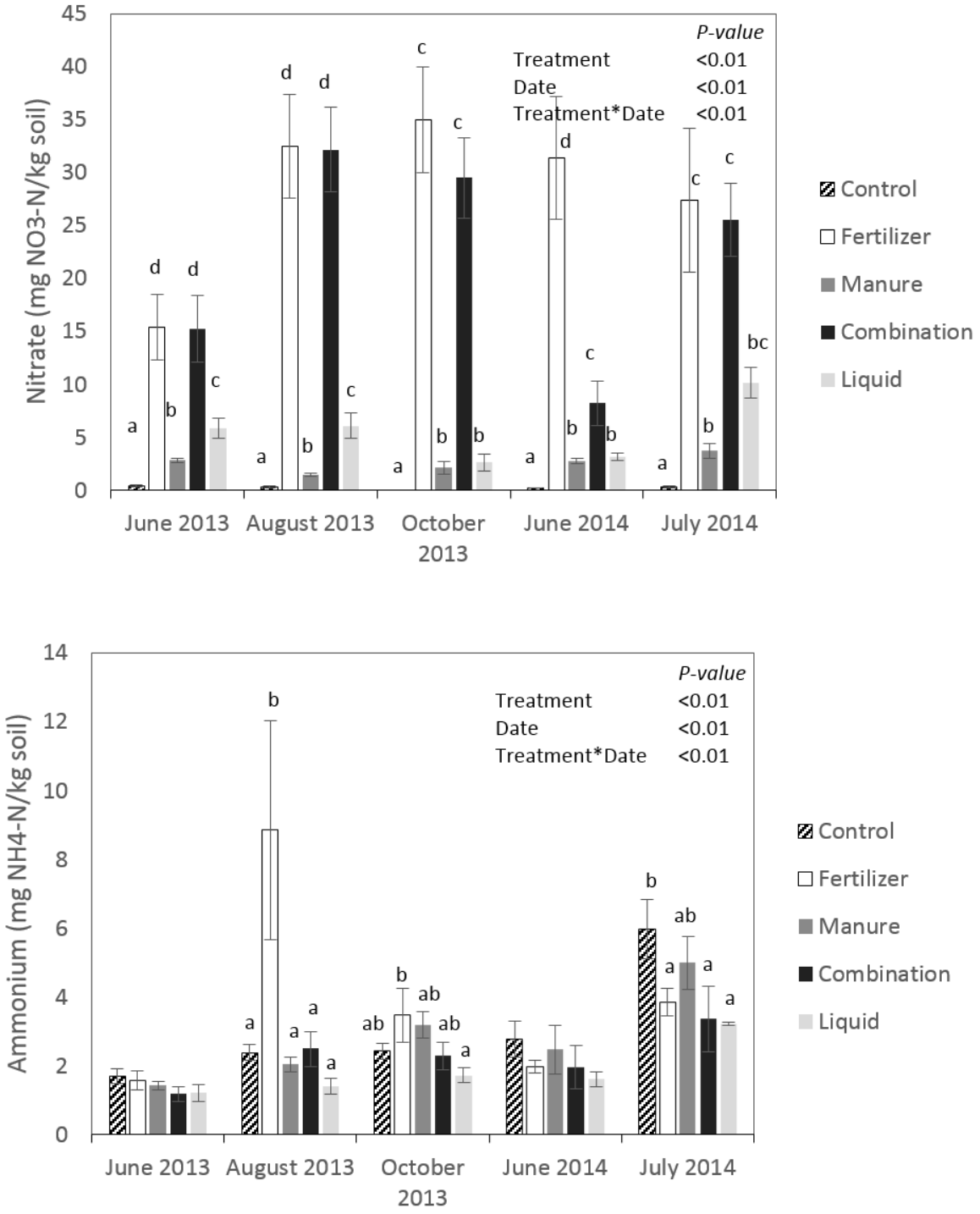


**Figure 9** Concentrations of soil nitrate (A) and ammonium (B) (mg N/kg soil) obtained from soil extracts in 2013 and 2014. Bars represent treatment means (n=4) ± one standard error. Treatments with the same letter are not significantly different ($p<0.05$).

**Table 4** Soil nitrogen pools and transformations in 2013 and 2014 after long-term application of fertilizer, combination, liquid fraction, and no amendment; n=20 for all variables. ANOVAs on soil $NO_3^-$/$NH_4^+$ at individual dates (due to treatment*date interaction), as well as averaged winter resin strip extraction (due to non-normality of residuals in the original model), were run with only treatment as a fixed effect, therefore Date and Treatment*Date effects are not applicable (N/A). Values in bold represent significance at $p<0.05$.

| Variable | Treatment | | Date | | Treatment*Date | |
|---|---|---|---|---|---|---|
| | F | p-value | F | p-value | F | p-value |
| Total Soil Nitrogen | **6.54** | **<0.01** | N/A | N/A | N/A | N/A |
| Microbial Biomass Nitrogen | **9.37** | **<0.01** | **11.94** | **<0.01** | 1.78 | 0.07 |
| Soil Nitrate | **356.42** | **<0.01** | **7.31** | **<0.01** | **6.68** | **<0.01** |
| June 2013 | **81.55** | **<0.01** | N/A | N/A | N/A | N/A |
| August 2013 | **71.71** | **<0.01** | N/A | N/A | N/A | N/A |
| October 2013 | **110.36** | **<0.01** | N/A | N/A | N/A | N/A |
| June 2014 | **65.51** | **<0.01** | N/A | N/A | N/A | N/A |
| July 2014 | **50.52** | **<0.01** | N/A | N/A | N/A | N/A |
| Soil Ammonium | **9.51** | **<0.01** | **25.87** | **<0.01** | **2.62** | **<0.01** |
| June 2013 | 2.93 | 0.06 | N/A | N/A | N/A | N/A |
| August 2013 | **10.13** | **<0.01** | N/A | N/A | N/A | N/A |
| October 2013 | 3.22 | 0.05 | N/A | N/A | N/A | N/A |
| June 2014 | 1.31 | 0.32 | N/A | N/A | N/A | N/A |
| July 2014 | **4.92** | **0.01** | N/A | N/A | N/A | N/A |
| Resin Strip Nitrate | | **<0.01** | | **<0.01** | | **<0.01** |
| Winter 2013 Average Resin Strip | **36.07** | **<0.01** | N/A | N/A | N/A | N/A |

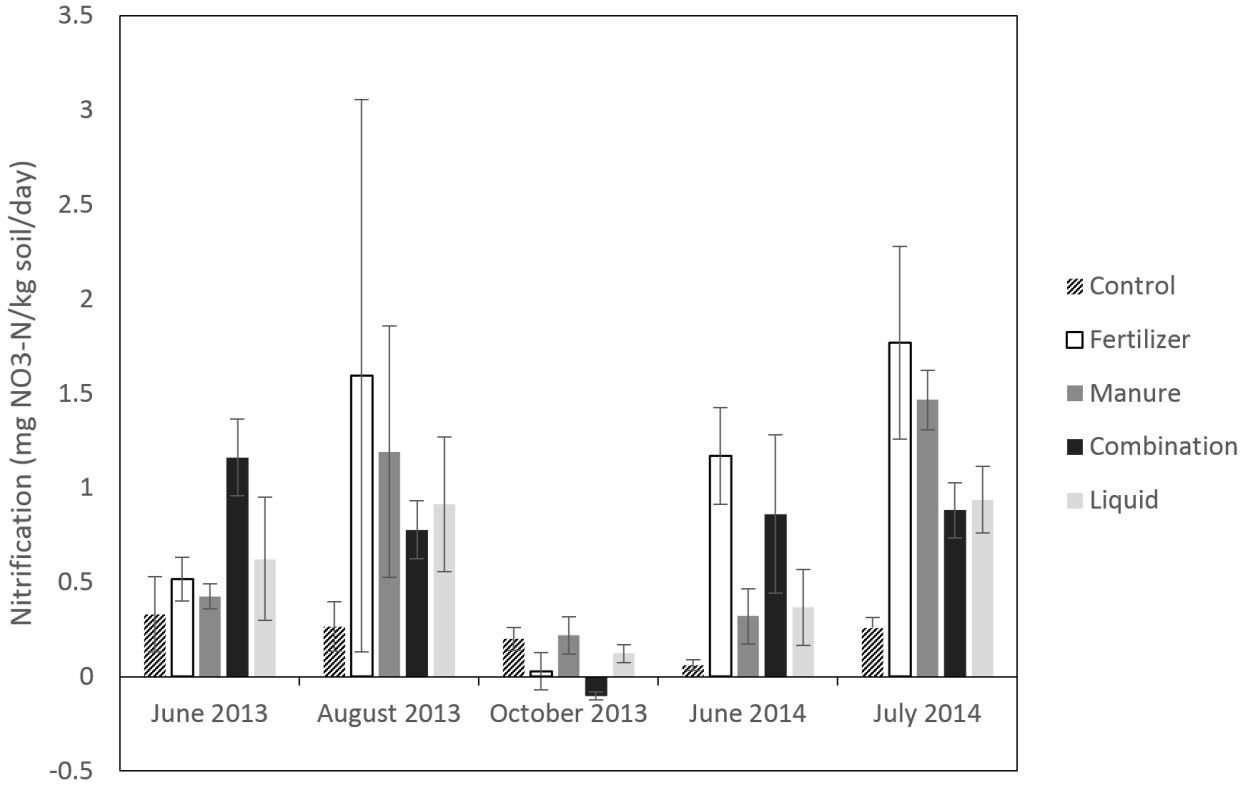


**Figure 10** Net nitrification rates (mg $NO_3$-N/g soil/21 days) in soils in control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) plots over 1 year. Treatments with the same letter are not significantly different ($p<0.05$).

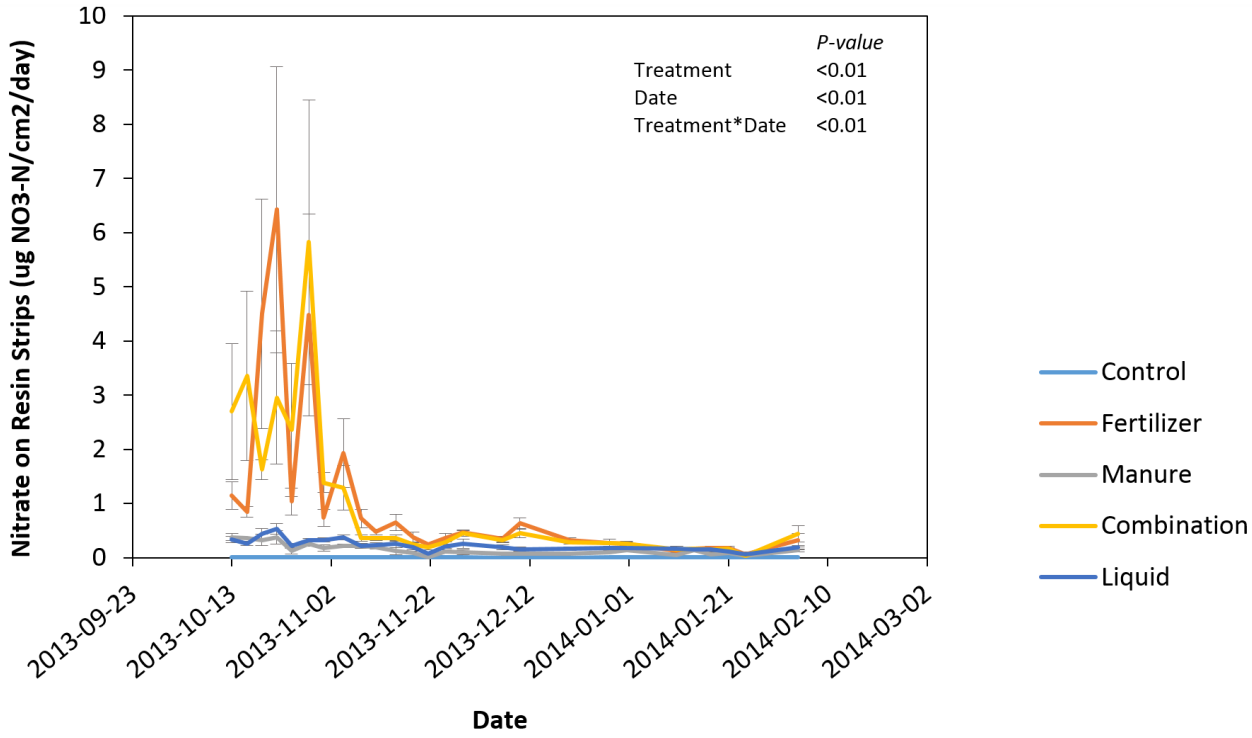


**Figure 11** Nitrate collected on resin strips (µg $NO_3$–N/$cm^3$/day) measured over the winter of 2013. Lines represent mean (n=1-4) of treatments ± one standard error.

### 3.3.2. Nitrogen Losses

*Greenhouse Gas Emissions*

In general, nutrient applications that included inorganic N (*fertilizer* and *combination*) had higher $N_2O$ emissions. Both treatment and date were significant for $N_2O$ losses from the soil, and there was a significant interaction among the terms ($p<0.05$). When averaged for the three-day sampling periods in the early and late summer 2013, *fertilizer* and the *combination* had the highest emissions, followed by *liquid* ($p<0.05$) (Figure 12). In the winter of 2013/2014, the *combination* had the highest emissions, and all other amendments were not different from each other (though still higher than the control) ($p<0.05$).

**Table 5** Potential N losses ($N_2O$ emissions, $NO_3^-$ leachate concentrations in lysimeters) over 2 years from control, fertilizer, combination, and liquid fraction. ANOVAs on averaged seasonal $N_2O$ and $NO_3^-$ leachate (run due to non-normality in residuals of original models) were run with only treatment as a fixed effect, therefore Date and Treatment*Date effects are not applicable (N/A); n=20. Values in bold represent significance at $p<0.05$.

| Variable | Treatment | | Date | | Treatment*Date | |
|---|---|---|---|---|---|---|
| | F | p-value | F | p-value | F | p-value |
| Nitrous Oxide | | **<0.01** | | **<0.01** | | **<0.01** |
| Early Summer 2013 Average Nitrous Oxide | **43.32** | **<0.01** | N/A | N/A | N/A | N/A |
| Late Summer 2013 Average Nitrous Oxide | **9.01** | **<0.01** | N/A | N/A | N/A | N/A |
| Winter 2013 Average Nitrous Oxide | **37.14** | **<0.01** | N/A | N/A | N/A | N/A |
| | | | | | | |
| Nitrate from lysimeters | | **<0.01** | | **<0.01** | | **<0.01** |
| Early Summer 2013 Average Nitrate | **3.05** | **0.1** | N/A | N/A | N/A | N/A |
| Late Summer 2013 Average Nitrate | **7.72** | **0.02** | N/A | N/A | N/A | N/A |
| Winter 2013 Average Nitrate | **31.1** | **<0.01** | N/A | N/A | N/A | N/A |
| Early Summer 2014 Average Nitrate | **1.11** | **0.4** | N/A | N/A | N/A | N/A |
| Late Summer 2014 Average Nitrate | **2.49** | **0.09** | N/A | N/A | N/A | N/A |

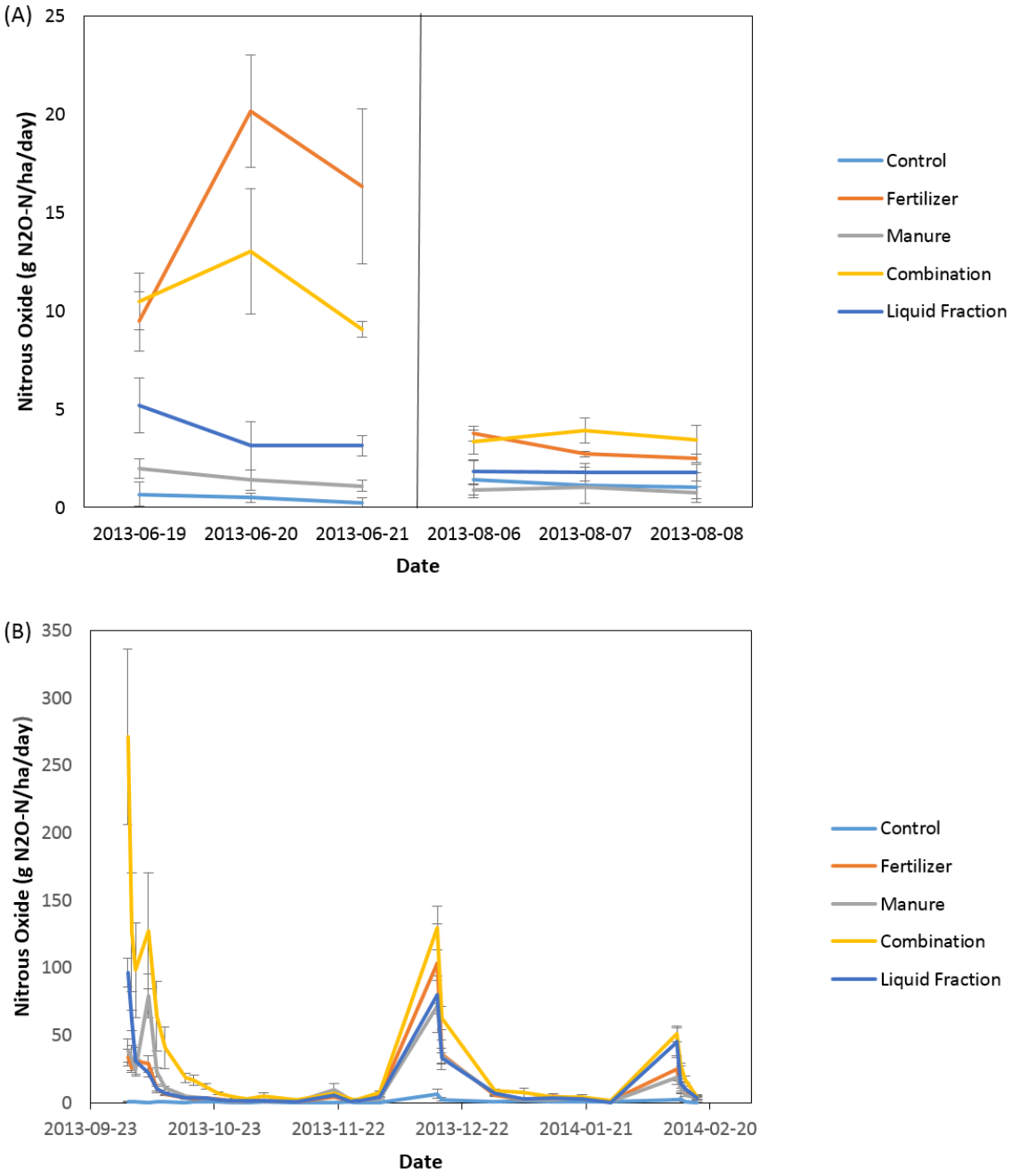


**Figure 12** Nitrous Oxide emissions (g $N_2O$-N/ha/day) measured in (A) summer 2013 and (B) winter 2013. Lines represent mean (n=4) of treatments, and error bars are ± one standard error.

*Nitrogen Leaching*

Both treatment and date had a significant effect on the concentration of $NO_3^-$ found in the soil solution, as measured using the suction cup lysimeters. Again, there was a significant interaction between treatment and date of sampling ($p<0.05$). Data from the summer of 2013 was analyzed separately, as two treatments (*fertilizer* and *combination*) were missing from these dates. In early summer 2013, there were no significant differences in $NO_3^-$ leaching from the different treatments, but in late summer 2013 the *liquid* had more $NO_3^-$ leachate than *control* and *manure* ($p<0.05$) (Figure 13). When averaged across the winter of 2013, the *combination, fertilizer*, and *liquid* had more $NO_3^-$ leachate than the *control*, with *combination* and *fertilizer* the highest ($p<0.05$) (Figure 13). There were no significant differences between $NO_3^-$ leaching from different treatments in the summer of 2013, likely due to low rainfall and low volumes of leachate.

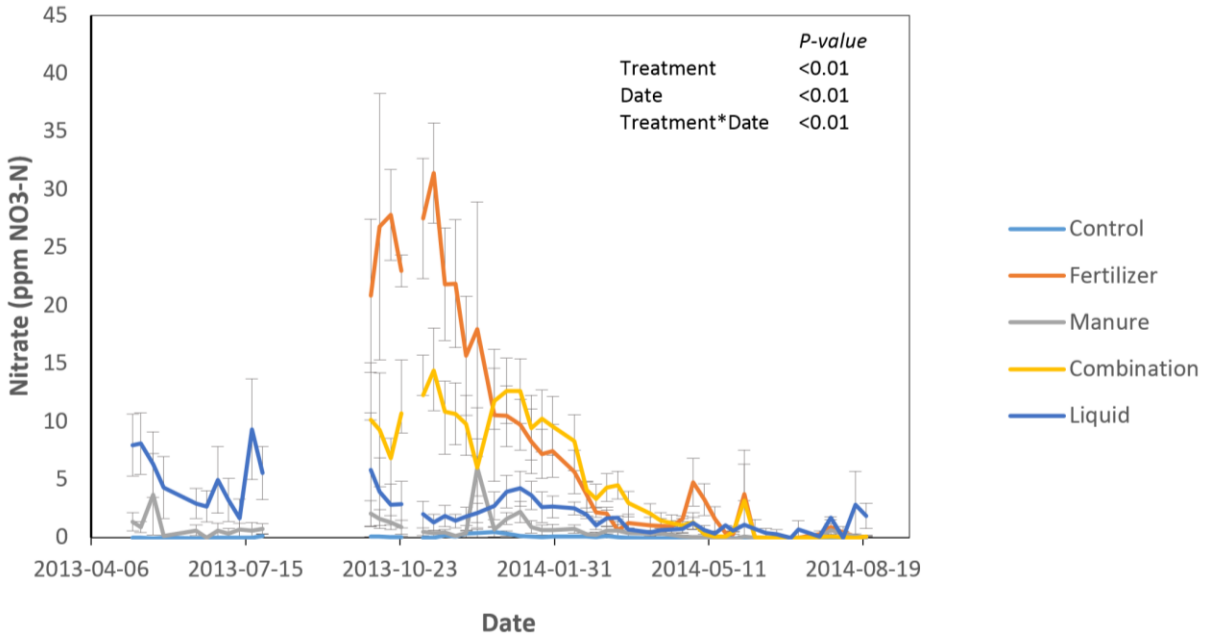


**Figure 13** Nitrate (ppm) retrieved from lysimeter water measured over one year. In the summer of 2013, fertilizer and combination were not measured. Lines represent mean (n=1-4 due to samples missing on some dates) of treatments, and error bars are ± one standard error. The gap between July and October is due to dry soils and no leaching would have occurred. Leaching proceeded after that dry period.

### 3.3.3. Relationships Between Microbial Community Structure and Nitrogen Transformations

I found limited evidence of correlations between microbial community structure and N pools, transformations and losses. MBC and MBN were significantly positively correlated with total soil N across all dates (except for winter 2013, when they were not measured) (Figures 14-16). MBN was negatively correlated with soil $NH_4^+$ in early summer, but positively correlated with soil $NO_3^-$ and soluble N in late summer 2013. In early summer 2014, both MBC and MBN were negatively correlated with net N mineralization and nitrification (Figure 16).

Soil $NH_4^+$ had generally negative relationships with MBC, MBN, actinobacteria and total bacteria. Gram-positive bacteria were positively correlated with $NH_4^+$ in 2013, but

negatively correlated in 2014. In contrast, Gram-negative bacteria were negatively correlated in late summer 2013 but positively (NS) correlated in 2014. Soil $NO_3^-$, similarly, was correlated only with Gram-positive bacteria only at one date, this time in early summer of 2013. Microbial biomass N was positively correlated with $NO_3^-$ in late summer 2013; but uncorrelated across all other dates.

In terms of $N_2O$ emissions, there was no consistent relationship with overall microbial biomass. However, total bacterial abundance was consistently positively correlated with $N_2O$ emissions (significant in early summer 2013) and actinobacteria (significant in early summer 2013; no relationship in late summer 2013) (Figure 14, Figure 15). $N_2O$ emissions were generally negatively correlated with fungal biomarkers (significant in early summer 2013 and fall/winter 2013). $NO_3^-$ in the leachate (no3leach) was positively correlated with Gram-positive and total bacteria in early summer 2014, but not in late summer 2014 (Figure 16).

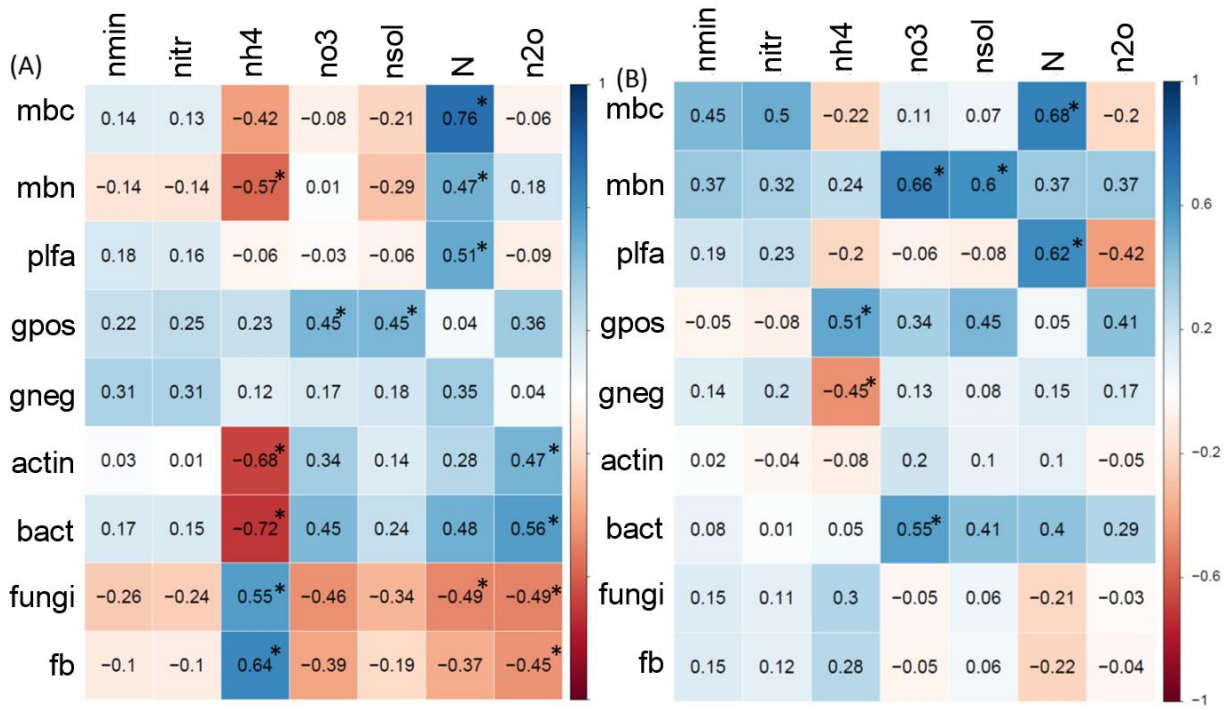


**Figure 14** 2013 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, and (B) late summer 2013. Variables included were microbial biomass carbon (mbc), microbial biomass nitrogen (mbn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin), bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil organic soluble N (nsol), and soil total N (N) in in mg N/kg soil, and $N_2O$ emissions (n2o) in g $N_2O$-N/ha/day. Values with stars reresent significant correlations ($p<0.05$).

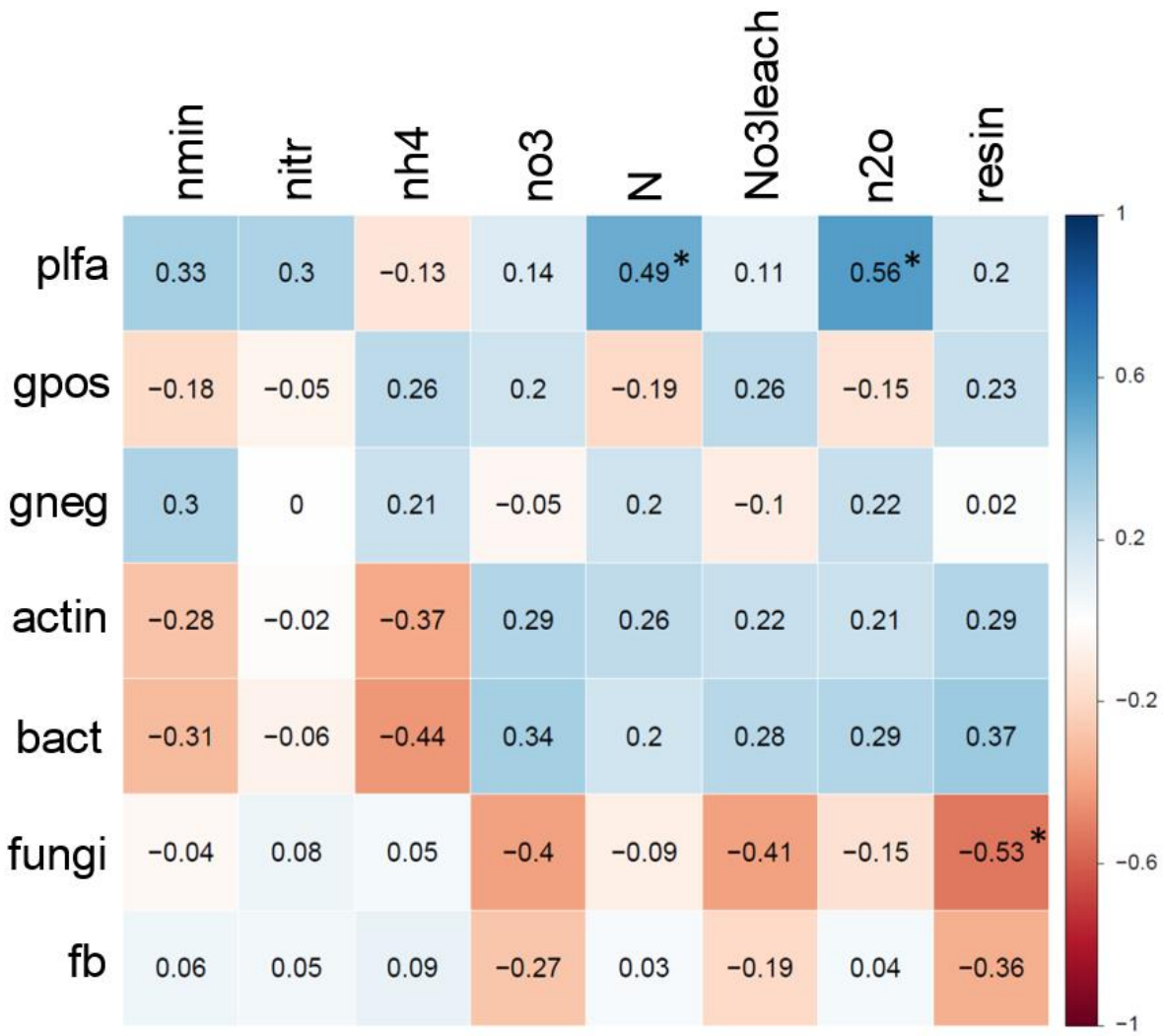


**Figure 15** Fall/winter 2013/2014 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R). Variables included were total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin), bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), and soil total N (N) in in mg N/kg soil, $NO_3^-$ in leachate (no3leach) in mg N/L, $N_2O$ emissions (n2o) in g $N_2O$-N/ha/day, and $NO_3^-$ taken up by resin strips (resin) in ug/$cm^2$/day . Values with stars rerpesent significant correlations ($p<0.05$).

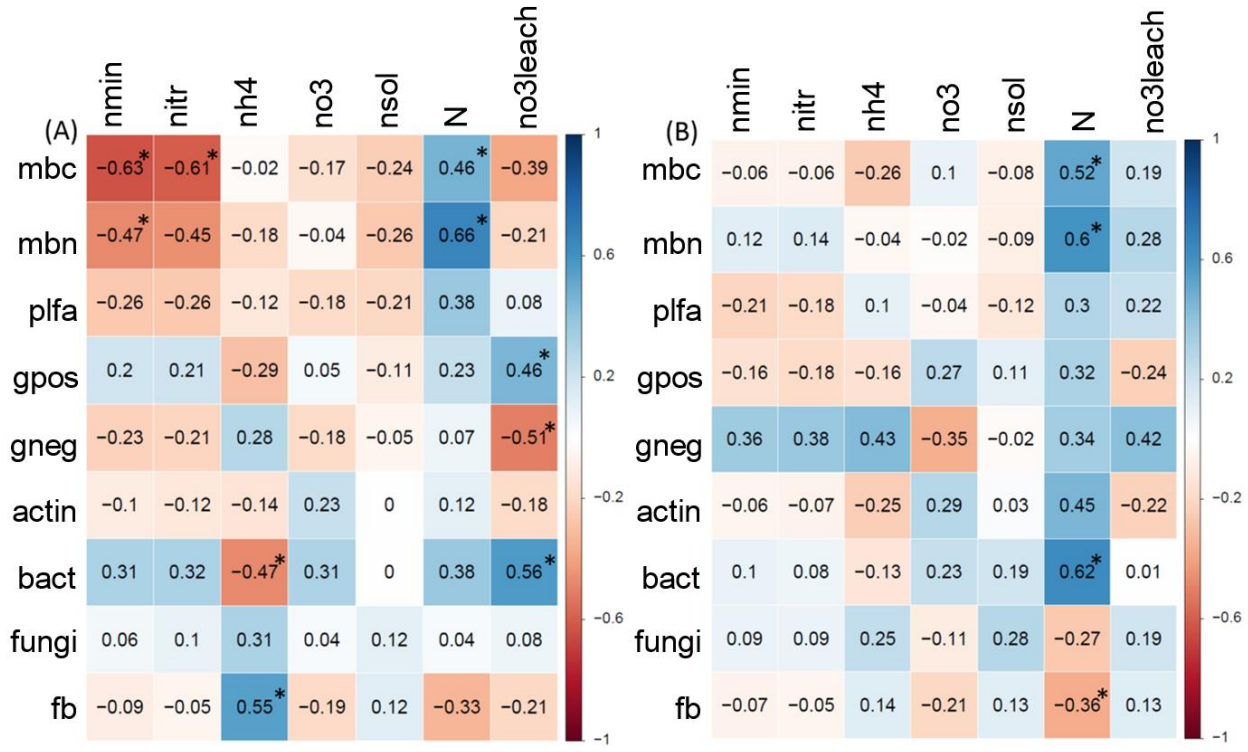


**Figure 16** 2014 Spearman's correlation matrices between microbial community structure and nitrogen pools and fluxes. Values represent positive or negative correlation coefficients (R) for (A) early summer 2014, and (B) late summer 2014. Variables included were microbial biomass carbon (mbc), microbial biomass nitrogen (mbn), total PLFA biomass in nmol/g soil (plfa), and biomarkers in mol % (Gram-positive (gpos), Gram-negative (gnet), actinobacteria (actin), bacteria (bac), fungi (fungi), and fungal:bacterial ratios (fb). Nitrogen variables included net nitrogen mineralization (nmin) and nitrification (nitr) in mg N/g soil/day, soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil soluble organic N (nsol), and soil total N (N) in in mg N/kg soil, and $NO_3^-$ in leachate (no3leach) in mg N/L. Values with stars reresent significant correlations ($p<0.05$).

Correlations between microbial activity and soil N pools were more consistent (Figure 17). N mineralization was related to soil N pools more strongly in the early summer (2013 and 2014) than other dates. In early summer 2013, N mineralization and nitrification were positively correlated with soil $NO_3^-$, soluble N, and total soil N. In early summer 2014, N mineralization and nitrification were positively correlated with only soil $NO_3^-$ and $NO_3^-$ leaching.

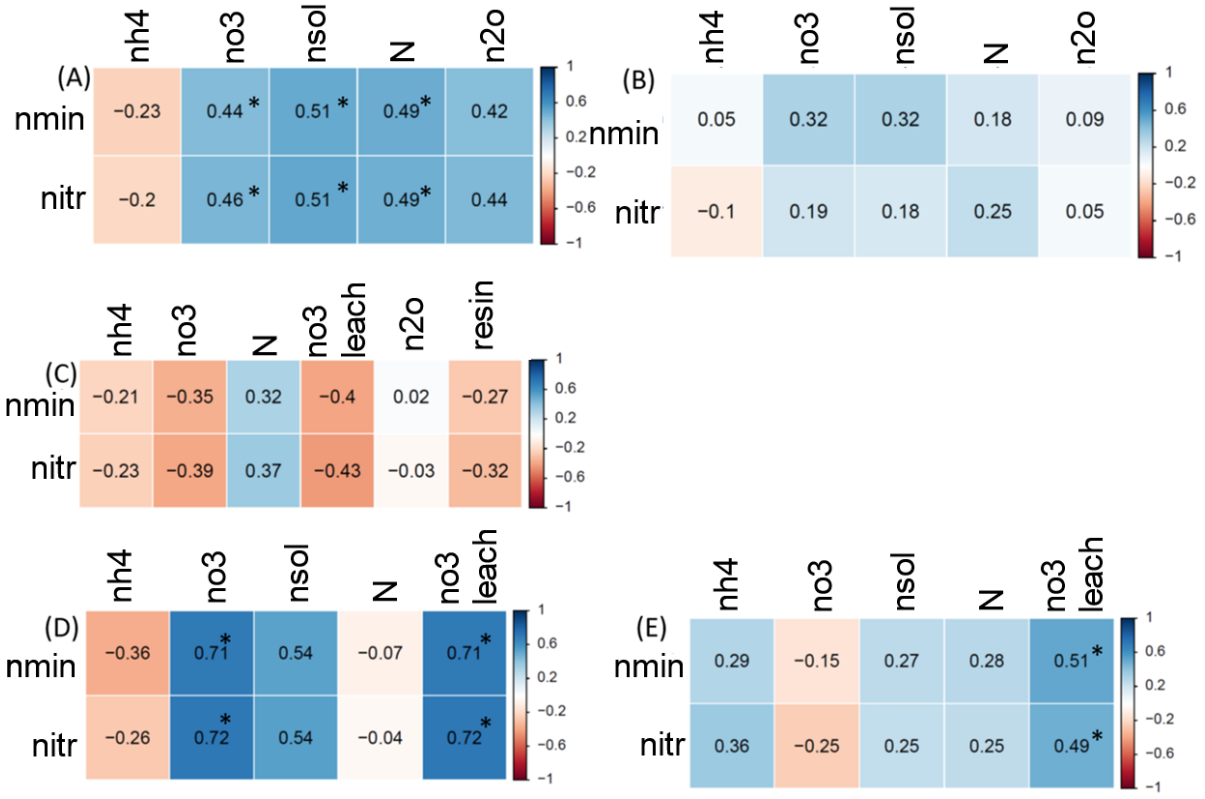


**Figure 17** Spearman's correlation matrices between microbial nitrogen fluxes and soil N pools. Values represent positive or negative correlation coefficients (R) for (A) early summer 2013, (B) late summer 2013, (C) fall/winter 2013/14, (D) early sumer 2014, and (E) late summer 2014. Variables included were net N mineralization (nmin) and nitrification (nitr) in mg N/g soil/day. Nitrogen variables included soil $NH_4^+$ (nh4) and $NO_3^-$ (no3), soil soluble organic N (nsol), and soil total N (N) in in mg N/kg soil, and $NO_3^-$ in leachate (no3leach) in mg N/L. Values with stars repesent significant correlations ($p<0.05$).

## 3.4. Discussion

My hypotheses about organic soil N pools being higher after applications of dairy slurry and liquid fraction dairy slurry than after fertilizer treatments were supported; both manure treatments increased total soil N and MBN. Despite lower applications of organic N from the *liquid* treatment there were no differences between *manure* and *liquid* in terms of N or MBN content. Inorganic pools of soil N also supported my hypotheses; highest values of soil $NO_3^-$ were found in the *fertilizer* plots (and *combination*), and *liquid* (with higher application of inorganic N) was higher than *manure*

in 2013. Most inorganic N was found as $NO_3^-$ in these soils, indicating that nitrification is usually more rapid than uptake by plants and microbes in this region with humid maritime climate. This was supported by the high correlation between nitrogen mineralization and nitrification in this study. Similarly, Zhang et al. (2012), in a grassland soil (Calcisorthic Aridisol according to the United States Soil Taxonomy classification) in Inner Mongolia, China, (mean annual soil temperature 5.3$^o$C, mean annual precipitation 401 mm) found that N mineralization rates and nitrification rates were closely connected, and concluded that there was a prevalence of nitrifying bacteria in their soils.

Rates of net mineralization and nitrification were not impacted by management (in contrast with my hypothesis), but showed only a date effect (being much lower in the winter period). N mineralization rates were much lower in a California grassland (0.03 ± 0.02 mg N/kg soil/day) compared with my values (0.54 ± 0.77 mg N/kg soil/day, across all treatments and dates) (in a much drier mediterranean climate, soil type Argixeroll according to the United States Soil Taxonomy classification) (Parker and Schimel, 2011). In a grassland in Inner Mongolia, China, ranges were more similar to my study (-0.08 to 0.52 mg N/kg soil/day; nitrification similar at -0.03 to 0.45 mg $NO_3^-$-N/kg soil/day) (Zhang et al., 2012). Both of these systems were in more arid conditions than those in my study, and had lower rates of N addition (annual precipitation ~700 mm/yr and 401 mm/yr vs. 1720 mm/yr, respectively; fertilization rates none and 20 to 40 kg urea-N/ha, respectively). Zhang et al. (2012) concluded that mineralization and nitrification were both controlled by temperature, and reached peak rates at the warmest part of the summer (July). Similarly, I saw that net rates of mineralizaton and nitrification were

lower over the winter period, when temperatures were lower. N mineralization and nitrification were consistently positively correlated with soil N pools ($NO_3^-$, total soil N) and $NO_3^-$ leaching.

In support of my N loss hypothesis, rates of potential N loss were higher for treatments with long-term fertilizer application than long-term manure application. Emissions of nitrous oxide ranged from 0-27 g $N_2O$-N/ha/day in early summer 2013, 0-5 g $N_2O$-N/ha/day in late summer 2013, and 0 – 451 g $N_2O$-N/ha/day in winter 2013. This is comparable with Bhandral et al. (2008) who found maximum emissions of 124 g $N_2O$-N/ha/day in the March-April period following application of whole dairy manure on bare soil or grass at the same study location (Agassiz, BC). $N_2O$ emissions were higher from soils receiving *fertilizer* than *manure* or *control* during the summer period with *liquid* being intermediate. In the winter, $N_2O$ emissions were equal in all treatments receiving nutrient amendments. In the summer of 2013, $N_2O$ emissions were correlated with soil $NO_3^-$ content particularly, suggesting soil $NO_3^-$ controlled $N_2O$ emissions at my sites during the summer. Despite fertilizer amended plots having relatively high soil $NO_3^-$ concentrations in October, there were no differences in winter $N_2O$ emissions between fertilizer and any manure treatments. In Quebec, loam soils were C-limited, and thus manure applications (with addition of organic matter) stimulated $N_2O$ production compared with fertilizer applications (Chantigny et al., 2010, 2007; P Rochette et al., 2008). However, on a clay soil in the same location with the same climate, $N_2O$ emissions were higher after fertilizer applications than manure applications (Chantigny et al., 2010). The clay soils had much higher organic C content than the loam soils; thus $N_2O$ was controlled by $NO_3^-$ content in the clay soil instead of C content as in the loam.

While my study was on a loam soil, I did not see that organic matter additions in the form of manure increased $N_2O$ emissions. Thus, C was likely not limiting in my soils; it may be that due to the perennial grass cover in this system, high amounts of labile C from plant roots stimulate $N_2O$ production in fertilizer as well as manure in my study. The Quebec studies were short-term (established <5 years previously) and on annual maize (Chantigny et al., 2010), or perennial crops established the year of sampling (timothy (Chantigny et al., 2007), and grass (P Rochette et al., 2008); thus they may not have the amount of organic matter additions from plants as my long-term study.

My hypothesis that $NO_3^-$ leaching would be lower from *manure* and *liquid* treatments than from *fertilizer* and *combination*, with all higher than *control,* was supported. My results were similar to those found by Di et al. (1999) in New Zealand pastures; I found that $NO_3^-$ leaching was higher under pastures receiving fertilizer. Concentration of $NO_3^-$ in leachate was also correlated to soil $NO_3^-$ content. In the summer of 2013, there was a higher concentration of lysimeter $NO_3^-$ in plots receiving liquid dairy slurry than in whole dairy slurry or the control. Low rates of precipitation in the summer (310 mm in 2013; 206 mm in 2014) mean that in the summertime $NO_3^-$ may not be leached to the depth of the lysimeters (45 cm), or lost from the system. The 45 cm depth of the lysimeters is likely below the root zone, and so $NO_3^-$ is unavailable for plant uptake at this depth; however, in order for this $NO_3^-$ to be actually leached from the system, there must be more precipitation to wash it away. In the winter, *fertilizer* and *combination* had a generally much higher concentrations of $NO_3^-$ in lysimeters than other treatments, with *liquid* intermediate. This may not be characteristic of all fertilizer application; the current rate (400 kg/ha/year) may be too high, or poorly timed with an application in October,

creating higher N loss than if it were better timed to match plant uptake. Higher rates of precipitation in the wintertime (1495mm in 2013; 1234 mm in 2014) make it very likely that $NO_3^-$ in the lysimeters in the winter was washed out of the system. Beckwith et al. (1998) found more $NO_3^-$- (mg/L) than my plots; even in unmanured soils, nitrate concentration was around 10 mg/L (compared to my 0-5 mg/L in control plots), and plots receiving manure had 60-80 mg/L of nitrate in the leachate on a sandy loam in the UK under fallow and winter rye. In contrast, in my soils, there was no significant increase in $NO_3^-$ leachate in manured over control plots, suggesting that any available $NO_3^-$ in the soil was either nitrified, denitrified, immobilized into microbial biomass, or taken up by the plants (Sørensen, 2004).

My third hypothesis, that microbial community characteristics could be correlated to certain soil N cycling processes was supported by several processes. Total microbial biomass was positively correlated with soil N; this could be driven by soil N (providing N sources to allow for a high microbial biomass), or higher MBC in the manured plots could allow more N to build up in the soil (Bittman et al., 2005).

In terms of soil nitrification, bacteria had negative relationships with $NH_4^+$ and positive relationships with $NO_3^-$, which may suggest a correspondence in total bacterial populations and the conversion of $NH_4^+$ to $NO_3^-$. However, here there were no significant relationships between bacteria and nitrification rates. Mineralization and nitrification rates did not appear to be correlated with any particular broad microbial groups that can be distinguished using PLFA (or total biomass), although they were correlated with soil N pools ($NO_3^-$, soluble N, and total soil N). This is likely an indication that the analysis did not have a high enough resolution to differentiate the key microbial

communities that mediate these processes such as bacterial nitrifiers in the soil. Although ammonia-oxidizing bacteria are Gram-negative bacteria, a study in coastal wetland soils in China found that, while total PLFA content was correlated with soil N, Gram-negative biomarker concentrations were not correlated with populations of ammonia-oxidizing bacteria (Jin et al., 2012). It may be that ammonia-oxidizing archaea, which have been shown to control nitrification in a variety of soils, were active in my soils; I did not have any measurements for soil archaea, but they could have had an impact on nitrification rates (Schauss et al., 2009).

$N_2O$ emissions were similarly uncorrelated with total microbial biomass. However, in terms of different microbial groups, I found that bacteria were generally positively correlated with $N_2O$ emissions, and fungi were either negatively correlated or uncorrelated. Therefore, it is unlikely that $N_2O$ production at my sites is controlled by fungal populations. In a clay loam Chernozem and a sandy loam Podzol in in Russia, Blagodatskiy et al. (2008) found that fungal:bacterial ratios impacted $N_2O$ production and consumption, and in particular that acidic soils (Podzols) had an inverse relationship of $N_2O$ to bacteria, potentially due to increased $N_2O$-reducing activity in these acid soils. Herold et al. (2012) found that fungal denitrification contributed 18% to total potential denitrification, and bacterial denitrification contributed 56%, by using selective inhibition of these microbial groups. They found no correlation between fungal and bacterial fatty acids and total denitrification; however, when bacterial denitrification was isolated (by inhibiting fungi) they found several fatty acids (a15:0,16:1ω7, 17:1ω8) were correlated. Thus, it is likely that a range of organisms are contributing to denitrification in my soils. In addition, as denitrifying organisms may take up only 5% of

the total microbial community (Philippot et al., 2007), it is possible that changes in denitrifying communities may be masked by changes in other microbial groups. In my soils, I do have an indication that bacterial populations may be correlated with (and controlling) $N_2O$ emissions, in agreement with the traditional models of denitrification (Hallin et al., 2009). This could be confirmed with selective inhibition studies investigating the relative contributions of bacteria or fungi, or through genetic work to isolate functional genes for denitrification.

### 3.5. Conclusions

N cycling dynamics in the soil were impacted by nutrient applications; higher organic amendments (whole dairy slurry) led to increased MBN and organic N in soil, and higher inorganic amendments led to more inorganic N, primarily $NO_3^-$, in these sandy loam soils. There was no correlation between treatment or microbial community on net N mineralization or nitrification rates. Nitrous oxide emissions and $NO_3^-$ leachate concentrations were generally highest after fertilizer application, in agreement with my hypothesis. The liquid dairy slurry amendment had N pools and cycling rates that were intermediate between whole dairy slurry manure and fertilizer, due to its reduced organic C and N and increased inorganic N content. Total microbial biomass was correlated with N immobilized in biomass and total soil N. Bacterial populations were consistently (but not always significantly) correlated with $N_2O$ emissions, while fungi were not. Thus, while any population of denitrifiers is likely only a subset of the population, it is more likely that $N_2O$ emissions are controlled by bacteria in this soil. In long-term experiments such as this, the use of molecular techniques could further link specific populations of microbes to activity rates, to better understand controls on

microbial activity in these perennial grass ecosystems. Despite finding some correlations between microbial biomass and soil N, as well as bacteria and $N_2O$ losses, these relationships were not always consistent or significant. Thus, their usefulness as indicators of N cycling may be limited.

In addition to building organic matter and increasing microbial biomass, whole and liquid dairy slurry manure had lower rates of $NO_3^-$ leaching than fertilizer after long-term applications. Thus, they are a good option over fertilizer for providing nutrients to crops while reducing N loss.

# 4. Potential of Nitrapyrin® to Reduce Soil N Losses and Effects on Microbial Community Structure

## 4.1. Introduction

Leaching of nitrate ($NO_3^-$) into aquatic ecosystems and denitrification (production of $N_2O$) are serious concerns due to mobilization of $NO_3^-$ in the soil after the growing season in the humid maritime climate of the Fraser Valley, BC. (Kowalenko, 2000). Due to large volumes of dairy manure produced in the Fraser Valley, application of manure in the fall period would be beneficial to farmers who otherwise must store large volumes of manure over the winter. However, the vulnerability of any nitrogen (N) applied to fields in the fall to nitrification and subsequent loss to the environment is currently very high (Kowalenko, 2000).

Nitrification inhibitors are products designed to slow or reduce the transformation of ammonia ($NH_4^+$) to $NO_3^-$ and are used world-wide. Nitrapyrin® is the most common inhibitor in the United States (US), and is designed to reduce the activity of the ammonia mono-oxygenase enzyme ($NH_4^+$ to $NO_2^-$) by targeting (and killing) ammonia-oxidizing bacteria (Trenkel, 1997). It has been shown to reduce nitrification rates and increase yields, especially on annual crops such as corn, in many instances across the US (Parkin and Hatfield, 2010). However, Nitrapyrin® has also shown phytotoxicity to plants (Maftoun et al., 1982), persistence in the soil (Sander and Barker, 1978), suppression of $CH_4$ oxidation (leading to increased $CH_4$ emissions) (Bronson et al., 1992), and decreased N mineralization rates (Roberts et al., 2003), yet few studies have investigated the effects of Nitrapyrin® on microbial community structure.

Microbial communities are essential to soil functions such as nutrient cycling and retention, C sequestration and decomposition, and providing soil structure. In addition,

the soil microbial community is also important for providing resistance and resilience to disturbances (Griffiths & Philippot, 2013). Different microbial communities may respond differently to disturbances, and may be more or less resilient. The long-term application of manure has consistently been linked to an increase in soil biodiversity over inorganic fertilizer applications (Enwall et al., 2007; Hu et al., 2011; Sun et al., 2004). Increased biodiversity may lead to increased stability (Griffiths and Philippot, 2013) and therefore, soils receiving manure in the long-term may be more resilient to disturbance than those receiving fertilizer (Ng et al., 2015).

The objective of this study was to establish if Nitrapyrin® is effective in reducing nitrification and subsequent losses of N due to $NO_3^-$ leaching and $N_2O$ emissions, from long-term pasture plots amended with either dairy slurry, separated liquid dairy slurry or fertilizer. In addition, the effect of Nitrapyrin® on the total soil microbial community was assessed. My hypotheses were that (1.) Nitrapyrin® would reduce $NO_3^-$ in the soil, reducing $N_2O$ emissions, and $NO_3^-$ leaching from the soil. (2.) Nitrapyrin® would reduce bacterial abundance and total microbial biomass in the soil, and (3.) Nitrapyrin® would have different magnitudes of effect on microbial community structure, having more effect on communities in plots receiving fertilizer> separated liquid dairy slurry>dairy slurry, due to increased resilience in manure plots.

## 4.2. Materials and Methods

### 4.2.1. Study Site

The study took place in the long-term soil amendment experimental plots at the Agriculture and Agri-food Canada research station in Agassiz, British Columbia (See chapter 2 for details).

### 4.2.2. Application of Nitrapyrin®

Nitrapyrin® was applied on October 1, 2013, concurrently with the final manure and fertilizer application of the year. Nitrapyrin® was applied to one sub-plot within each plot (plots were split crosswise into 3 equal sub-plots; Nitrapyrin® was applied to one, and the other two were used for all other analyses). In order to account for potential gradients across the field area, applications were alternated between the front and the back subplots in the field; block 1 received Nitrapyrin® in the front subplot, block 2 in the back, block 3 in the front, and block 4 in the back, in order to make sure that all treatments received equal front/back treatment.

Nitrapyrin® was obtained from Dow AgroSciences in the formulation eNtrench®. It was applied according to label recommendations at a target rate of 5.12 L/ha. Nitrapyrin® was applied manually, by hand, in a 15 L backpack sprayer equipped with a 1.5 m boom spray arm within 5 hours after fertilizer/manure application (October 1, 2013). Walk rate and spray speed was calibrated beforehand, and Nitrapyrin® was diluted into the backpack sprayer to reach the target rate. I conducted three complete passes over the 3x15 m area, spraying just above the sward height, in order to cover the area completely and with as little heterogeneity as possible. Label recommendations for the effective use of Nitrapyrin® suggest that in order to avoid manual incorporation, it must rain 12.5 mm within 10 days of application. At my site, it rained 5.9 mm October 1, 6.4 mm October 2 and 48.0 mm on October 9/10 of 2013, in theory ensuring infiltration of Nitrapyrin® into the soil.

### 4.2.3. Soil Measurements

Soil sampling was carried out on October 17, 2013; 17 days after manure and Nitrapyrin® application. 250 g of soil (4-6 cores) were taken from each subplot at a depth of 0-15 cm (for a total of 40 samples). The cores were taken at random intervals across the plot, and composited in the field. The soil was stored at 4$^{o}$C until preparation, then sieved through a 2 mm sieve. 10 g was freeze-dried for phospholipid fatty acid analysis, 50 g was oven-dried at 105$^{o}$C until constant weight for moisture content determination, the rest of the soil sample was kept at 4$^{o}$C for other analyses, carried out within a week of sampling. $NO_3^-$ leachate and $N_2O$ measurements were initiated immediately after application (October 2, 2013) and continued through to 2014.

### 4.2.4. Soil Nitrogen Pools and Transformations

*Soil Nitrogen Pools*

I determined soil inorganic nitrogen ($NH_4^+$, $NO_3^-$) according to methods outlined in Chapter 2.

*Soil Nitrogen Transformations*

Net rates of N mineralization and nitrification, as well as $NO_3^-$ availability using resin strips, were measured according to methods outlined in Chapter 3. Due to lower soil temperatures in the winter, mineralization and nitrification were measured in bags buried for 3 months, instead of 21 days.

### 4.2.5. Soil Nitrogen Losses

*Greenhouse Gas Emissions*

Greenhouse gas fluxes were measured using techniques described in Chapter 3. $N_2O$ is reported continuously from October 2013 to February 2013. $N_2O$ was measured at changing intervals over the sample period in order to account for freeze-thaw sampling events, but at least bi-weekly over the sampling period. $N_2O$ emissions in Nitrapyrin® and non-Nitrapyrin® plots were always measured concurrently. To compare between plots, seasonal averages were taken over the winter period.

*Nitrogen Leaching*

Estimates of dissolved $NO_3^-$ in leachate were measured as described in Chapter 3 every 5-15 days between October 2013 and August 2014. A single lysimeter was installed in each plot previous to installation of the current study; a second set of lysimeters was installed into the subplots in August/September of 2013; and measurement commenced in October 2013, following application of the nitrification inhibitor. To compare between plots, seasonal averages were taken over the entire sampling period.

### 4.2.6. Microbial Community Structure

Microbial community structure was characterized using phospholipid fatty acids (PLFA) as outlined in Chapter 2.

### 4.2.7. Statistical Analysis

Variables were tested for normality using the Shapiro-Wilk test, and if normal were analyzed for differences between Nitrapyrin® and non-Nitrapyrin® using a linear mixed

effects model, R package lmerTest with nutrient treatment and Nitrapyrin® as fixed effects and replication as a random effect. An ANOVA was used to determine significant effects, with a Tukey's HSD to evaluate differences where applicable. When non-normal (as in the case of $NO_3^-$ concentrations, resin strips, $N_2O$ emissions and $NO_3^-$ leaching), seasonal averages were taken. Values were averaged for each treatment for the fall/winter (October – May) of 2013/2014 period, and subsequently analyzed using the mixed effects model with nutrient treatment and Nitrapyrin® as fixed effects. In the case that the averaged variables still were not normal ($N_2O$ emissions, $NO_3^-$ leaching), the variables were analyzed separately by treatment using the same mixed model. To investigate relative changes in PLFA abundance, the percentage difference between Nitrapyrin® and non-Nitrapyrin® subplots was calculated, and analysed with a linear mixed effects model and Tukey's HSD. Analyses were considered significant at $p<0.05$. All analyses were carried out in R (R Foundation for Statistical Computing. 2014).

## 4.3. Results

### 4.3.1. Soil Nitrogen Pools

Soil $NH_4^+$ concentrations were lowest in the *liquid* treatment; however, there was no impact of Nitrapyrin® on soil $NH_4^+$ (Figure 18). Similarly, while *fertilizer* and *combination* had extremely high levels of soil $NO_3^-$ compared with all other treatments, Nitrapyrin® application similarly did not significantly impact soil $NO_3^-$ (Figure 18).

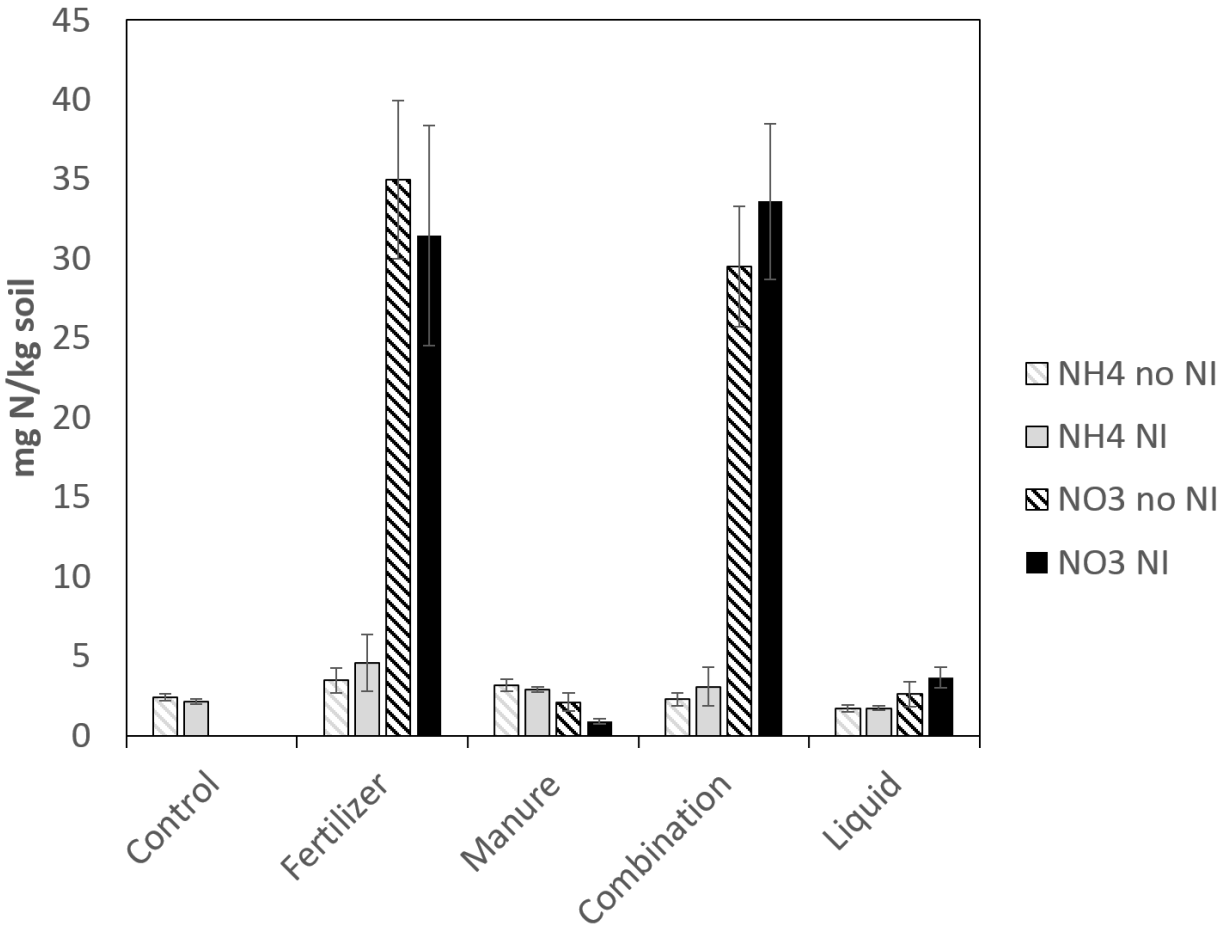


**Figure 18** Soil $NH_4^-$ and $NO_3^-$ concentrations 17 days following manure/fertilizer application without (noNI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Bars represent means (n=4) ± one standard error.

### 4.3.2. Soil Nitrogen Transformations

There was no impact of Nitrapyrin® on N mineralization. Contrary to expectations, there was also no difference in nitrification rates after application of Nitrapyrin® (Figure 19). While nitrification was significantly lower in *fertilizer* and *combination* treatments than the *manure* treatment, Nitrapyrin® did not lower nitrification rates in any of the treatments (Figure 19). There was a non-significant trend towards increased mineralization and nitrification following Nitrapyrin® application (in all treatments except for the *control*).

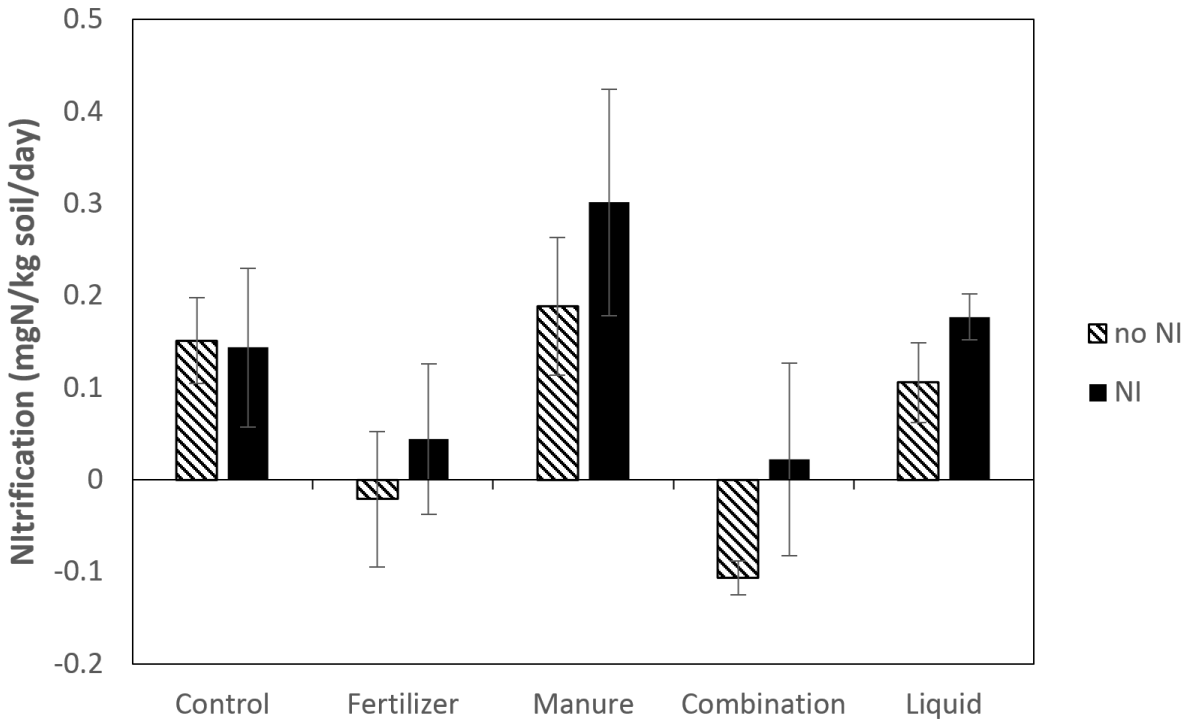


**Figure 19** Net rates of nitrification 17 days following manure/fertilizer application without (no NI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Bars represent means (n=4) ± one standard error.

$NO_3^-$ released onto resin strips was not significantly different for any treatment. Although not significant (p=0.11), $NO_3^-$ released onto resin strips was visually lower following Nitrapyrin® application in the manure treatment only (Figure 20).

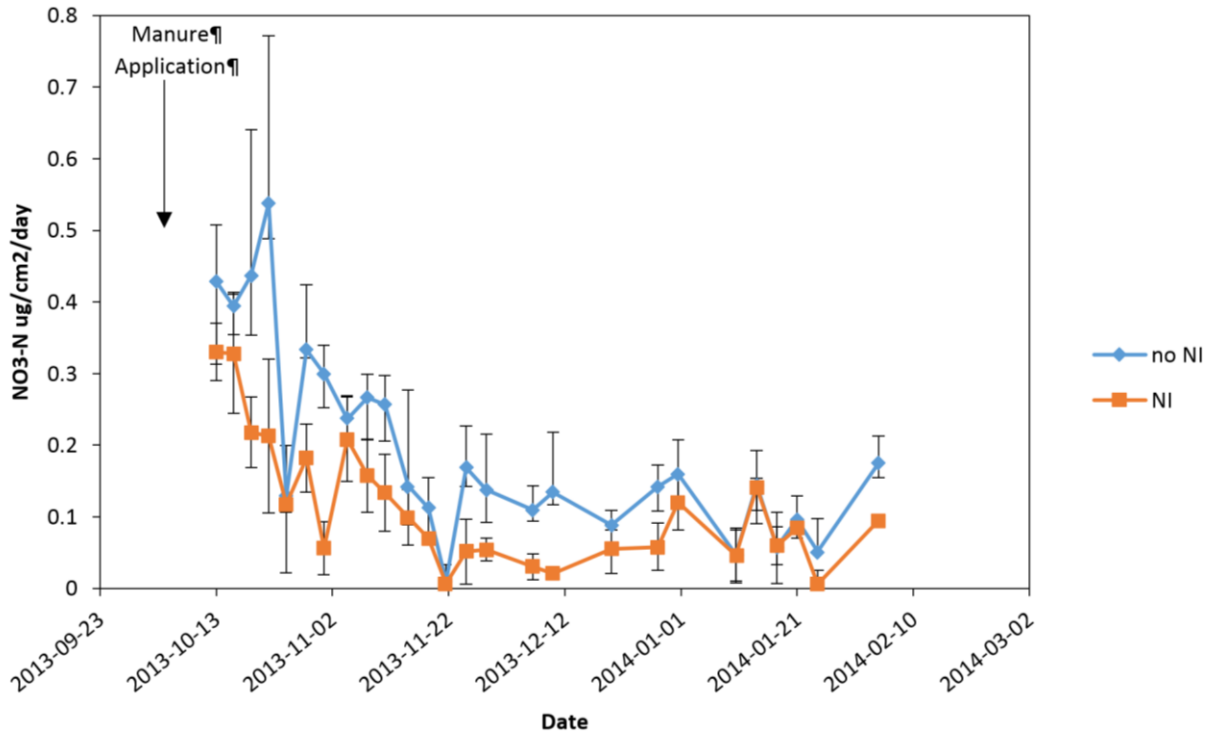


**Figure 20** Nitrate released onto resin strips in the winter of 2013, following whole slurry dairy manure application only, without (no NI) and with (NI) application of the nitrification inhibitor Nitrapyrin®. Lines represent means (n=4) ± one standard error.

### 4.3.3. Nitrogen Losses

Similarly, $N_2O$ emissions were not significantly reduced by Nitrapyrin® application to any of the treatments; however, the trend indicated slightly decreased $N_2O$ emissions with Nitrapyrin® addition, particularly in the manure treatment ($p=0.057$) (Figure 21).

In contrast, $NO_3^-$ in the leachate was consistently higher after Nitrapyrin® application ($p<0.05$) (Figure 22). This was surprising, as we predicted the opposite (an decrease in potential $NO_3^-$ leaching following Nitrapyrin®).

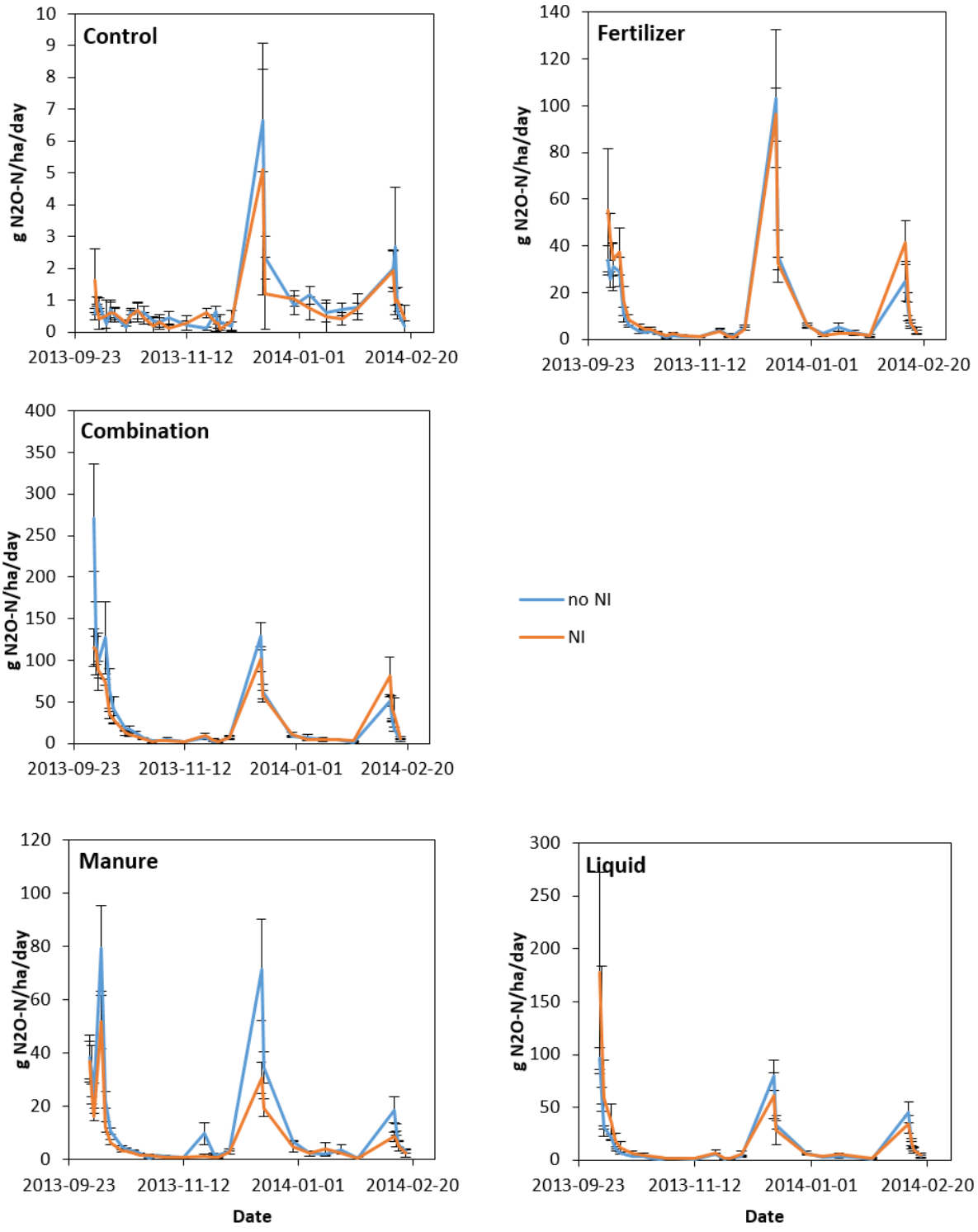


**Figure 21** $N_2O$ emissions for October 2013 – February 2014 following eleven years of fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4).

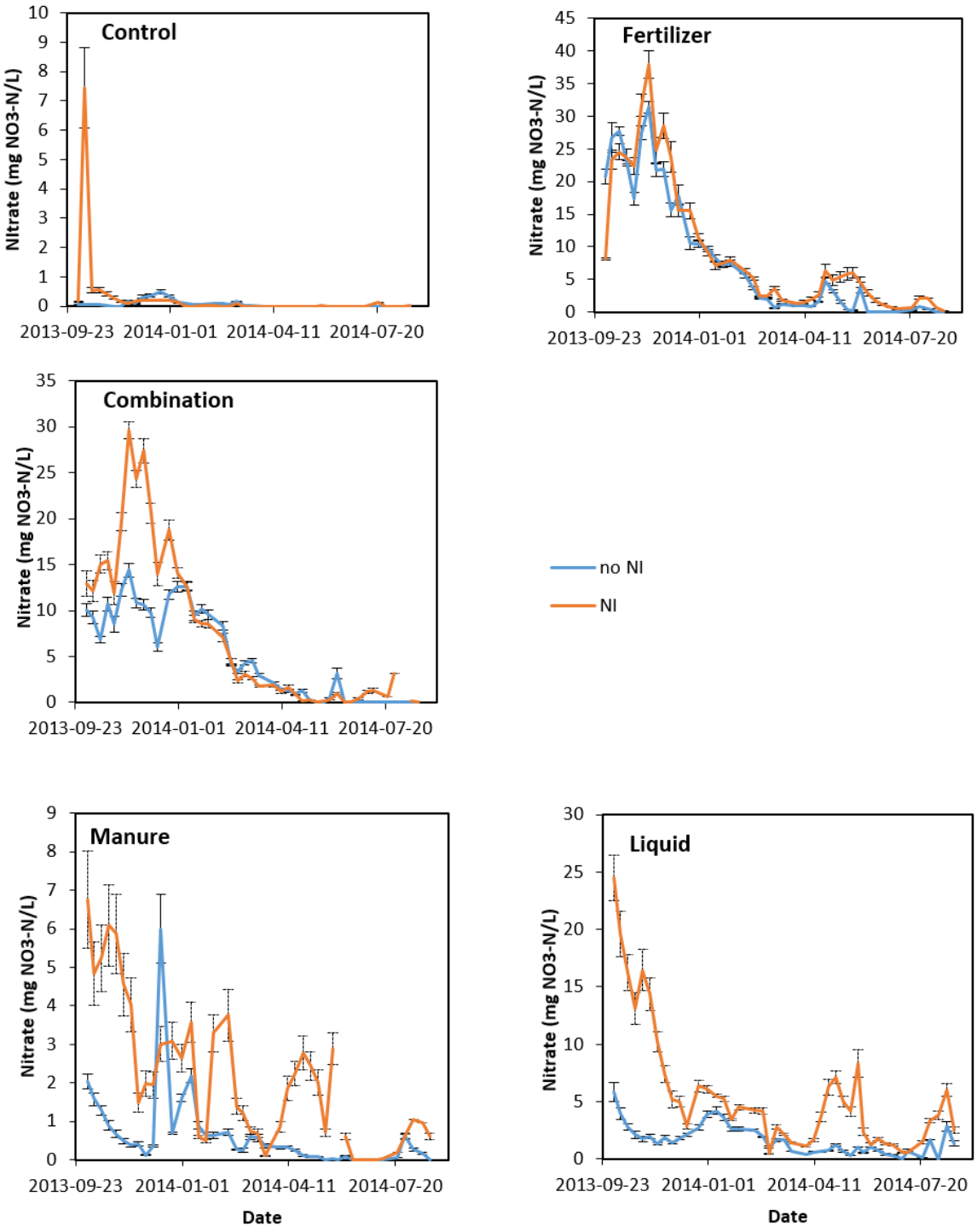


**Figure 22** $NO_3^-$ concentrations in suction cup lysimeters for October 2013 – August 2014, following eleven years of fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4) ± one standard error.

### 4.3.4. Microbial Community Structure

Despite the fact that Nitrapyrin® had no significant influence on its target process (nitrification), there was a significant effect of nitrapyrin on soil microbial community structure (PLFA). In fact, total PLFA abundance was greater in plots that received Nitrapyrin® than in plots that did not (Figure 23). The microbial community was also changed; a PCA on PLFA microbial biomarkers described 72% of the variance in the first two dimensions (Figure 24). Gram-negative bacteria were correlated with both axes, while Gram-positive bacteria were negatively correlated; this corresponded with Gram-negative bacteria being relatively more abundant after Nitrapyrin® application than Gram-positive bacteria (Figure 24). Fungal abundance was also stimulated by nitrapyrin addition to soil (Figures 23, 24). I calculated the percentage increase in PLFA concentrations by subtracting the non-Nitrapyrin® plots from the Nitrapyrin® plots, and compared these by plot. There were no differences in the magnitude of increase in PLFA concentrations depending on nutrient amendment, due to very high variability (some paired treatments had 0% or negative change, while others had PLFA concentrations up to 80% higher) (Figure 25). Visually, the pattern did follow expectations, with manure seeing the lowest % change, liquid fraction intermediate, and manure and combination both seeing high % of change after nitrapyrin.

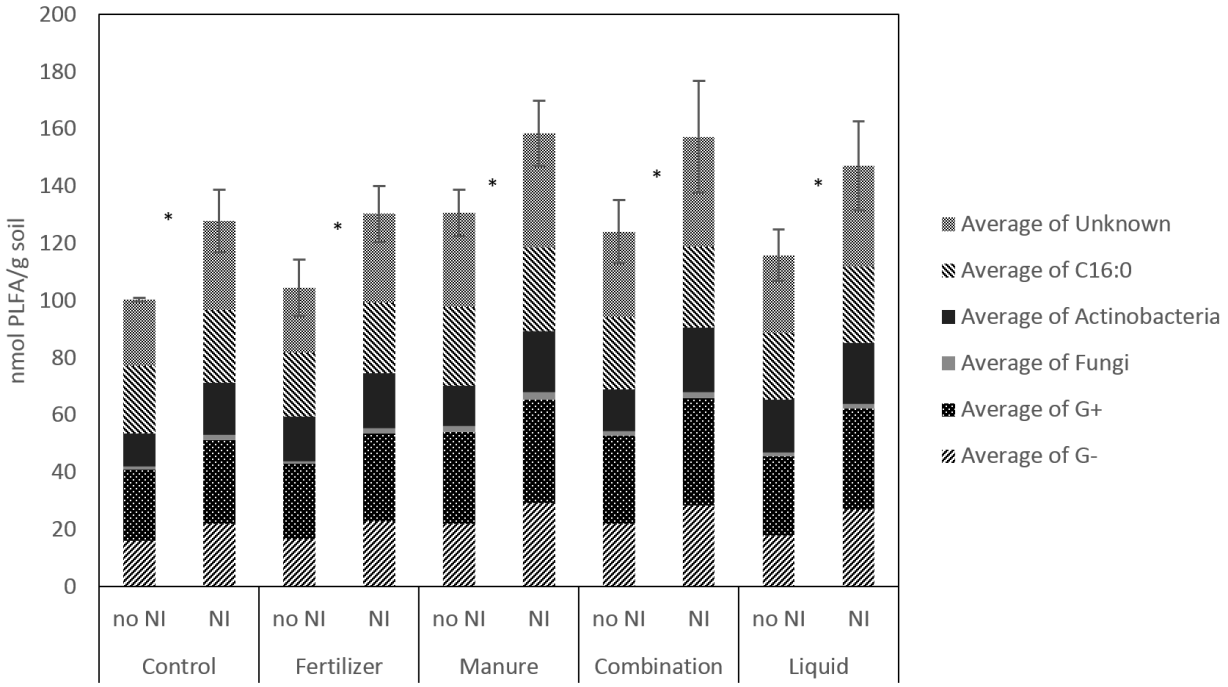


**Figure 23** PLFA concentrations in soils in October 2013 following long-term fertilizer, whole and liquid fraction dairy slurry, and a combination of fertilizer and manure, with (NI) and without (no NI) fall-applied Nitrapyrin®. Lines represent treatment means (n=4) ± one standard error. Asterisks represent significant differences between NI and no NI.

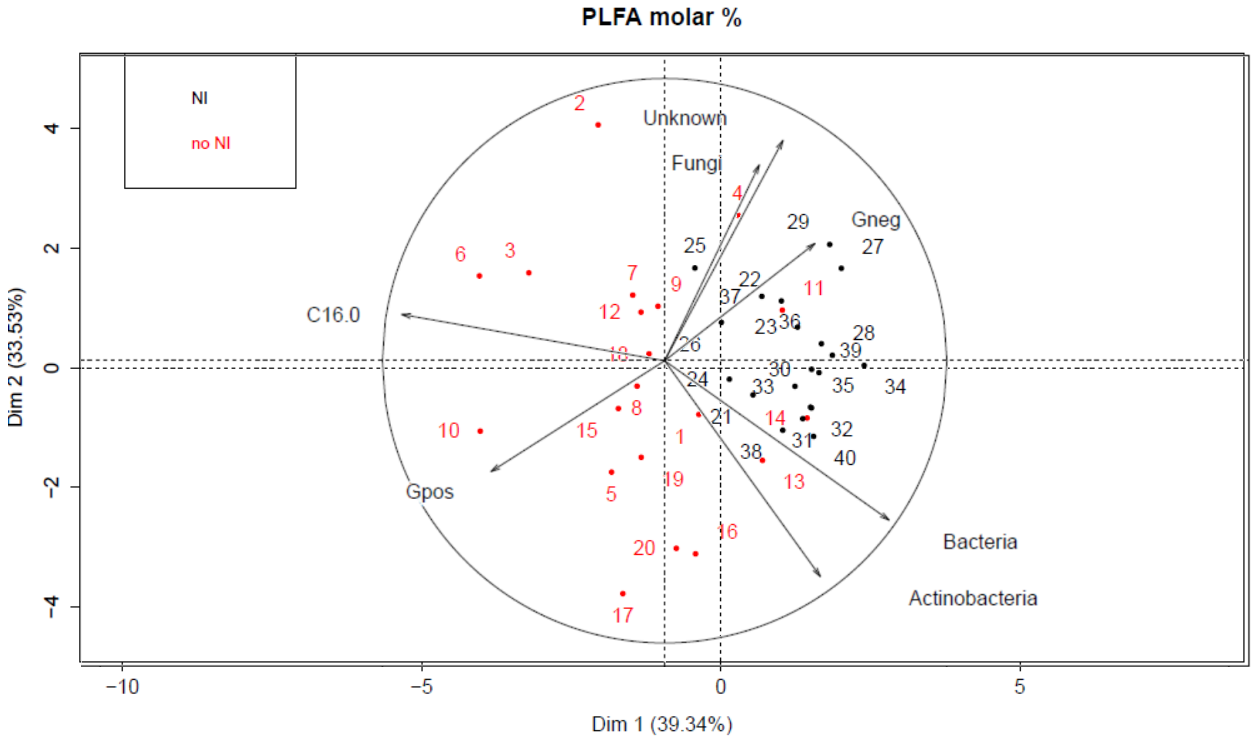


**Figure 24** Results of a principal components analysis (PCA) between molar % of PLFA biomarker groups. n=40. Points (with adjacent numbers) represent individual samples and are colour-coded by treatment. Labelled arrows represent the association of each microbial variable with each Principal Component (Dim 1 or Dim 2).

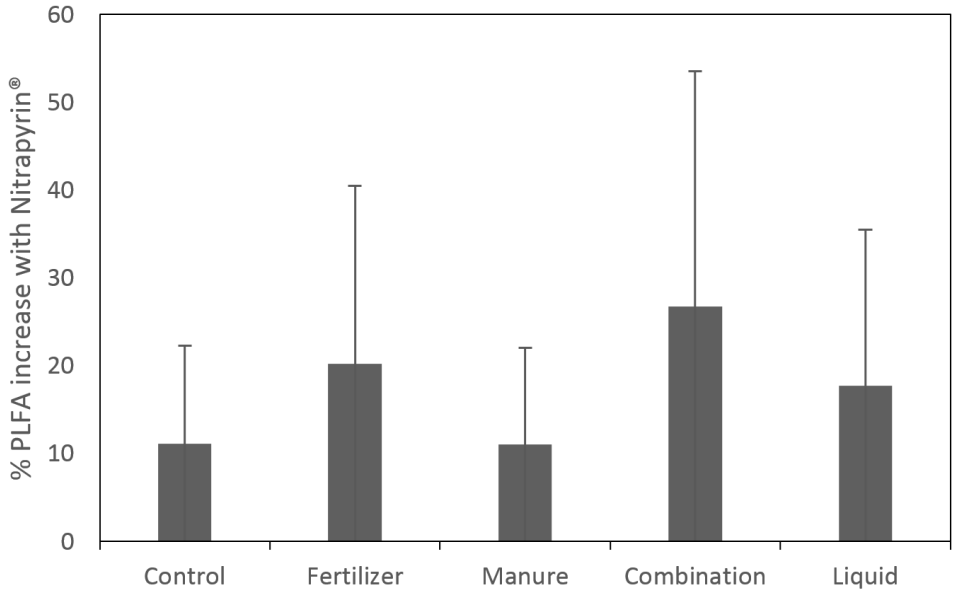


**Figure 25** Percentage increase in total PLFA concentration in soils after Nitrapyrin® addition. Bars represent mean (n=4) ± one standard error.

## 4.4. Discussion

Surprisingly, Nitrapyrin® did not cause any significant changes in soil $NH_4^+$ or $NO_3^-$, nitrification (or mineralization) rates, $NO_3^-$ released onto resin strips, or $N_2O$ emissions, despite some indications that nitrapyrin decreased $NO_3^-$ released onto resin strips and $N_2O$ emissions in the *manure* treatment. Surprisingly, there was also a trend of increased $NO_3^-$ in the leachate of all treatments with Nitrapyrin® addition. Therefore, for all intents and purposes, the Nitrapyrin® did not work. This is unexpected, as Nitrapyrin® has been shown to reduce $N_2O$ emissions on multiple soil types. For example,$N_2O$ was decreased following Nitrapyrin® application on two soil types under soybeans (Watanabe, 2006)), on silty loams with urea fertilizer on corn (Omonode and Vyn, 2013) and with manure in a laboratory environment (Calderon et al., 2005). However, there are fewer studies of the effect of Nitrapyrin® on perennial grasses. A trial in Edinburgh, Scotland (McTaggart et al., 1997) found that Nitrapyrin® was less effective than dicyandiamide (DCD) on reducing $N_2O$ emissions under perennial ryegrass. As the recommendation is also to mix the Nitrapyrin® with the fertilizer or manure being applied, it is possible that the backpack sprayer method was not effective in ensuring that Nitrapyrin® had contact with $NH_4^+$ in the manure in time to prevent nitrification through interception by the grass canopy or drift due to wind, although this technique was developed in consultation with Dow AgroSciences. Repeating the study and mixing the Nitrapyrin® into the manure and fertilizer would be beneficial to see if the small reductions in $NO_3^-$ released to resin strips and $N_2O$ emissions would be more pronounced in a second year.

I did observe significant differences in net N mineralization and nitrification between treatments in this October date, in contrast with the results of Chapter 3 (no treatment effect on net N mineralization and nitrification). Net mineralization and nitrification rates in October were significantly lower than at all other dates, and so when compared in conjunction with other dates, they did not contribute very strongly to the overall treatment analysis. In addition, the patterns from October mineralization and nitrification were not consistent with other dates. This may be because a longer incubation period in this October measurement (153 days vs 21 days in main study) combined with high initial amounts of $NO_3^-$ resulted in net N immobilization in the fertilizer and combination plots.

Surprisingly, I also found that potential $NO_3^-$ leaching was increased following Nitrapyrin® applications. This is the opposite of what we would expect to happen, and was not reflected in soil $NO_3^-$, rates of release of $NO_3^-$ onto resin strips, or $N_2O$ emissions. This was surprising, but discrepancies between these variables could be due to differences in depth of measurement: $NO_3^-$ released onto resin strips was measured at 5 cm depth, and $N_2O$ release may occur primarily in the top 5-10 cm of soil (Webb et al., 2010), while I saw more $NO_3^-$ in the leachate collected at 45 cm depth. There was a trend of increased nitrification following Nitrapyrin® additions, measured at 0-15cm depth, which could have released more $NO_3^-$ to deeper layers. As the microbial communities were changed following Nitrapyrin® application, this could have changed unmeasured process rates which resulted in greater loss of $NO_3^-$. Alternatively, temporal differences in the time of measurement may show the dynamic nature of Nitrapyrin® in my system; all soil and microbial measurements were taken 17 days following nutrient

and Nitrapyrin® application. We do not have measurements of these variables immediately following Nitrapyrin® application. In addition, water and $NO_3^-$ take time to move through the soil profile, and therefore concurrent measurements of surface $NO_3^-$ and subsurface $NO_3^-$ at 45 cm may not reflect each other.

Despite no or contradictory impact on the targeted effects, I did see an increase in total soil PLFA content, and a corresponding change in microcial community structure. In fact, Gram-negative bacteria, which are r-strategist bacteria, showed an increase in relative proportion as compared to plots that did not receive Nitrapyrin®, suggesting the Nitrapyrin® may have been used as a nutrient source (for C and N). The stimulation of heterotrophic bacterial growth by Nitrapyrin® has previously been reported (Kangatharalingham & Priscu, 1993). Increases in biomass of different microbial populations is not an uncommon response to the addition of agricultural chemicals. A review by Lo (2010) found that pesticides can have varying effects on the soil microbial community; while some inhibit populations, some stimulated only the bacterial community (including glyphosate), while some stimulated fungal populations. In fact, another nitrification inhibitor, dicyandiamide, increased gross N mineralization and turnover in an incubation on grassland loam soils, suggesting that microbial activity was stimulated, however, net N mineralization/nitrification rates were unaffected by nitrapyrin in this study (Ernfors et al., 2014).

### 4.5. Conclusions

I cannot conclude that Nitrapyrin® was effective at reducing nitrification, and subsequent losses of N, under forage grass in the Fraser Valley of BC. Contrary to expectations, Nitrapyrin® did stimulate total soil PLFA concentrations, and increased

$NO_3^-$ in the leachate at 45 cm depth. This study would be worth repeating for a second year to investigate if changes are consistent, or if they are due to ineffective mixing of inhibitor with amendment. Conclusions reached in this study illustrate the unpredictable response of the microbial community to novel management in the short-term. Despite the fact that I saw no significant differences in magnitude of response, this study illustrates the importance of understanding soil microbial community dynamics, how they are impacted by long-term nutrient application, and how they may in turn respond to short-term changes in management.

# 5. General Summary and Conclusions

## 5.1. Research Conclusions

In the current study, I examined changes in microbial biomass, microbial community structure, and activity after eleven years of *fertilizer, manure, combination, and liquid* treatments to plots of tall fescue in the field. I compared relationships between soil microbial community structure and activity and soil physical and chemical characteristics, and investigated linkages with N cycling processes and dynamics. In addition, I assessed the potential of a nitrification inhibitor, Nitrapyrin®, to reduce N losses from these systems.

### 5.1.1. Effects of Nutrient Amendments on Microbial Community Structure and Activity

As expected, I found higher microbial biomass C after *manure* and *liquid* treatments, and a combination of fertilizer and manure application to plots than after *fertilizer* application or *control*, although it cannot be ruled out that some of this biomass came directly from the manure. Soil fungal populations were negatively affected by *fertilizer* as well as the *liquid* amendment, indicating that *liquid* had a similar effect on the microbial community structure to *fertilizer*, despite having total biomass similar to *manure*. C cycling enzyme activities were higher after all forms of amendment (vs. no amendment), including inorganic fertilizer, suggesting either that these enzymes are bound to mineral particles in soil and operating independently from microbial biomass, or that populations of organisms producing C cycling enzymes are proportionately more prolific in fertilizer over manured plots, potentially due to labile organic compounds released from grass roots. No form of nutrient application impacted the activity of P, N, or S-cycling enzymes. Overall,

*manure, liquid,* and *combination* increased microbial biomass and promoted fungal populations in the soil, but did not otherwise affect microbial community structure or activity.

### 5.1.2. Effects of Nutrient Amendments on Nitrogen Cycling and links to Microbial Communities

The impact of long-term nutrient applications on the contents of N pools in the soil met my expectations; organic N was higher after manure and liquid treatments than fertilizer or no amendment, and fertilizer had much higher soil $NO_3^-$ concentrations. However, this did not lead to the expected higher rates of mineralization (or nitrification) in manure treatments. No form of nutrient application affected rates of net N mineralization and nitrification. However, net N mineralization and nitrification were correlated with total soil N and soil $NO_3^-$. Overall higher microbial biomass was found where there was more inorganic N; however, enzyme activity was not correlated with either total biomass or N cycling rates. Therefore, it is likely that total microbial biomass cannot be used to represent organisms that are mineralizing and nitrifying the soil. Nitrous oxide emissions were greater from fertilizer and combination treated soils. $N_2O$ emissions were more strongly correlated with bacterial biomarkers over fungal biomarkers, suggesting that bacterial denitrification (or nitrification) are stronger producers of $N_2O$ than fungal denitrifiers in my soil. Overall, organic amendments change N cycling dynamics by retaining more N in organic forms in the soil, but at the broad resolution of microbial community analysis in this study, changes in biomass do not relate closely to N transformations or losses. Therefore, the ability of PLFA measurements to make conclusions about N cycling in the soil is limited.

### 5.1.3. Potential of the Nitrification Inhibitor, Nitrapyrin, to Prevent N Losses after Nutrient Amendment

The nitrification inhibitor nitrapyrin did not reduce rates of nitrification, $NO_3^-$ leaching, or $N_2O$ emissions in any treatment. In fact, it increased potential rates of $NO_3^-$ leaching. However, total PLFA concentrations were higher, and community structure was changed, across all nutrient treatments after application of nitrapyrin. The lack of an inhibitory effect of nitrapyrin were contrary to expectations, although the stimulation of heterotrophic microbial growth by nitrapyrin has previously been reported.

## 5.2. Strengths and Contribution to the Field of Study

This study contributes to the body of literature linking microbial community structure and function. While soil microbial communities are increasingly being recognized as an essential component of soil quality, there is still a gap in our understanding of how microbial populations influence soil characteristics and processes. This study provides a glimpse of how broad microbial groups may (or may not) be linked to N cycling pools and processes. It is often asserted that 'microbial communities are essential to nutrient cycling in the soil', however, there are currently few studies which specifically compare measures of broad resolution soil microbial communities and N-cycling processes. This study shows that even higher resolution microbial measurements do not always lead to an increased ability to predict soil processes.

In addition, this study has been able to further investigate the impacts of long-term dairy manure and $NH_4NO_3$ fertilizer on microbial community structure, activity, and N-cycling processes. This study was initiated out after 11 years of management. Long-term, replicated trials such as this, where the management history is known, are rare,

especially in the Fraser Valley of Canada. Due to the rich history of research at the Agriculture and Agri-Food Research Centre in Agassiz, external factors were carefully accounted for, and experimental design was well laid-out. There was a wealth of information from previous, similar trials at the same study site to draw on. This study contributed a comprehensive look at baseline microbial communities in this ecosystem, and how they are influenced by management.

### 5.3. Limitations and Directions for Future Research

Unfortunately, microbial measurements were not taken on establishment of the treatments in this study, which did not allow for a comparison of microbial or soil C and N changes over time; this may have provided some insights into whether the microbial community changed after treatments only in the short-term (but became more similar in the long-term). I have shown that there is some, but not large, differentiation of microbial communities after long-term management, and increased C content of the soil in conjunction with higher microbial biomass; thus, it would be interesting to evaluate these management strategies from when they are initiated to calculate rates of change of microbial biomass and fungal populations, in comparison to other soil characteristics such as total soil C and N.

Secondly, it would likely have been beneficial to include molecular analyses in my study. The resolution of PLFA measurements is comparably low, and I were not able to quantify populations of, for example, nitrifying or denitrifying bacteria, which would be done with specific genetic primers. Actual differences in microbial community structure may have been masked by non-specific fatty acids in the PLFA measurements. Future

research to characterize these specific microbial groups could provide more insight into the community changes after long-term nutrient application.

I measured net rates of nitrification and N mineralization which were not significantly different between treatments; except in the winter of 2013, when fertilizer and combination showed net N immobilization, likely due to high initial concentrations of $NO_3^-$ and a longer incubation time. However, the fact that $NO_3^-$ concentrations were greater in soils amended with fertilizer suggests nitrification processes were greater in these soils. Use of $^{15}N$ isotope dilution methods (Muller 2007) to measure gross rates of these processes in future would be an improvement.

Other limitations of the study included the coordination of this study with other, ongoing work at the same site. For example, $N_2O$ emissions were taken frequently over the winter of 2013/2014, but only in 3-day campaigns during the summer of 2013.

Finally, I found that enzyme activity rates were highly variable in this study. Due to small soil weights (0.1g) and the potential for spatial variability, obtaining a 'true' representative sample for enzyme analysis is difficult (Wallenius et al., 2011). Some research has shown that separating soil enzyme activity within aggregates ( Liu et al., 2013), or between the rhizosphere and bulk soil (Pathan et al., 2014), may improve our understanding of soil enzyme activity. In a grass-dominated system such as the current one, with many plant roots, these may be beneficial approaches.

## 5.4. Implications of this Study for the Dairy and Agriculture Industry in the Fraser Valley

Ultimately, information on the impacts of manure management strategies can be used to recommend practices to dairy farmers in the Fraser Valley of southwest BC. Based on the results of this study, the whole dairy slurry, liquid fraction dairy slurry and a combination of fertilizer and whole slurry had higher microbial biomass and higher organic C and N compared to the fertilizer treatment. Both the whole and liquid fraction dairy slurry had lower summertime $N_2O$ emissions and much lower $NO_3^-$ leaching than the combination or the fertilizer alone. While I did not measure yield in this study, previous work has shown that the liquid fraction improves yield compared with whole fraction dairy slurry (Bittman et al., 2011). Thus, the liquid fraction is a good option for long-term nutrient application in order to increase soil C and microbial biomass and decrease N losses compared to fertilizer, while increasing yield compared to whole manure.

# References


Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol. Biochem. 64, 68–79. doi:10.1016/j.soilbio.2013.03.034

Andrews, S.S., Karlen, D.L., Cambardella, C.A., 2004. The Soil Management Assessment Framework : A Quantitative Soil Quality Evaluation Method. Soil Sci. Soc. Am. J. 68, 1945–1962.

Arshad, M.A., Martin, S., 2002. Identifying critical limits for soil quality indicators in agro-ecosystems. Agric. Ecosyst. Environ. 88, 153–160. doi:10.1016/S0167-8809(01)00252-3

Bach, E.M., Hofmockel, K.S., 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biol. Biochem. 69, 54–62. doi:10.1016/j.soilbio.2013.10.033

Baggs, E.M., 2011. Soil microbial sources of nitrous oxide: Recent advances in knowledge, emerging challenges and future direction. Curr. Opin. Environ. Sustain. 3, 321–327. doi:10.1016/j.cosust.2011.08.011

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479.

Basso, B., Ritchie, J.T., 2005. Impact of compost, manure and inorganic fertilizer on nitrate leaching and yield for a 6-year maize-alfalfa rotation in Michigan. Agric. Ecosyst. Environ. 108, 329–341. doi:10.1016/j.agee.2005.01.011

Bastida, F., Zsolnay, A., Hernandez, T., Garcia, C., 2008. Past, present and future of soil quality indices: A biological perspective. Geoderma 147, 159–171. doi:10.1016/j.geoderma.2008.08.007

Beauregard, M.S., Hamel, C., Atul-Nayyar, St-Arnaud, M., 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. Microb. Ecol. 59, 379–89. doi:10.1007/s00248-009-9583-z

Beckwith, C.P., Cooper, J., Smith, K.A., Shepherd, M.A., 1998. Nitrate leaching loss following application of organic manures to sandy soils in arable cropping. I. Effects of application time, manure type, overwinter crop cover and nitrification inhibition. Soil Use Manag. 14, 123–130.

Bhandral, R., Bittman, S., Kowalenko, C.G., Friesen, A., Hunt, D.E., 2008. Emissions of nitrous oxide after application of dairy slurry on bare soil and perennial grass in a maritime climate. Can. J. Soil Sci.

Binkley, D., Matson, P., 1983. Ion exchange resin bag method for assessing forest soil nitrogen availability. Soil Sci. Soc. Am. J. 47, 1050–1052. doi:10.2136/sssaj1983.03615995004700050045x

Bittman, S., Forge, T., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem. 37, 613–623. doi:10.1016/j.soilbio.2004.07.038

Bittman, S., Hunt, D.E., Kowalenko, C.G., Chantigny, M., Buckley, K., Bounaix, F., 2011. Removing Solids Improves Response of Grass to Surface-Banded Dairy Manure Slurry: A Multiyear Study. J. Environ. Qual. 40, 393–401. doi:10.2134/jeq2010.0177

Bittman, S., Kowalenko, C.G., Forge, T., Hunt, D.E., Bounaix, F., Patni, N., 2007. Agronomic effects of multi-year surface-banding of dairy slurry on grass. Bioresour. Technol. 98, 3249–3258. doi:10.1016/j.biortech.2006.07.016

Blagodatskiy, S. a., Avksent'ev, a. a., Davydova, M. a., Blagodatskaya, E. V., Kurakov, a. V., 2008. Nitrous oxide production in soils and the ratio of the fungal to bacterial biomass. Eurasian Soil Sci. 41, 1448–1455. doi:10.1134/S1064229308130140

Blake, G., 1986. Chapter 30: Bulk Density, in: Klute, E.A. (Ed.), Methods of Soil Analysis Part 1. Physical and Mineralogical Methods. American Society of Agronomy and Soil Science Society of America, pp. 374–390.

Bohme, L., Langer, U., Bohme, F., 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ. 109, 141–152. doi:10.1016/j.agee.2005.01.017

Bol, R., Kandeler, E., Amelung, W., Glaser, B., Marx, M.C., Preedy, N., Lorenz, K., 2003. Short-term effects of dairy slurry amendment on carbon sequestration and enzyme activities in a temperate grassland. Soil Biol. Biochem. 35, 1411–1421. doi:10.1016/S0038-0717(03)00235-9

Börjesson, G., Menichetti, L., Kirchmann, H., Kätterer, T., 2012. Soil microbial community structure affected by 53 years of nitrogen fertilisation and different organic amendments. Biol. Fertil. Soils 48, 245–257. doi:10.1007/s00374-011-0623-8

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of Soil Microbial Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty Acid Profiles. Microb. Ecol. 36, 1–12.

Brockett, B., 2008. Patterns in Forest Soil Microbial Community Composition across a range of regional climates in Western Canada. (Unpublished masters thesis) University of British Columbia.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol. Biochem. 44, 9–20. doi:10.1016/j.soilbio.2011.09.003

Bronson, K.F., Mosier, a. R., 1994. Suppression of methane oxidation in aerobic soil by nitrogen fertilizers, nitrification inhibitors, and urease inhibitors. Biol. Fertil. Soils 17, 263–268. doi:10.1007/BF00383979

Bronson, K.F., Mosier, A.R., Bishnoi, S.R., 1992. Nitrous Oxide Emissions in Irrigated Corn as Affected by Nitrification Inhibitors. Soil Sci. Soc. Am. J. 56, 161–165.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58, 216–234. doi:10.1016/j.soilbio.2012.11.009

Calderon, F.J., McCarty, G.W., Reeves, J.B., 2005. Nitrapyrin Delays Denitrification on Manured Soils. Soil Sci. 170, 350–359. doi:10.1097/01.ss.0000169905.94861.c7

Carter, M.R., Gregorich, E.G., Anderson, D.W., Doran, J.W., Janzen, H.H., Pierce, F.J., 1997. Concepts of Soil Quality and their Significance, in: Soil Quality for Crop Production and Ecosystem Health, Developments in Soil Science. Elsevier, pp. 1–19. doi:10.1016/S0166-2481(97)80028-1

Chantigny, M.H., Angers, D.A., Rochette, P., Bélanger, G., Massé, D., Côté, D., 2007. Gaseous nitrogen emissions and forage nitrogen uptake on soils fertilized with raw and treated swine manure. J. Environ. Qual. 36, 1864–1872. doi:10.2134/jeq2007.0083

Chantigny, M.H., Rochette, P., Angers, D.A., Bittman, S., Buckley, K., Massé, D., Bélanger, G., Eriksen-Hamel, N., Gasser, M.-O., 2010. Soil nitrous oxide emissions following band-incorporation of fertilizer nitrogen and swine manure. J. Environ. Qual. 39, 1545–1553. doi:10.2134/jeq2009.0482

Chen, X., Li, Z., Liu, M., Jiang, C., Che, Y., 2015. Microbial community and functional diversity associated with different aggregate fractions of a paddy soil fertilized with organic manure and/or NPK fertilizer for 20 years. J. Soils Sediments 15, 292–301. doi:10.1007/s11368-014-0981-6

Condron, L., Stark, C., Callaghan, M.O., 2010. The Role of Microbial Communities in the Formation and Decomposition of Soil Organic Matter, in: Dixon, G.R., Tilston, E.L. (Eds.), Soil Microbiology and Sustainable Crop Production. Springer Netherlands, Dordrecht, pp. 81–118. doi:10.1007/978-90-481-9479-7

Cruz, A.F., Hamel, C., Hanson, K., Selles, F., Zentner, R.P., 2008. Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a Brown Chernozem. Plant Soil 315, 173–184. doi:10.1007/s11104-008-9742-x

Di, H.J., Cameron, K.C., Moore, S., Smith, N.P., 1999. Contributions to nitrogen leaching and pasture uptake by autumn-applied dairy effluent and ammonium fertilizer labeled with 15N isotope. Plant Soil 210, 189–198. doi:10.1023/A:1004677902049

Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenström, J., Hallin, S., 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biol. Biochem. 39, 106–115. doi:10.1016/j.soilbio.2006.06.015

Ernfors, M., Brennan, F.P., Richards, K.G., Mcgeough, K.L., Griffiths, B.S., Laughlin, R.J., Watson, C.J., Philippot, L., Grant, J., Minet, E.P., Moynihan, E., Müller, C., 2014. The nitrification inhibitor dicyandiamide increases mineralization–immobilization turnover in slurry-amended grassland soil. J. Agric. Sci. 152, 137–149. doi:10.1017/S0021859613000907

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273–284. doi:10.1016/j.agee.2006.05.022

Forge, T., Bittman, S., Kowalenko, C.G., 2005. Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem. 37, 1751–1762. doi:10.1016/j.soilbio.2004.11.013

Fraser Valley Regional District, 2011. Agricultural Economy in the Fraser Valley Regional District; Regional Snapshot Series. Chilliwack, BC.

Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 4, 151–163.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625. doi:10.1016/j.soilbio.2010.11.021

García-Ruiz, R., Ochoa, V., Viñegla, B., Hinojosa, M.B., Peña-Santiago, R., Liébanas, G., Linares, J.C., Carreira, J.A., 2009. Soil enzymes, nematode community and selected physico-chemical properties as soil quality indicators in organic and conventional olive oil farming: Influence of seasonality and site features. Appl. Soil Ecol. 41, 305–314. doi:10.1016/j.apsoil.2008.12.004

Gasser, M.-O., Chantigny, M.H., Angers, D.A., Bittman, S., Buckley, K.E., Rochette, P., Massé, D., 2012. Plant-available and water-soluble phosphorus in soils amended

with separated manure solids. J. Environ. Qual. 41, 1290–300. doi:10.2134/jeq2011.0290

Gattinger, A., Gunther, A., Schloter, M., Munch, J.C., 2003. Characterisation of Archaea in Soils by Polar Lipid Analysis. Acta Biotechnol. 23, 21–28.

Ginting, D., Kessavalou, A., Eghball, B., Doran, J.W., 2003. Greenhouse gas emissions and soil indicators four years after manure and compost applications. J. Environ. Qual. 32, 23–32.

Gómez-Rey, M.X., Couto-Vázquez, A., González-Prieto, S.J., 2012. Nitrogen transformation rates and nutrient availability under conventional plough and conservation tillage. Soil Tillage Res. 124, 144–152. doi:10.1016/j.still.2012.05.010

Grant, C., Wu, R., 2008. Enhanced-Efficiency Fertilizers for Use on the Canadian Prairies Crop Management. Plant Manag. Netw. doi:10.1094/CM-2008-0730-01-RV.Abstract

Grayston, S.J., Campbell, C.D., Bardgett, R., Mawdsley, J.L., Clegg, C.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., Millard, P., 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl. Soil Ecol. 25, 63–84. doi:10.1016/S0929-1393(03)00098-2

Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L., Whitmore, A.P., 2009. The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185. doi:10.1016/j.geoderma.2009.08.002

Griffiths, B.S., Kuan, H.L., Ritz, K., Glover, L.A., McCaig, A.E., Fenwick, C., 2004. The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. Microb. Ecol. 47, 104–13. doi:10.1007/s00248-002-2043-7

Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37, 112–29. doi:10.1111/j.1574-6976.2012.00343.x

Gu, Y., Zhang, X., Tu, S., Lindström, K., 2009. Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. Eur. J. Soil Biol. 45, 239–246. doi:10.1016/j.ejsobi.2009.02.005

Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3, 597–605. doi:10.1038/ismej.2008.128

Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen Mineralization, Immobilization, and Nitrification, in: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis Part 2 - Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 985–1007.

Herold, M.B., Baggs, E.M., Daniell, T.J., 2012. Fungal and bacterial denitrification are differently affected by long-term pH amendment and cultivation of arable soil. Soil Biol. Biochem. 54, 25–35. doi:10.1016/j.soilbio.2012.04.031

Hofman, G., Van Cleemput, O., 2004. Soil and Plant Nitrogen. Paris, France.

Hu, J., Lin, X., Wang, J., Dai, J., Chen, R., Zhang, J., Wong, M.H., 2011. Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer. J. Soils Sediments 11, 271–280. doi:10.1007/s11368-010-0308-1

Jin, T., Shimizu, M., Marutani, S., Desyatkin, A.R., Iizuka, N., Hata, H., Hatano, R., 2010. Effect of chemical fertilizer and manure application on N2O emission from reed canary grassland in Hokkaido, Japan. Soil Sci. Plant Nutr. 56, 53–65. doi:10.1111/j.1747-0765.2010.00447.x

Jin, X., Huang, J., Zhou, Y., 2012. Impact of coastal wetland cultivation on microbial biomass, ammonia-oxidizing bacteria, gross N transformation and N2O and NO potential production. Biol. Fertil. Soils 48, 363–369. doi:10.1007/s00374-011-0631-8

Jirout, J., Šimek, M., Elhottová, D., 2013. Fungal contribution to nitrous oxide emissions from cattle impacted soils. Chemosphere 90, 565–572. doi:10.1016/j.chemosphere.2012.08.031

Kemper, W.D., Rosenau, R.C., 1986. Aggregate Stability and Size Distribution., in: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis Part 1. Physical and Mineralogical Methods. American Society of Agronomy and Soil Science Society of America, Madison, pp. 425–441.

Kleineidam, K., Košmrlj, K., Kublik, S., Palmer, I., Pfab, H., Ruser, R., Fiedler, S., Schloter, M., 2011. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. Chemosphere 84, 182–6. doi:10.1016/j.chemosphere.2011.02.086

Kowalenko, C.G., 2000. Nitrogen pools and processes in agricultural systems of Coastal British Columbia - A review of published research. Can. J. Plant Sci. 80, 1–10.

Kowalenko, C.G., 2001. Assessment of Leco CNS-2000 analyzer for simultaneously measuring total carbon, nitrogen, and sulphur in soil. Commun. Soil Sci. Plant Anal. 32, 2065–2078. doi:10.1081/CSS-120000269

Kowalenko, C.G., Babuin, D., 2003. Re-evaluation of Factors Affecting Total Nitrogen Measurements in Soil Extract Solutions Using Microwave-Assisted Persulfate Oxidation. Commun. Soil Sci. Plant Anal. 34, 2745–2762. doi:10.1081/CSS-120025201

Liu, L., Zhang, T., Gilliam, F.S., Gundersen, P., Zhang, W., Chen, H., Mo, J., 2013. Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLoS One 8, e61188. doi:10.1371/journal.pone.0061188

Liu, Y.-R., Li, X., Shen, Q.-R., Xu, Y.-C., 2013. Enzyme Activity in Water-Stable Soil Aggregates as Affected by Long-Term Application of Organic Manure and Chemical Fertiliser. Pedosphere 23, 111–119. doi:10.1016/S1002-0160(12)60086-1

Lo, C.-C., 2010. Effect of pesticides on soil microbial community. J. Environ. Sci. Health. B. 45, 348–359. doi:10.1080/10934520903467873

Maftoun, M., Sameni, A.M., Darbeheshti, M., Yasrebi, J., Hojjati, S.M., 1982. Toxicity of nitrapyrin to sunflower under different N nutrition regimes. Plant Soil 65, 411–414. doi:10.1007/BF02375061

McTaggart, I.P., Parker, J., Swan, L., Clayton, H., Smith, K.A., 1997. Nitrous oxide emissions from grassland and spring barley, following N fertiliser application with and without nitrification inhibitors. Biol. Fertil. Soils 25, 261–268. doi:10.1007/s003740050312

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2009. Denitrifier Community Dynamics in Soil Aggregates under Permanent Grassland and Arable Cropping Systems. Soil Sci. Soc. Am. J. 73, 1843. doi:10.2136/sssaj2008.0357

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2009. Influence of Liquid Manure on Soil Denitrifier Abundance, Denitrification, and Nitrous Oxide Emissions. Soil Sci. Soc. Am. J. 73, 760. doi:10.2136/sssaj2008.0059

Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., Siebert, B.D., Osmond, G., 1998. Effects of soil compaction on phosphorus uptake and growth of Trifolium subterraneum colonized by four species of vesicular – arbuscular mycorrhizal fungi. New Phytol. 140, 155–165.

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: classical and molecular approaches. Biol. Fertil. Soils 48, 743–762. doi:10.1007/s00374-012-0723-0

Nelson, M.A., Griffith, S.M., Steiner, J.J., 2006. Tillage Effects on Nitrogen Dynamics and Grass Seed Crop Production in Western Oregon, USA. Soil Sci. Soc. Am. J. 70, 825–831. doi:10.2136/sssaj2005.0248

Ng, E.L., Patti, A.F., Rose, M.T., Schefe, C.R., Smernik, R.J., Cavagnaro, T.R., 2015. Do organic inputs alter resistance and resilience of soil microbial community to drying? Soil Biol. Biochem. 81, 58–66. doi:10.1016/j.soilbio.2014.10.028

Omonode, R.A., Vyn, T.J., 2013. Nitrification kinetics and nitrous oxide emissions when nitrapyrin is coapplied with urea-ammonium nitrate. Agron. J. 105, 1475–1486. doi:10.2134/agronj2013.0184

Parham, J., Deng, S.P., Raun, W.R., Johnson, G. V, 2002. Long-term cattle manure application in soil 1. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. Biol. Fertil. Soils 35, 328–337. doi:10.1007/s00374-002-0476-2

Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Appl. Soil Ecol. 48, 185–192. doi:10.1016/j.apsoil.2011.03.007

Parkin, T.B., Hatfield, J.L., 2010. Influence of nitrapyrin on N2O losses from soil receiving fall-applied anhydrous ammonia. Agric. Ecosyst. Environ. 136, 81–86. doi:10.1016/j.agee.2009.11.014

Pathan, S.I., Ceccherini, M.T., Pietramellara, G., Puschenreiter, M., Giagnoni, L., Arenella, M., Varanini, Z., Nannpieri, P., Renella, G., 2014. Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. Plant Soil 387, 413–424. doi:10.1007/s11104-014-2306-3

Paul, J.W., Beauchamp, E.G., 1989. Effect of Carbon Constituents in Manure on Denitrification in Soil. Can. J. Soil Sci. 61, 49–61.

Paul, J.W., Zebarth, B.J., 1997a. Denitrification during the growing season following dairy cattle slurry and fertilizer application for silage corn. Can. J. Soil Sci. 545, 0–7.

Paul, J.W., Zebarth, B.J., 1997b. Denitrification and nitrate leaching during the fall and winter following dairy cattle slurry application. Can. J. Soil Sci. 77, 231–240.

Peacock, A.D., Mullen, M.D., Ringelberg, D.B., Tyler, D.D., Hedrick, D.B., 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Plant Soil 33, 1011–1019.

Philippot, L., Hallin, S., 2005. Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. Curr. Opin. Microbiol. 8, 234–9. doi:10.1016/j.mib.2005.04.003

Philippot, L., Hallin, S., Schloter, M., 2007. Ecology of Denitrifying Prokaryotes in Agricultural Soil 96, 249–305. doi:10.1016/S0065-2113(07)96003-4

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., Wood, C., 2009. Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. Ecol. Indic. 9, 1212–1221. doi:10.1016/j.ecolind.2009.02.009

Roberts, G.C., Peurou, F., Penwell, A.J., 2003. Assessing the toxic effect of nitrapyrin on nitrogen transformation in soil. Soil Biol. Biochem. 35, 479–481. doi:10.1016/S0038-0717(02)00289-4

Rochette, P., Angers, D.A., Chantigny, M.H., Gagnon, B., Bertrand, N., 2008. N2O fluxes in soils of contrasting textures fertilized with liquid and solid dairy cattle manures. Can. J. Soil Sci. 88, 175–187. doi:10.4141/CJSS06016

Rochette, P., Worth, D.E., Lemke, R.L., Mcconkey, B.G., Pennock, D.J., Wagner-riddle, C., Desjardins, R.L., 2008. Estimation of N2O emissions from agricultural soils in Canada I. Development of a country-specific methodology. Can. J. Anim. Sci. 3, 641–654.

Sánchez-Moreno, S., Smukler, S., Ferris, H., O'Geen, A.T., Jackson, L.E., 2007. Nematode diversity, food web condition, and chemical and physical properties in different soil habitats of an organic farm. Biol. Fertil. Soils 44, 727–744. doi:10.1007/s00374-007-0256-0

Sander, D.J., Barker, A. V, 1978. Comparative Toxicity of Nitrapyrin and 6-Chloropicolinic Acid to Radish and Cucumber under Different N Nutrition Regimes (AJ). Agron. J. 70, 295–298.

Schauss, K., Focks, A., Leininger, S., Kotzerke, A., Heuer, H., Thiele-Bruhn, S., Sharma, S., Wilke, B.-M., Matthies, M., Smalla, K., Munch, J.C., Amelung, W., Kaupenjohann, M., Schloter, M., Schleper, C., 2009. Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environ. Microbiol. 11, 446–456. doi:10.1111/j.1462-2920.2008.01783.x

Schloter, M., Bach, H.J., Metz, S., Sehy, U., Munch, J.C., 2003. Influence of precision farming on the microbial community structure and functions in nitrogen turnover. Agric. Ecosyst. Environ. 98, 295–304. doi:10.1016/S0167-8809(03)00089-6

Sheppard, S.C., Bittman, S., Swift, M.L., Beaulieu, M., Sheppard, M.I., 2011. Ecoregion and farm size differences in dairy feed and manure nitrogen management : A survey. Can. J. Anim. Sci. 91, 459–473. doi:10.4141/CJAS2010-004

Shoun, H., Kano, M., Baba, I., Takaya, N., Matsuo, M., 1998. Denitrification by actinomycetes and purification of dissimilatory nitrite reductase and azurin from Streptomyces thioluteus. J. Bacteriol. 180, 4413–4415.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264. doi:10.1111/j.1461-0248.2008.01245.x

Sinsabaugh, R.L., Saiya-Cork, K., Long, T., Osgood, M.P., Neher, D.A., Zak, D.R., Norby, R.J., 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO2-driven increases in primary production. Appl. Soil Ecol. 24, 263–271. doi:10.1016/S0929-1393(03)00002-7

Slangen, J., Kerkhoff, P., 1984. Nitrification inhibitors in agriculture and horticulture: A literature review, in: Fertilizer Research.

Smil, V., 1999. Nitrogen in crop production : An account of global flows. Global Biogeochem. Cycles 13, 647–662.

Sørensen, P., 2004. Immobilisation, remineralisation and residual effects in subsequent crops of dairy cattle slurry nitrogen compared to mineral fertiliser nitrogen. Plant Soil 267, 285–296. doi:10.1007/s11104-005-0121-6

Steenwerth, K., Jackson, L.E., Caldero, F.J., Stromberg, M.R., L, Scow, K.M., 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 34, 1599–1611.

Stoddard, C.S., Grove, J.H., Coyne, M.S., Thom, W.O., 2005. Fertilizer, tillage, and dairy manure contributions to nitrate and herbicide leaching. J. Environ. Qual. 34, 1354–1362. doi:10.2134/jeq2004.0226

Stott, D.E., Andrews, S.S., Liebig, M.A., Wienhold, B.J., Karlen, D.L., 2010. Evaluation of β-Glucosidase Activity as a Soil Quality Indicator for the Soil Management Assessment Framework. Soil Sci. Soc. Am. J. 74, 107–119. doi:10.2136/sssaj2009.0029

Štursová, M., Baldrian, P., 2010. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant Soil 338, 99–110. doi:10.1007/s11104-010-0296-3

Sun, H.Y., Deng, S.P., Raun, W.R., 2004. Bacterial Community Structure and Diversity in a Century-Old Manure-Treated Agroecosystem. Appl. Environ. Microbiol. 70, 5868–5874. doi:10.1128/AEM.70.10.5868

Thiessen, H., Moir, J.O., 1993. Total and Organic Carbon, in: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis. CRC Press LLC.

Thomsen, I., Hansen, J., Kjellerup, V., Christensen, C., 1993. Effects of cropping system and rates of nitrogen in animal slurry and mineral fertilizer on nitrate leaching from a sandy loam. Soil Use Manag. 9, 53–58. doi:10.1111/j.1475-2743.1993.tb00929.x

Tian, L., Dell, E., Shi, W., 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. Appl. Soil Ecol. 46, 426–435. doi:10.1016/j.apsoil.2010.09.007

Tian, X., Hu, H., Ding, Q., 2013. Influence of nitrogen fertilization on soil ammonia oxidizer and denitrifier abundance , microbial biomass , and enzyme activities in an alpine meadow. Biol. Fertil. Soils 50, 703–713. doi:10.1007/s00374-013-0889-0

Topp, E., 1993. Effects of selected agrochemicals on methane oxidation by an organic agricultural soil. Can. J. Soil Sci. 287–291.

Toyota, K., Kuninaga, S., 2006. Comparison of soil microbial community between soils amended with or without farmyard manure. Appl. Soil Ecol. 33, 39–48. doi:10.1016/j.apsoil.2005.09.002

Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F., 2008. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil Biol. Biochem. 40, 2146–2155. doi:10.1016/j.soilbio.2008.03.015

Trenkel, M.E., 1997. Controlled-Release and Stabilized Fertilizers in Agriculture. International Fertilizer Industry Association. Report for the Food and Agriculture Organization of the United Nations, Paris.

Truu, M., Truu, J., Ivask, M., 2008. Soil microbiological and biochemical properties for assessing the effect of agricultural management practices in Estonian cultivated soils. Eur. J. Soil Biol. 44, 231–237. doi:10.1016/j.ejsobi.2007.12.003

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biol. Biochem. 19, 703–707.

Vitousek, P., Cassman, K., Cleveland, C., 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57, 1–45. doi:10.1023/A:1015798428743

Voroney, R.P., Brookes, P.C., Beyaert, R., 2008. Soil Microbial Biomass C, N, P, and S, in: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis. CRC Press LLC.

Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindström, K., Hartikainen, H., Raateland, A., Niemi, R.M., 2011. Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. Soil Biol. Biochem. 43, 1464–1473. doi:10.1016/j.soilbio.2011.03.018

Watanabe, T., 2006. Influence of 2-chloro-6 (trichloromethyl) pyridine and diciandiamide on nitrous oxide emission under different soil conditions. Soil Sci. Plant Nutr. 52, 226–232. doi:10.1111/j.1747-0765.

Watts, D.B., Torbert, H.A., Feng, Y., Prior, S.A., 2010. Soil Microbial Community Dynamics as Influenced by Composted Dairy Manure, Soil Properties, and Landscape Position. Soil Sci. 175, 474–486. doi:10.1097/SS.0b013e3181f7964f

Webb, J., Pain, B., Bittman, S., Morgan, J., 2010. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—A review. Agric. Ecosyst. Environ. 137, 39–46. doi:10.1016/j.agee.2010.01.001

Weiske, A., Benckiser, G., Herbert, T., Ottow, J.C.G., 2001. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol. Fertil. Soils 34, 109–117. doi:10.1007/s003740100386

Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P.G., Schmidt, T.M., Zhou, J., 2013. Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. Appl. Environ. Microbiol. 79, 1284–1292. doi:10.1128/AEM.03393-12

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A., 2001. 3,4-Dimethylpyrazole phosphate (DMPP) - a new nitrification inhibitor for agriculture and horticulture. Biol. Fertil. Soils 34, 79–84. doi:10.1007/s003740100380

Zhang, Q.C., Shamsi, I.H., Xu, D.T., Wang, G.H., Lin, X.Y., Jilani, G., Hussain, N., Chaudhry, A.N., 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. Appl. Soil Ecol. 57, 1–8. doi:10.1016/j.apsoil.2012.02.012

Zhang, X., Wang, Q., Gilliam, F.S., Bai, W., Han, X., Li, L., 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. Grass Forage Sci. 67, 219–230. doi:10.1111/j.1365-2494.2011.00836.x

Zumft, W.G., 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. 61, 533–616.

# Appendix

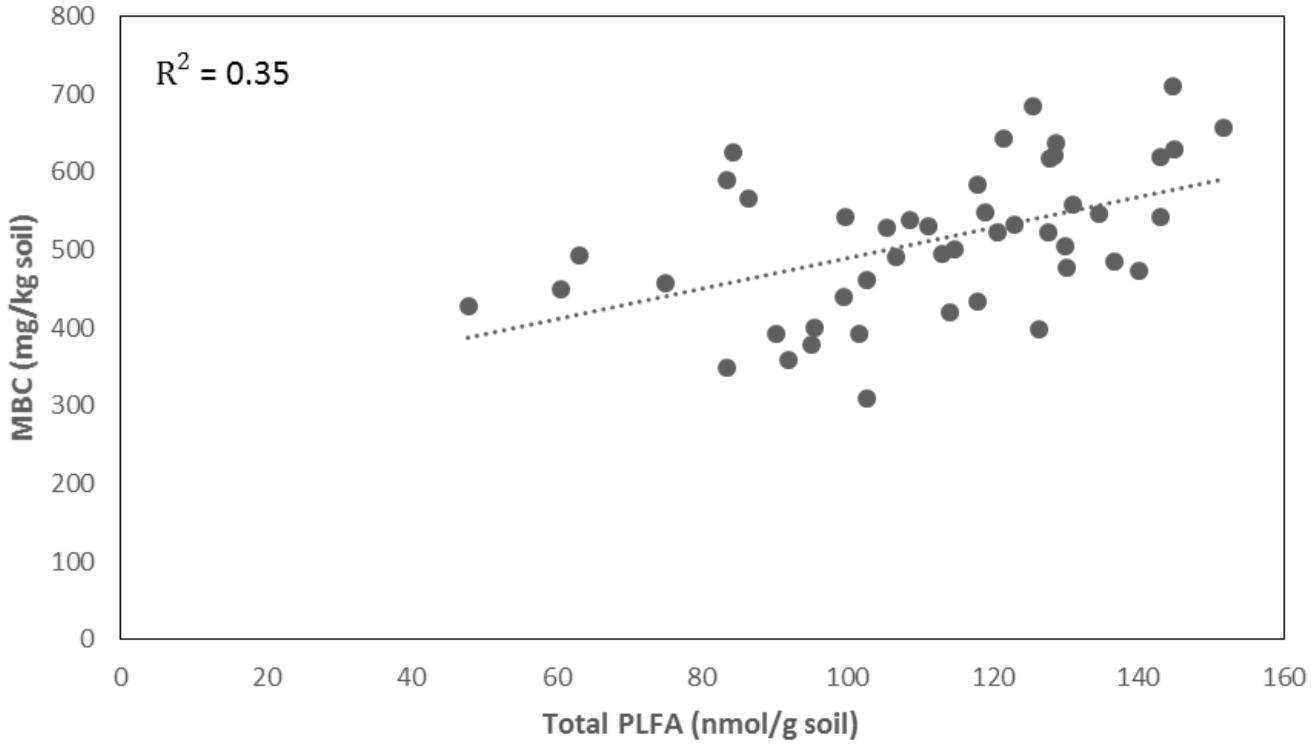


**Figure 26** Relationship between microbial biomass carbon (MBC) and total Phopsholipid Fatty Acids (nmol/g soil).

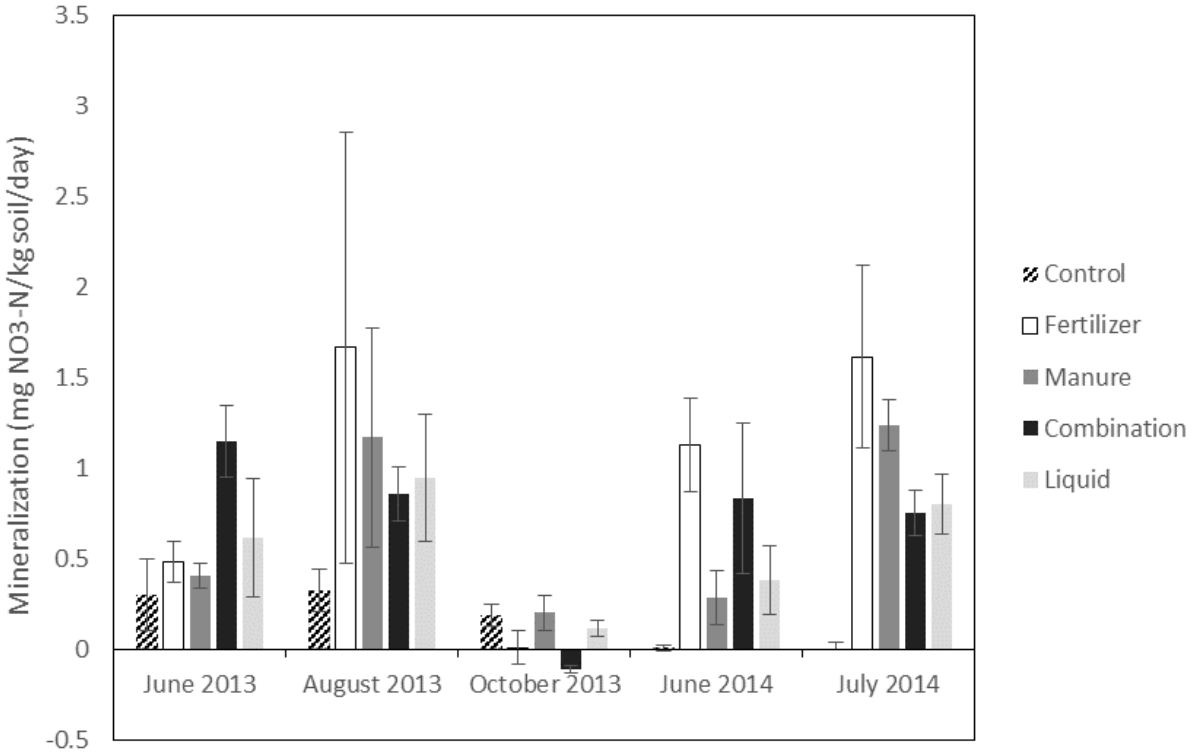


**Figure 27** Net soil N mineralization rates (mg inorganic N/g soil/day) in soils in control, fertilizer, whole manure (manure), whole manure/fertilizer combination (combination) and liquid fraction (liquid) plots over 2 years.