Effects of cover crop and fertilizer incorporation on the structure and function of microbial communities in soils under long-term organic management

A THESIS SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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October 2015

Abstract

Incorporation of organic materials, including cover crop residues and biological fertilizers, is an important element of organic farming practices. These amendments can affect the composition of soil microbial communities that carry out nutrient cycling and perform other functions crucial to crop health and growth. These changes may be detectable through use of 16S rDNA profiling of soil bacterial community composition. We conducted a field experiment at three southern Minnesota sites under long-term organic management to determine the effects of cover crop and organic fertilizer treatments applied in fall 2012 on soil bacterial community structure, nutrient cycling functions, and physicochemical properties during the 2013 growing season. Illumina sequencing of 16S rDNA revealed diverse communities encompassing 45 bacterial phyla in bulk and rhizosphere soil. Cover crop and fertilizer application tended to lower OTU richness and diversity in May 2013 samples; however, these treatments increased diversity in some July 2013 samples compared to a no-amendment control. Fertilizer treatments tended to decrease relative abundance of the ammonia-oxidizing family *Nitrosomonadaceae*, as did some cover crops. Fertilizers that produced large increases in soluble N levels also decreased relative abundance of *Rhizobiales*. Soil functional profiles were more strongly predicted by location than by treatment. Location differences were strongly associated with variance in soil physicochemical parameters. Both treatment and location effects were mediated by soil physicochemical properties including pH, moisture, soil organic matter, and nutrient levels. While cover crop and fertilizer application affected several soil functions, including increases in respiration and enzyme activities in cover crop treatment, these effects were not consistent across locations and sampling time points. Differences in soil function were better explained by using both soil physicochemical test values and community structure data than using soil physicochemical tests alone. Strong associations were observed in both bulk and rhizosphere soil between functional profiles and the families *Cytophagaceae* and *Micrococcaceae*. The *Actinobacteria* overall emerged as the phylum most significantly predictive of functional profiles. We also observed correlations between soil physicochemical parameters and nutrient cycling functions and bacterial family abundances, including strong correlations between pH and several phylogenetically disparate families including *Xanthobacteraceae*, *Pseudonocardiodaceae*, *Propionibacteraceae*, and *Sinobacteraceae*. Strong positive and negative associations with bacterial families were observed for β-glucosidase activity. Bayesian analysis inferred no directional relationships between specific functional activities and particular bacterial families or physicochemical parameters. However, concentrations of NO_3-N, SO_4-S, and OM were each inferred to have parent relationships to the abundance of at least one family. OTU richness (Chao1) had both parent and child relationships to bacterial family abundances. Moisture and pH, although generally highly predictive of bacterial community composition, did not show directional relationships with taxa abundances in the Bayesian analysis. Our findings indicate that cover crop and fertilizer application practices are capable of affecting the microbial environment encountered by subsequent crops. Our results also support the use of sequencing-based microbial community structure profiling as an important tool for increasing our understanding of community structure-function relationships, with the goal of improving our ability to predict the effects of agricultural management practices on important soil functions.

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Introduction

The advent of high-throughput DNA sequencing has enabled researchers to obtain

unprecedented quantities of information about the phylogenetic composition of microbial

communities in environmental samples. Recent years have brought an explosion of research

into the microbiomes of environments ranging from river water and acid mine drainage to

hospital room air, and a surge of scientific and popular interest in the role of the body

microbiome in almost every aspect of human health (Staley et al., 2014; Kuang et al., 2013;

Kembel et al., 2012; Human Microbiome Project Consortium, 2012). Soil, however, is the most

microbially diverse of these habitats, and translating the new wealth of raw information about

the composition of soil communities into insights about the soil functions on which natural and

agricultural ecosystems depend is a complex and challenging task.

Soil microorganisms are responsible for a wide range of functions essential to plant

health and growth. While microbes can sometimes be antagonistic to plants, immobilizing

nutrients or acting as pathogens, microbial action also promotes plant growth through a variety

of crucial functions, including breakdown of plant residues and other organic substrates,

transformation of nutrients into plant-available forms and facilitation of nutrient uptake by

plant roots, suppression of disease-causing organisms, and formation of soil aggregates and

stabilized organic matter (van der Heijden et al., 2008; Ollivier et al., 2011; Miransari, 2013;

Bezemer and van Dam, 2005; Hayat et al., 2010; Lützow et al., 2006; Bronick and Lal, 2005).

The roles of microorganisms in soil function and plant growth are of particular interest

to organic farmers. The foundational tenet of organic farming is that soil health is essential to

crop production, and the USDA's organic standards require growers to choose practices that

"maintain or improve the physical, chemical, and biological condition of soil" as a requirement

of organic certification (National Organic Program, 2015). Many organic farmers also express

both professional interest in and curiosity about soil microorganisms and their activity. "Soil health/biology" was rated as the top research priority in a 2010 survey of organic farmers in Minnesota (Moynihan, 2010). Key management tools used by organic farmers, including tillage, manure, and cover crops, are known to affect both the composition and function of soil communities (Wortman et al., 2013, Carrera et al., 2007)

Researchers working in organic agricultural systems have been quick to explore the application of microbial community sequencing to these soils (Bowles et al., 2014; Gardner et al., 2011; Li et al., 2012). A better ability to predict how management practices will affect both the structure of soil microbial communities ("who's living there?") and their functions ("what are they doing?") will provide a powerful tool for organic growers as they design rotations and systems for soil building. However, the complexity and diversity of soil communities, and their heterogeneity at every scale, increases the challenge of distinguishing the effects of management practices. Our earlier sequencing-based investigation of organic soil (Fernandez et al., 2014), in which we generated bacterial community profiles of samples from six sites where long-term organic and conventional management had been practiced in neighboring fields on the same underlying soil type, underscored this challenge. Despite the unique opportunity presented by these paired soils, bacterial community structure differences caused by organic and conventional management were largely obscured by the high degree of unexplained variability among the samples. Nonetheless, the results of this early experiment suggested that certain bacterial taxa were more susceptible than others to differences in management, inviting further research into the identity and functional significance of taxa affected by farming practices.

The goals of the project described in this thesis were to investigate the effects of cover cropping and organic fertilizer application on bacterial community structure and soil nutrient-

cycling function, and to explore the relationships between soil functions and bacterial community composition and diversity. We used Illumina sequencing of 16S rDNA to generate phylogenetic profiles of bacterial communities in bulk and corn rhizosphere soil following cover crop and fertilizer application at three sites that have been under long-term organic management. We also assayed nutrient cycling functions in these soils, including enzyme activity, soil respiration, and net N mineralization. We tested the effects of our cover crop and fertilizer treatments on these measures of microbial community structure and function, and used a variety of multivariate analyses to explore relationships between structure and function. Our ultimate aim is to improve our understanding how fertility management practices can influence the microbial populations that growers rely on to carry out the functions that support crop growth.

As high-throughput sequencing and the analysis of phylogenetic data become increasingly routine and affordable, it is plausible that soil community profiling could become available to growers as a management tool, alongside the familiar tests of soil nutrient levels and other physicochemical parameters. In this project, we found that many effects of organic material incorporation were inconsistent across sites. The reasons for this inconsistency likely include differences in the microbial communities initially present at these sites before treatment; however, the limited number of sites used in this experiment did not allow us to associate specific pre-treatment microbial community differences with variation in responses to treatment. As new research continues to deepen our understanding of the roles of soil microbial communities in crop production, potential uses of routine farm-field microbial community profiling could include providing site-specific predictions, based on existing microbial communities, of how soils and crops are likely to respond to nutrient addition—for example, phosphorus response, which is notoriously difficult to predict using conventional soil tests

(Heckman et al., 2006; Rosen et al., 2014)—or tillage, inoculation, or other management practices. Microbial community profiling could also be used to retrospectively assess the effects of longer-term cropping and management history on soil biodiversity and function. However, this kind of data will be useful for management decision making only if it is accompanied by a clearer understanding of how differences in community composition can be expected to be reflected in soil functions. Although the findings described in this thesis are not, by themselves, immediately translatable into recommendations for growers, it is my hope that they will contribute to a growing body of research that can clarify how management decisions affect soil microbial communities, and how these effects can be efficiently measured and predicted.

Chapter 1: Structure of soil bacterial communities following cover crop and organic fertilizer incorporation

Abstract

Incorporation of organic materials including cover crop residues and biological fertilizers is an important element of organic farming practice. These amendments can affect the composition of the soil bacterial communities that carry out nutrient cycling and other functions crucial to crop health and growth. We conducted a field experiment at three southern Minnesota sites to determine the effects of cover crops and fertilizers on bacterial community structure in agricultural soils under long-term organic management. Illumina sequencing of 16S rDNA revealed diverse communities encompassing 45 bacterial phyla in corn rhizosphere and bulk field soil. Community structure was most affected by location and bulk vs. rhizosphere soil, followed by sampling time and amendment treatment. Both treatment and location effects were largely mediated by soil physicochemical properties including pH, moisture, soil organic matter, and nutrient levels. Changes in soil bacterial community structure associated with organic matter addition were transient to some extent. However, treatment differences were still apparent in both bulk and rhizosphere soils at the time of peak corn growth in the season following cover crop and fertilizer application, indicating that these practices are capable of affecting the microbial environment encountered by subsequent crops. Cover crop and fertilizer treatments applied in fall 2012 tended to lower OTU richness and diversity in May 2013 samples; however, winter rye, oilseed radish, and buckwheat cover crops increased diversity in some July 2013 samples compared to a no-amendment control. Fertilizer treatments tended to decrease relative abundance of the ammonia-oxidizing family *Nitrosomonadaceae*, as did some cover crops. Pelleted poultry manure and Sustane (commercial fertilizer), which produced large

increases in soluble N levels, decreased relative abundance of *Rhizobiales*. Our findings highlight

the importance of site differences and soil heterogeneity in structuring bacterial communities,

and point to a need for future research exploring the how the influence of cover crops on

bacterial communities differs with biomass composition and quantity, as well as research on

how existing soil conditions and microbial community composition can influence how soil

microbial populations will respond to agricultural management practices.

Introduction

Cover cropping and the incorporation of biological fertilizers are crucial aspects of

management of soil quality and fertility in organic systems. Cover crops are valued for their

ability to provide physical protection of soil, increase soil organic matter, protect soil nutrients

from leaching, and, in the case of legumes, provide biologically-fixed N (Wyland, 1996; Snapp et

al., 2005). In addition to direct effects on soil structure and nutrients, cover crops and organic

fertilizers can affect soil function through changes in soil microbial communities, which are

responsible for crucial soil functions, including nutrient cycling and availability, transformation

and breakdown of crop residues, and disease suppression or susceptibility (van der Heijden et

al., 2008; Garbeva et al., 2004).

Other studies have investigated the impacts of organic matter incorporation on changes

in soil microbial communities, often using profiling techniques that are culture-independent but

not sequencing-based, such as phospholipid fatty acid (PLFA) or terminal restriction fragment

length polymorphism (T-RFLP) analyses. Organic fertilizers and soil amendments have been

shown to have effects on soil microbial communities distinct from those produced by synthetic

fertilizers, and organic material addition in general tends to produce increases in total microbial

activity and rates of nutrient cycling, and can sometimes increase microbial diversity (Fließbach et al., 2007; Stark et al., 2008; Marchner et al., 2003, Li et al., 2012; Ngosong et al., 2010). Amendments of differing composition can produce various changes in soil microbial communities, including parameters such as total microbial activity and ratios of gram+:gram- bacteria and bacterial:fungal ratios (Pieta and Kesik, 2008; Buyer et al., 2010; Bowles et al., 2014; Wortman et al., 2013).

However, the availability of high-throughput DNA sequencing technology now allows these effects to be explored in unprecedented detail. New studies using sequencing of soil microbial community DNA extracts have begun to elucidate differences between organic- and conventionally-managed agricultural soils, including the relative abundance of bacterial groups at various taxonomic levels, total community diversity, and physicochemical drivers associated with differences in community composition (Li et al., 2012; Gardner et al., 2011; Acosta-Martinez et al., 2008; 2010) However, much remains to be learned about the effects of specific practices within these systems. The sequencing of 16S rDNA provides only a partial window into the whole soil community, as it does not detect fungal or other eukaryotic organisms. However, the data provided by this method may be particularly relevant in agricultural soils, as high-intensity agricultural management is generally considered to lead to greater dominance by bacteria relative to fungi (Bailey et al., 2002). There is also an increasing understanding that farm soils under long-term organic and conventional management may show different responses to the same practices, highlighting the need for research on organic practices to be conducted within organic systems, preferably working farms (Stark et al., 2008; Fliessbach et al., 2000).

Here we investigated the influence of four cover crops, with very different growth habits and chemical properties – hairy vetch (*Vicia villosa*), winter rye (*Secale cereale*), buckwheat

(*Fagopyrum esculentum*), and oilseed radish (*Raphanus sativus*)--on soil microbial community structure. We also examined how three organic fertilizers, with contrasting chemical and microbial compositions – beef manure, pelleted poultry manure, and bagged commercial organic fertilizer--affected microbial community structure and diversity. These treatments were designed to represent practices available to organic farmers in the Upper Midwest. Illumina sequencing of soil 16s rRNA was used to measure changes in composition and diversity of bacterial communities as a result of amendment treatments. The overall goal of these studies is to improve our understanding of how cover cropping and fertility management practices can influence the microbial populations that growers rely on to carry out important soil functions – those that affect every aspect of crop health, from nutrient cycling and uptake, to disease susceptibility and suppression.

Recent research in microbial biogeography indicates that a vast "seedbank" of bacterial taxa exists in environmental samples, from which particular taxa may rise to abundance as conditions favor their predominance (Lennon and Jones, 2011; Nemergut et al., 2013; Fierer and Lennon, 2011). Agricultural management practices can change the microbial community of a given soil, and its associated functions. This occurs through their effects on biotic and abiotic conditions prevailing in that soil, opening the possibility of manipulating microbial communities to achieve improvements in soil function and crop production. As high-throughput DNA sequencing becomes much more affordable and big-data analysis becomes routine, high-resolution microbial community profiling may one day be added to the suite of tools available to farmers for management decision-making.

Materials and Methods

Field experiment

Study fields were established at Farmington, Lamberton, and Madison, MN. The Farmington and Madison sites were on the land of collaborating farmers, and the Lamberton site was on the University of MN's Southwest Research and Outreach Station. Site details are shown in Table 1.1. All sites had at least seven years under organic certification and at least fifteen years in organic management. Experimental design was a randomized complete block with four replications at each site. Eight treatments were used: four cover crops, three fertilizers, and a no-amendment control. 6.1 x 9.1 m with 6.1 m alleys to prevent transport of soil among experimental plots. Plot layout was established and study fields were planted to barley between March 28 and April 7, 2012. Barley was removed and fields were tilled in July of 2012, then cultivated to remove volunteer barley immediately before cover crop planting. Cover crops were planted August 13-15, 2012.

Cover crop treatments were hairy vetch (*Vicia villosa*); winter rye (*Secale cereale*); oilseed radish (*Raphanus sativus*); and buckwheat (*Fagopyrum esculentum*). Hairy vetch (HV), winter rye (WR), oilseed radish (OR), and buckwheat (BW) seeding rates were 22, 112, 22, and 56 kg ha^{-1}, respectively. Fertilizer treatments were pelleted chicken manure (CP), beef manure (BM), and Sustane (SU). Percent N-P-K content was 5-3-2 for CP, 1-1-1 for BM, and 8-2-4 for Sustane. A no-amendment control treatment was used (NC). Fertilizers were applied October 30-November 1, 2012. Application rates were 10.5, 7.7, and 3.9 Mg ha^{-1} for chicken manure, beef manure, and Sustane, respectively. Fertilizer plots were tilled to incorporate immediately after application. Fields were tilled in April 2013 to incorporate cover crop residue and terminate overwintered rye and vetch, and were planted to corn (*Zea mays*) in May 2013.

Tillage operations were conducted perpendicular to wide alleys to prevent transport of soil among experimental plots.

Due to very dry conditions, cover crop establishment in fall of 2012 was not consistent. Cover crop plots that produced less than 20% ground cover in fall 2012 were considered treatment failures and were excluded from analysis. Due to these establishment failures, the HV treatment was included in analysis only at the Farmington site, and BW was included only at Farmington and Lamberton.

Soil sampling

Samples of bulk field soil were collected in August 2013 immediately following cultivation and prior to planting of cover crop treatments, in May 2014 immediately before corn planting, and in July 2013 when corn was at the V13 (late vegetative) stage. Ten cores per plot at a depth of 10 cm were collected using a 2-cm diameter hand probe. Cores were randomly distributed across the plot, avoiding corn root zones in the case of July 2013 sampling. Cores were bulked at the time of sampling in August 2012. For May 2013 and July 2013 samplings, each core was placed in a separate bag for use in enzyme assays and sequencing. An additional ten cores were collected from each plot and placed in a single bag; these were later homogenized and used for N mineralization and soil respiration assays and for chemical analysis. Samples were transported on ice, and were stored at -20 C upon arrival in the laboratory.

Corn rhizosphere soil was collected at the July 2013 sampling date. Whole roots of three plants were taken from each plot and shaken to remove non-adhering soil. Roots were coarsely chopped and approximately 30 mL of root pieces with adhering soil were agitated on a shaker table for 30 minutes in a $(NH_3)_4PO_4$ and 1% gelatin buffer (Kingsley and Bohlool, 1981). Root

pieces were removed and the soil suspension was centrifuged at 10,000xg for 20 minutes. The

supernatant was discarded and the pelleted soil + microbial fraction was stored at -20 C.

Samples were submitted for analysis to the University of Minnesota Research Analytical

laboratory (protocols are available at http://ral.cfans.umn.edu/soil-analysis-and-methods/).

Parameters analyzed (Table 1.2) were: Bray-P, K, NO_3-N, SO_4-S, organic matter (OM),

exchangeable cations (Mg, Na, and Ca), and pH.

16S rDNA sequencing

For the August 2012 bulk soil samples, the ten soil cores from each plot were

homogenized to produce a single plot sample. For the May and July 2013 samples, three

subsamples were used, each consisting of three homogenized cores. DNA was extracted from

homogenized bulk soil samples and from pelleted rhizosphere soil using MoBio PowerSoil kits

(MoBio Laboratories, Carlsbad, CA). Amplicon preparation and sequencing were performed by

the University of MN Genomics Center. 16S rDNA from the V5-V6 hypervariable region was PCR

amplified using Nextera primers and barcode indexed as previously described by Staley et al.

(2014). Amplicons were paired-end sequenced on Illumina MiSeq at a read length of 2x300 bp.

Sequence data were processed and analyzed using mothur (Schloss et al., 2009).

Sequences were trimmed to 160 bp, paired-end joined, and screened for quality. Sequences

were removed if they had a quality score < 35 over a window of 50 nt, had a mismatch to a

barcode sequence, had >1 mismatch to a primer sequence, had homopolymers > 8 nt, or had an

ambiguous base (N). Singleton sequences and sequences identified as chimeras by UCHIME

(Edgar et al., 2011) were removed. Sequences were aligned to the SILVA database (Quast et al.,

2013). Sequence read numbers were standardized by random subsampling to 35,724 reads per

sample, and sequences corresponding to chloroplast lineages were removed. Sequences were

clustered into OTUs using the average-neighbor algorithm at 97% similarity. Due to dataset size, OTU clustering was performed within locations and sampling timepoints. OTUs were classified to the SILVA database. For May and July 2013 bulk samples, reads from the three subsamples were merged to create a single composite sample per plot, which was normalized to 97,124 (May) or 91,684 (July) reads per sample. Sequences will be deposited to the National Center for Biotechnology Information's GenBank Sequence Read Archive when an accession number is assigned.

Data analysis

For all mothur analyses, unless otherwise stated, replicates were maintained as separate samples and grouped by using the .design files. The significance of treatment effects was tested using analysis of molecular variance (amova) on Yue and Clayton distance matrices, which is similar to a non-parametric analysis of variance (Excoffier et al., 1992). Principal component analysis, principal coordinate analysis, and discriminant function analysis (DFA) were performed using XLStat software (Addinsoft Co., New York, NY). Redundancy analysis and analysis of variance were performed in R using packages vegan, nlme, and lmerTest (R core team, 2014; Oksanen et al., 2015; Pinhiero et al., 2015; Kuznetsova et al., 2014). All statistical analyses were done at α = 0.05.

Results and Discussion

Composition of bulk and rhizosphere bacterial communities

Mean numbers of OTUs identified per sample were 1971 ± 1394 for October 2012 bulk soil, 4258 ± 1248 for May 2013 bulk soil, 3945 ± 1157 for July 2013 bulk soil, and 1549 ± 866 for

July 2013 rhizosphere soil (Table 1.3). OTUs were classified to 45 bacterial phyla, with 0.01% of

reads unclassified at the phylum level. The bacterial community in bulk soils was predominantly

composed of members of the phyla *Actinobacteria* (35.0% and 50.1% of reads in October 2012

and July 2013, respectively), *Proteobacteria* (32.0 and 23.3%), *Bacteroidetes* (9.7% and 5.0%),

and *Acidobacteria* (6.3% and 6.0%). This is a greater predominance of *Actinobacteria* than

generally reported for soil samples, likely due to increasingly dry conditions over the sampling

period (Barnard et al, 2013). Rhizosphere soils were dominated by members of phyla

Proteobacteria (63.3% in July 2013), *Actinobacteria* (19.7%), *Bacteroidetes* (5.8%), and

Acidobacteria (2.9%).

Factors affecting bacterial community composition

　　　　Overall variation in bacterial community structure among samples was associated

strongly with bulk-rhizosphere differences and sampling locations. Smaller, but notable, levels of

variation were associated with treatments (Figure 1). These patterns were consistent across

analyses at various taxonomic levels; variation in family composition is shown here. The

predominant effects of location, which represented quite distinct underlying soil types, and

bulk-rhizosphere differences, are consistent with previous findings (Girvan et al., 2003; Bossio et

al., 1998). The greater similarity of taxa from the Madison and Lamberton samples to one

another compared to taxa from the Farmington site is consistent with the similarity of these

sites in latitude and rainfall, as well as their classification by soil type, with Madison and

Lamberton situated on finer-textured soils compared to the relatively sandy Farmington site.

This is reflected in differing compositions of the bacterial communities, evident at the phylum

level, at the three sites before treatments were applied (Figure 2).

Effects of amendment treatments

Discriminant analysis was used to generate axes consisting of linear and quadratic combinations of predictor variables (bacterial family abundances) along which treatment centroids were maximally dispersed. Although amendment treatments were responsible for a relatively small portion of variation among samples, treatments could be significantly dispersed by bacterial community composition in both bulk and rhizosphere post-treatment soils (Wilks λ p=0.011 for rhizosphere, p<0.0001 for bulk). Bacterial families making the greatest contributions to dispersion among treatments differed in the bulk compared to the rhizosphere soils (Table 1.4). The *Bacillaceae* (p=0.001), *Sphingobacteriaceae* (p=0.002), and *Pseudonocardiaceae* (p=0.002) were most associated with treatment dispersion in the bulk soil, while the *Sanguibacteraceae* (p<0.001), *Microbacteriaceae* (p<0.001), and *Sphingobacteriaceae* (p<0.001) were most associated with dispersion in the rhizosphere soil.

The significance of amendment treatment effects was confirmed by amova comparisons of OTU composition within timepoints and locations (Table 1.5). OTU composition was significantly affected by treatment at Farmington (p<0.001), Lamberton (p=0.047), and Madison (p=0.02) in May 2013. However, by July 2013, treatment effects were no longer significant in bulk soil at Lamberton and Madison. In contrast, the rhizosphere community at these locations was affected by treatment (p=0.044 and p=0.048 at Lamberton and Madison, respectively), but did not differ significantly with treatment (p=0.118) at Farmington. This indicates that organic material additions may affect the immediate microbial environment encountered by crop roots even if those effects are not detectable in the bulk field soil. CP consistently differed from the no-amendment control, and appeared most frequently in differing treatment pairs designated by amova, confirming the indication by principal component analysis (Figure 1) and redundancy analysis (Figure 3) that CP was the treatment that most strongly altered the bacterial

community. As described more fully in Chapter 2, this treatment was also the one that produced the most dramatic changes in nutrient and pH levels. Of the cover crops, WR differed most frequently from other treatments, likely related to the relatively large biomass production of this crop.

Relationship of treatment effects to soil physicochemical changes

Variance partitioning by redundancy analysis indicated that treatment and location effects were largely mediated by the soil physiochemical properties outlined in Table 1.2. As we will discuss in Chapter 2, all parameters differed markedly among locations, while pH and NO_3-N were significantly affected by amendment treatments. Of the total variance in bacterial family composition that was explainable by amendment treatments and soil physicochemical test values, soil physicochemical parameters explained 79.2% of variance, while only 4.9% was uniquely attributable to treatment differences, and 15.9% was collinear between treatment and physicochemical parameters. For location effects, 39.9% of total explainable variance in bacterial community structure was unique to physicochemical parameters, 3.3% was unique to location, and 56.7% was collinear between location and physicochemical parameters. This indicates that the tested parameters, including pH, moisture, OM, and nutrients, were likely the main mechanisms driving the observed difference in community structure among locations and treatments.

OTU richness and diversity in bulk and rhizosphere soils

Treatments affected OTU richness and diversity of both bulk and rhizosphere soils (Table 1.6). Much greater OTU richness and diversity were found in bulk soil than in rhizosphere soil. In most cases, where amendments significantly differed from control, species richness

and/or diversity was lowered by amendments. This was particularly evident in the CP treatment, although CP increased diversity in the July sampling of Lamberton bulk soil. This contradicts the common assertion that organic matter addition promotes soil biological diversity, suggesting that instead, incorporated material may reduce evenness in the short term by favoring coptiotrophic taxa (Fierer et al., 2012). This is also consistent with our observation that the CP and SU treatments produced some effects that were similar to other forms of highly available N fertilizers, including inorganic fertilizers: very large, but not sustained, spikes in soil NO_3-N, and slight decreases in pH, as described more fully in Chapter 2.

Some increases in OTU richness and diversity were measured in July that were not present in May, possibly indicating increasingly diverse substrate availability as organic material was broken down by soil microbiota. Microbial diversity is generally considered to be advantageous to soil health and function, and is a particularly desired goal among organic growers that is often implied to be a part of the "package" of soil quality benefits promoted by organic practices (Sullivan, 1999). However, within the short timeframe encompassed by this study, it was not clear that organic amendments had a consistent effect on community diversity, nor what the likely duration of that effect would be.

Differences in community composition between individual treatments as assessed by amova did not always correspond to differences in overall OTU richness and diversity. This is not unexpected, as it is not unusual for diversity to remain similar while community structure differs with management (Wu et al., 2008). Although some treatment effects were transient, as indicated by amova tests of OTU composition, they were sufficiently persistent to produce differences in rhizosphere communities in July 2013, reflecting the immediate biotic environment experienced by crop roots at the time of peak nutrient uptake, and therefore likely

the most applicable measure of the potential of these management treatments to affect

biological functions relative to crop production.

Amendment effects on functionally significant taxa

Amendment treatments were associated with differences in abundance of key taxa

associated with important soil function (Table 1.7). As with diversity and total composition,

effects observed in the May 2013 bulk soil samples had largely faded by July 2013, although

differences were apparent in the July 2013 rhizosphere samples. Fertilizer treatments,

particularly CP, tended to decrease relative abundance of the ammonia-oxidizing family

Nitrosomonadaceae, as did cover crops in some cases at Farmington. Reduced abundance of

these organisms is associated with reduced denitrification activity (Wessen et al., 2011). Relative

abundance of members of the class *Rhizobiales*, which includes the symbiotic nitrogen fixing

root nodule bacteria *Rhizobium* and *Bradyrhizobium*, was indistinguishable from the control in

cover crop treatments, including hairy vetch. However, *Rhizobiales* abundance was reduced in

both bulk (at Farmington and Madison) and rhizosphere (at Madison) soils in CP, suggesting that

large soluble N additions may be directly detrimental to populations of potential microbial

symbionts even when a legume host is not present, in addition to decreasing the effectiveness

of symbiotic N fixation when host plants are available. (Salvagiotti et al., 2008).

The phylum *Actinobacteria* includes many metabolically unique members, and its

prevalence, as well as those of subgroups *Actinomyces* and *Streptomyces,* has been associated

with disease suppressiveness (Mendes et al., 2011; Pankhurst et al., 2002; Peng et al., 1999;

Wiggins and Kinkel, 2005). Relative abundance of *Actinobacteria* was not affected by treatment

in bulk soils; however, it was increased in the corn rhizosphere community in OR at Lamberton

and in WR at Madison. Rye and radish or mustard cover crops have been associated with

reduced crop disease incidence in some, but not all, studies (Larkin et al., 2010; Hartz et al., 2005; Hartwig and Ammon, 2002). This is in contrast with the findings of Fierer et al. (2012), who observed that *Actinobacteria* increased in abundance with N addition, as part of a broader shift toward copiotrophic taxa in communities in high-N environments. Prevalence of members of the phylum *Firmicutes*, also associated with disease suppression, was not affected by amendment treatment.

Phylogenetic characterization of core and treatment-affected OTUs

OTU composition of the July 2013 bulk and rhizosphere datasets was examined at each location, along with two subsets of OTUs: those appearing in every sample at a location regardless of treatment, which we have designated as "core" OTUs, and those whose abundance differed significantly (Kruskal-Wallis *p*<0.05) by treatment within a location, which we have designated as "treatment-affected" OTUs. The composition of these subsets was observed to differ at the phylum, order, and family levels from the overall dataset. In both bulk and rhizosphere soil, *Actinobacteria* in general and *Actinomycetacaea* in particular, and also *Rhizobiales,* are overrepresented among treatment-affected OTUs, while *Planctomycetaceae* were underrepresented in both core and treatment-affected OTUs. *Acidobacteriales* and *Burkholderiales* were overrepresented in core OTUs but not in treatment-affected OTUs.

We suggest two possible explanations for these observations: 1) the representation of groups in core and treatment-affected OTUs may reflect the ecological or metabolic significance of these groups. The groups that are overrepresented in both core and treatment-affected OTUs, particularly *Actinomycetacea* and *Rhizobiales*, fill unique and important niches in the biotic community, niches which vary in their tendency to be affected by the treatments applied in this experiment. Conversely, the underrepresentation of the *Planctomycetes*, which are generally considered to be aquatic, may suggest that, rather than integral members of the

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community, they are residents of scattered microhabitats which happened to appear in some of the extracted soil portions. Groups overrepresented only in the core OTUs may be relatively cosmopolitan. 2) Alternatively, groups overrepresented in core or treatment-affected OTUs may be those whose 16S similarity tends to be better aligned with metabolic similarity, which would result in OTU clustering being a better identifier of ecologically coherent units. In that case, the patterns noted could be considered potentially an indicator of the mutation/horizontal gene transfer habits of those groups, but also to some degree a methodological artifact of OTU-based community profiling. Although determining the extent to which OTUs represent ecologically and metabolically coherent groupings is an identified challenge in the interpretation of environmental sequence data, much remains to be learned about how this coherence differs across phylogenetic units (Schmidt et al., 2014; Koeppel and Wu, 2013; Nowell et al, 2014; Kopac et al., 2014).

Conclusions

In field soils under long-term organic management, we observed differences in soil bacterial community structure associated with locations (soil types), time of sampling, bulk vs. rhizosphere soil, and cover crop and organic fertilizer treatment. Variance in bacterial community composition was strongly associated with soil physicochemical parameters, which explained much of the variance associated with location. Treatment effects were comparatively small, but still produced significant effects on bacterial community composition, including differences in OTU richness, diversity, and relative abundance of functionally significant taxa in both bulk and rhizosphere soils. Although effects were not consistent across sites, addition of highly available N, in the form of pelleted poultry manure, most sharply affected bacterial community structure, resulting in reduced diversity and decreased abundance of functionally

significant taxa. Cover crops, particularly winter rye, showed potential to increase bacterial diversity and the abundance of functionally significant taxa. However, these effects were inconsistent, and opposite effects were also observed in some cases.

Our results underscore the importance of soil type as a driver of bacterial community composition, as well as the high variability stemming from heterogeneity in the soil environment at all scales. Future research will be needed to further explore the influence of cover crop biomass composition and quantity on the type and persistence of bacterial community changes. Work will also be needed to refine techniques for extracting the "signal" of treatment effects on soil bacterial communities from the "noise" of sample and site heterogeneity, and to better understand how existing communities and conditions, shaped by soil type and site history, affect and predict the changes that management practices can produce in soil microbial community structure.

Chapter 2: Associations between soil bacterial community structure and soil nutrient cycling function in long-term organic farm soils following cover crop and organic fertilizer amendment

Abstract

Agricultural management practices can produce changes in soil bacterial populations whose functions are crucial to crop production. These changes may be detectable through profiling of soil bacterial community composition using high-throughput sequencing of 16S rDNA, which is becoming increasingly routine. However, if we hope to apply sequencing-derived bacterial community structure data to on-farm decision-making, we will need to better understand the complex and variable associations between soil microbial community structure and soil function. Here we used 16S sequencing to generate phylogenetic profiles of soil bacterial communities following the application of cover crop and organic fertilizer treatments in certified organic field crop systems. We measured the effects of these treatments on soil nutrient-cycling functions including enzyme activities, net N mineralization, and soil respiration; and soil physicochemical properties including nutrient levels, organic matter (OM) and pH. We then assessed the relationships between these functional and physicochemical parameters and soil bacterial community structure. Soil functional profiles were more strongly predicted by location than by treatment, and location differences were largely explained by soil physicochemical parameters. We observed treatment effects on several soil functions, including increases in N-acetyl-β-D-glucosaminidase and β-glucosidase activity associated with buckwheat and oilseed radish cover crops and pelleted poultry manure. However, these effects were not consistent across locations and sampling timepoints. Composition of soil bacterial communities at the family and phylum level was predictive of soil functional profiles, and individual correlations

were observed among functional parameters and relative abundances of individual bacterial families and phyla, although Bayesian analysis showed no inferred directional relationships between specific functional activities and particular bacterial families or physicochemical parameters. Differences in soil function were better explained using both soil physicochemical test values and bacterial community structure data than using soil tests alone. This indicates that pursuing a better understanding of bacterial community composition and how it is affected by farming practices is a promising avenue for increasing our ability to predict the effects of management on important soil functions.

1. Introduction

Soil is home to a diverse and heterogeneous range of microorganisms, which mutually interact with aboveground plant life in many ways. This is particularly important in agricultural soils, where microbes carry out soil functions that are critical to crop health and performance. Microbial communities are responsible for the decomposition of crop residues, formation of stabilized soil organic matter, and soil aggregate structure, affecting O_2 availability and water infiltration and retention (Lützow et al., 2006; Bronick and Lal, 2005). The microbial environment also influences key transformations governing the availability and uptake of most macro- and micronutrients, and the type, extent, and prevalence of plant disease (Bezemer and van Dam, 2005; Hayat et al., 2010; Miransari, 2013). Bacterial community structure is strongly shaped by abiotic soil characteristics, particularly pH, soil texture (affecting water holding capacity, and O_2 availability), and redox potential (de Vries et al., 2012; Lauber et al., 2009; Nemergut et al., 2013). However, inoculation or transplant of differing microbial communities within the same soil can also result in substantial functional differences (Strickland et al. 2009; Reed et al., 2013).

Recent advances in high-throughput DNA sequencing technology have provided insight into the composition of environmental microbial communities at an unprecedented level of resolution. Next-generation sequencing of 16S rDNA from soil samples is an attractive tool to investigate bacterial and archaeal community structure and function, as protocols for sequencing and pipelines for the analysis of 16S data are becoming increasingly well-established and, with sufficient sequencing depth, can yield detailed phylogenetic profiles including both abundant and rare taxa (Boon et al., 2014; Schmidt et al., 2014).

While it is conceivable that soil microbial community profiling could become available to crop advisors or growers as a management tool, 16S rDNA profiles do not provide direct information about the functional attributes of the microbial communities studied. In contrast, full metagenome sequencing and mRNA-based sequencing methods, which can directly investigate functional genes, require extensive sequencing and data analysis resources and/or specialized sample handling. If phylogenetic profiling of microbial communities is to be useful in management decision-making, the complex and variable associations between soil microbial community structure and soil function will need to be better understood, in order to interpret the significance and implications of the detailed community structure differences that are now observable using sequencing-based methods.

Because the enormous diversity within soil microbial communities may produce extensive functional redundancy among taxa, increases in microbial phylogenetic diversity may not correspond to increases in functional diversity, and changes in community structure and function do not necessarily co-occur (Fierer et al., 2012; Six et al., 2006). It is also not yet clear to what extent microbial community structure data can improve predictions of soil function, compared to routine soil tests (Bowles et al., 2013). Previous investigations of relationships between microbial community structure and function have generally explored communities that

were distinguished by large-scale differences in environment or land use, or had limited diversity (Kuramae et al., 2012; Lozupone and Knight, 2007; Fierer et al., 2012b; Keith et al., 2012; Drenovsky et al., 2010; Naether et al., 2012). Cropping systems present a distinct subject for research, as they are subject to intensive regimes of disturbance, nutrient management, and various chemical interventions, all of which have been shown to affect soil bacterial community structure in sequencing-based studies (Sengupta and Dick, 2015; Lavecchia et al., 2015, Jacobsen and Hjelmsø, 2014).

Established organic systems are also a specialized case, because these systems are subject to a distinct management regime that produces lasting soil differences that may affect the responses of soil microbial communities to specific practices (Fliessbach et al., 2000; Mallory and Griffin, 2007). Organic farmers, who depend on biologically-derived nutrient sources, choose among a wide range of animal and green manuring practices to add organic matter and nutrients to soils. Breakdown of organic material additions in agricultural soils, and the resulting release of nutrients in crop-available forms, depend on the composition of the added material and the microbial communities present in the soil (Hadas et al., 2004; Majumder and Kuzyakov, 2010). The composition and function of soil microbial communities are shaped by agricultural management practices, including crop and rotation selection, tillage regimes, and fertilization practices (Berg and Smalla, 2009; Gardner et al., 2011). The response of soil community structure and function to organic amendments is affected both by the quantity and composition of organic material applied, and also by the community present in the soil before amendment addition (Stark et al., 2008; Xue et al., 2013). Organic matter additions of differing chemical compositions, particularly differing C:N ratios, can have distinct effects on soil microbial diversity and activity (Bending et al., 2002; Carrera et al., 2007; Bowles et al., 2014). Within the context of longer-term management systems, these short-term applications of plant- and animal-derived

organic materials can affect microbial nutrient-cycling functions, thereby determining the rate and timing of nutrient immobilization, or release to crop plants (Stark et al., 2008; Monaco et al., 2008; Wortman and Francis, 2012). This was especially noted by Gardner et al. (2011), who found variation in nutrient cycling enzyme activity co-occurring with variation in microbial community structure that was associated with different crops in organic systems.

In general, the addition of either animal or green manures would be expected to increase soil activity levels; however, microbial community responses to organic matter addition are often different in organic systems, where additions of animal and green manures are routine and necessary components of fertility management, and baseline soil organic matter levels are generally higher (Marriott and Wander, 2006; Stark et al., 2008). Previous research in organic management, which in the U.S. has focused largely on vegetable production systems, cannot always be generalized to field crop systems, as production and crop rotation practices may be extremely different. The agronomic rotations practiced by organic farmers in the Corn Belt are a major contributor to organic production. To our knowledge, this is the first application of sequencing-based methods of microbial community investigation in these systems.

A field experiment was done at three Minnesota locations with at least 15 years of organic management history. The objectives of this work were to determine the 1) effects of cover crops and organic fertilizers on microbial community activity and nutrient cycling in established organic field cropping systems, and 2) relationships between soil bacterial community structure and nutrient-cycling functions in the context of organically-managed agricultural field soils. We also analyzed the effects of amendment treatments on soil physicochemical parameters, and the relationships between functional measures and these parameters, in order to explain mechanisms by which organic matter amendments can affect soil microbiological activity. Physicochemical parameters measured included pH, OM, moisture,

and nutrient levels. Soil functional profiles consisted of corn crop yield, and five measures of microbial activity: 1) total respiration, indicating general activity and substrate turnover; 2) net N mineralization; and 3) activity of three enzymes: β-glucosidase, N-acetyl-β-D-glucosaminidase (NAGase), and phosphatase. This study will help to establish whether 16S rDNA-generated bacterial community profiles have sufficient sensitivity and resolution to show functionally-relevant distinctions among soils within the relatively narrow range of variation generated by agricultural management practices.

2. Materials and Methods

2.1 Field experiment

A field plot experiment was conducted in 2012-2013 at the University of Minnesota's Southwest Research and Outreach Center in Lamberton, MN, and two cooperating farm sites in Farmington and Madison, MN. Soils at Farmington, Lamberton, and Madison were classified as Wadena loam (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls), Webster clay loam (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls), and Normania loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls), respectively. All sites were certified organic for at least seven years and under organic management for at least 15 years. At each site, a randomized complete block experimental design was used with four replicates. Eight experimental treatments were applied (Table 2.1), consisting of four cover crops, three organic fertilizer additions, and a no-amendment control. Cover crops were hairy vetch (*Vicia villosa*), winter rye (*Secale cereale*), oilseed radish (*Raphanus sativus*), and buckwheat (*Fagopyrum esculentum*). Fertilizers were beef manure, pelleted poultry manure

(Chickety Doo Doos^{TM}, Unlimited Renewables, Onalaska, WI), and Sustane® 8-2-4 (Sustane,

Cannon Falls, MN). Cover crops were planted in August 2012 and fertilizers were applied and

incorporated in October 2012. The sites were tilled and planted to corn in April 2013.

2.2 Sample collection

Soil samples were collected in August 2012 prior to treatment application, in May 2013

following corn emergence, and in July 2013 at the V13 (late vegetative) stage. Bulk and corn

rhizosphere soil samples were collected from each plot in July 2013. Corn rhizosphere soil was

obtained by shaking corn roots in a solution of 0.1 M $(NH_4)_3PO_4$ and 0.1% gelatin for 30 min to

elutriate adhering soil. The recovered suspension was centrifuged at 10,000 x g for 20 minutes

to obtain a pellet of rhizosphere soil and microbes. Bulk soil was sampled with a hand probe to a

depth of 10 cm. Two sets of bulk soil samples were collected from each plot. One set was frozen

at -20 C immediately upon arrival in the laboratory and was used for DNA extraction. The other

set was placed in refrigerated storage and submitted to the University of MN Research

Analytical Laboratory (St. Paul, MN) for chemical analysis. All measured variables are

summarized in Table 2.2. Experimental sites, plot layout, and timing and protocols for cover

crop and fertilizer application and soil sampling are described in detail in Chapter 1.

2.3 Nitrogen mineralization assay

Net N mineralization was measured using procedures similar to those described by Riggs

et al. (2015). Fifty g aliquots of soil were placed in specimen cups and covered with polyethylene

film. Cups were incubated at 20° C for 28 d. Soil was sprayed weekly with DI H_2O to restore to

initial sample moisture level. At 0, 7, and 28 d, 10g of soil was removed from incubations and

shaken in 1.0 M KCl solution for 30 min to extract soluble N. The solution was filtered to remove

suspended soil. The N content of KCl extracts was quantified using colorimetric salicylate

(ammonium) and vanadium (nitrate/nitrite) assays.

2.4 Soil respiration assay

Fifty g aliquots of soil were placed in 473-mL glass Mason jars and sealed with lids fitted

with rubber septa. Jars were incubated in the dark at 20°C for 48 h. Triplicate 5-mL samples of

headspace gas were drawn from the jars immediately after closing and following 24 and 48 h of

incubation. Gas samples were analyzed for CO_2 content using a gas chromatograph (Shimadzu

GC14, Shimadzu Scientific Instruments, Wood Dale, Illinois). Standards (0, 645, 1025, and 10,000

ppm CO_2) were used to construct a standard curve for calculating CO_2 evolution.

2.5 Enzyme activity assays

Methylumbelliferone (MUB)-linked substrates were used to measure potential enzyme

activity (Darrah and Harris, 1986). Phosphatase, N-acetyl-β-D-glucosaminidase (NAGase), and β-

glucosidase were measured for each subsample from the May 2013 and July 2013 timepoints. β-

glucosidase is involved in soil C cycling, N-acetyl-β-D-glucosaminidase (NAGase) is a chitinolytic

enzyme contributing to N and C cycling, and phosphatase is responsible for the release of plant-

available phosphate groups from larger organic molecules. Soil aliquots (0.5 g) were suspended

in 50 mL 100 mM maleic acid buffer (pH 6.8) and 0.25-mL assays were conducted in 96-well

plates. Sixteen analytical reps of the assay wells were used, and eight reps of the quench, soil

control, negative control, reference standard, and blank wells. A 25 μM MUB solution was used

as a reference standard and each assay contained 0.01 μmol substrate. Plates were incubated

for 1 h at 25° C for all substrates. Reactions were stopped by adding 10 μL of a 0.5 M NaOH

solution. Ten min after NaOH addition, fluorescence of the plate wells was read by a plate

reader at 365 nm emission and 450 nm fluorescence.

2.6 Bacterial community 16S rDNA sequencing

For the August 2012 bulk soil samples, the ten soil cores from each plot were

homogenized together to produce a single plot sample. For the May and July 2013 samples,

three subsamples were used, each consisting of three homogenized cores. DNA was extracted

from homogenized bulk soil samples and rhizosphere soil using MO BIO PowerSoil kits (MO BIO

Laboratories, Carlsbad, CA). Amplicons were prepared using Nextera primers. Barcode indexing

and sequencing of 16S rDNA (V5-V6 hypervariable region) were done by the University of MN

Genomics Center (St. Paul, MN). Amplicons were paired-end sequenced on an Illumina MiSeq

platform at a read length of 2 x 300 bp. Sequencing and sequence data processing were done as

described in chapter 1 and by Staley et al.(2014).

Sequence data was processed and analyzed using mothur (Schloss et al., 2012).

Sequences were trimmed to 160 bp, paired-end joined, and screened for quality. Singleton

sequences and those identified as chimeras by UCHIME (Edgar et al., 2011) were removed.

Sequences were aligned to the SILVA database (Quast et al., 2013). Sequence read number was

normalized by random subsampling to 35,724 reads per sample, and sequences corresponding

to chloroplast lineages were removed. Sequences were clustered into OTUs using the average-

neighbor algorithm at 97% similarity. OTUs were classified to the SILVA database. For May and

July 2013 bulk samples, reads from all subsamples were merged to create a single composite

sample. May and July composite samples were normalized to 97,124 reads and 91,684 reads,

respectively.

2.7 Statistical analysis

All determinations of significance were made at α=0.05. Discriminant analysis was

performed on the July 2013 data using XLStat software (Addinsoft Co., New York, NY), and

included only values from treatments that were present at every site; i.e. excluding hairy vetch

and buckwheat. Pearson correlation coefficients were used to evaluate relationships among soil

physicochemical and biological parameters, incorporating measurements from May and July

2013. Redundancy analysis and analysis of variance were done using R program v. 3.1.0 (R core

team, 2014). Treatment and location effects were evaluated using mixed models implemented

in the package *lme4* (Bates et al., 2014), and significance of fixed effects was tested using

Satterthwaite approximations of degrees of freedom as implemented in the package *lmerTest*

(Kuznetsova et al., 2014). Enzyme activity data were log-transformed to correct non-normality

of model residuals. Redundancy analysis was performed in R using the vegan package (Oksanen,

2014).

3. Results

3.1 Influence of treatments on soil nutrient, OM, and pH levels.

Results of soil tests are summarized in Table 2.3. Pelleted poultry manure and, to a

lesser degree, Sustane, produced large increases in NO_3-N in May 2013. These increases were

mostly depleted but still significant (p=0.02, p<0.001, and p<0.01 at Farmington, Lamberton, and

Rosemount, respectively) in July 2013 (Table 2.4). NO_3-N was higher in pelleted poultry manure

than in the no-amendment control by 120 and 62 ppm in May and July, respectively. NO_3-N was

higher in Sustane than in the control by 15 and 6 ppm in May and July, respectively. Beef

manure, in contrast, did not increase NO_3-N levels compared to the control. At Farmington, NO_3-

N was also higher in July in cover crop treatments than in control, but did not differ between

cover crops and control at the other sites. Pelleted poultry manure and Sustane lowered pH by

0.4-0.72 units compared to control at the May sampling time (p=0.002, p<0.001, and p=0.001 at

Farmington, Lamberton, and Madison, respectively). Pelleted poultry manure also increased

Bray-P values compared to control in May 2013 at Farmington and Lamberton by an average of

13.4 ppm (data not shown). Bray-P and pH did not differ between cover crop treatments and

control. Soil organic matter was not affected by treatment.

*3.2 Discrimination and prediction of soil functional profiles by treatment, location, and
physicochemical profiles*

Discriminant analysis, which disperses groups along axes consisting of linear and

quadratic combinations of predictor variables, indicated that treatments did not differ

significantly in their functional profiles (p=0.429), although they differed (p=0.010) in their soil

physicochemical profiles. Soil functional profiles differed (p<0.0001) with location. Redundancy

analysis (Figure 1) indicated that effects associated with location were largely explainable by

physicochemical differences. The overall functional profiles (enzyme activities, net N

mineralization, soil respiration, and corn yield) of soils sampled in July 2013 were significantly

predicted by soil physicochemical tests (p=0.001). Parameters most predictive of function were

moisture ($p<0.001$), organic matter ($p<0.001$), pH ($p<0.001$), and exchangeable Ca ($p=0.02$). Of the total variation in functional profiles that was explainable by location and soil physicochemical test values, only 5.9% was uniquely attributable to location independent of physicochemical values, while 50.6% was uniquely attributable to physicochemical values and 43.5% was collinear between location and physicochemical values.

3.3 Treatment effects on enzyme activities, soil respiration, and N mineralization

Activity of NAGase and β-glucosidase enzymes at the Madison site was greater in pelleted poultry manure-treated soils compared to the no-amendment control soils in May 2013 ($p<0.001$ and $p=0.005$ for NAGase and β-glucosidase, respectively), and β-glucosidase was higher in the oilseed radish treatment ($p=0.028$); however, no differences were observed in these enzymes in July 2013 (Table 2.5). At Farmington, no differences were observed in May in NAGase and β-glucosidase activity, but both were greater in winter rye ($p=0.015$ and $p<0.001$ for NAGase and β-glucosidase, respectively), and β-glucosidase activity was also greater in oilseed radish ($p=0.014$) and buckwheat ($p=0.014$), in July 2013. No effects of treatment on NAGase and β-glucosidase activity were observed at Lamberton. Interestingly, pelleted poultry manure consistently increased NAGase activity in May and July at all locations, although in most cases the increases were not significant at $\alpha=0.05$. Phosphatase activity was greater in all amendment treatments compared to the control in May 2013 at Lamberton; however, at Madison a reduction in May phosphatase activity was observed in the oilseed radish, beef manure, and Sustane treatments. No significant changes in phosphatase activity ($p=0.56$) were observed in the July 2013 soil samples, and N mineralization was not affected by treatment ($p=0.64$).

Soil respiration consistently decreased at all locations between the May and July

sampling times, including in the no-amendment control. This is likely attributable to increasingly

dry conditions as the growing season progressed. However, despite the overall decline, 101%

greater soil respiration (p<0.001) was observed in July in winter rye compared to the no-

amendment control at Farmington. This suggested that a somewhat delayed or sustained boost

in microbial activity may have been prompted by the ongoing breakdown of incorporated rye

biomass at this site (Bending et al., 2002). Other cover crops did not affect soil respiration.

Pelleted poultry manure increased soil respiration in the May samples by 84% and 104%

(p<0.001) at Lamberton and Madison, respectively, but this spike was short-lived, and was no

longer apparent by the July sampling date (Figure 2).

3.4 Intercorrelations among soil physicochemical parameters and functional characteristics

Enzyme activities and soil respiration were positively correlated with each other and

with soil moisture. However, they were not correlated with corn yields. When values were

recentered to location averages, corn yield was not associated with any soil test or measured

function. Although the correlation between yield and NO_3-N was not significant (Tables 2.7 and

2.8), at Farmington and Madison, treatments with increased yield generally corresponded to

those with increased NO_3-N levels (Tables 2.5 and 2.6).

Where measures of soil nutrient-cycling functions were associated with soil nutrient

levels, the correlations were generally positive. All enzyme activities were positively correlated

with SO_4-S (p<0.001, p<0.001, and p=0.012 for NAGase, β-glucosidase, and phosphatase,

respectively), and β-glucosidase activity was positively correlated (p<0.001) with all measured

nutrient levels except Bray-P and NO_3-N. Soil respiration was positively correlated (p<0.01) with

all nutrients tested, except for Bray-P. Exceptions to this pattern included a decrease in phosphatase activity with increases in Bray-P (p=0.022), and net N mineralization, which was negatively correlated with SO_4-S (p=0.005) and exchangeable Ca and Mg (p<0.001). R-values are shown in Table 2.7.

3.5 Prediction of soil functional profiles by bacterial phylum and family abundance

The composition of bacterial communities in these soils and the effects of over crop and fertilizer treatments on community structure are described in Chapter 1. Briefly, averaged across locations, we identified a mean of 4258, 3945, and 1549 OTUs in samples of May 2013 bulk soil, July 2013 bulk soil, and July 2013 rhizosphere soil, respectively, encompassing 45 bacterial phyla. The bacterial community was predominantly composed of members of the phyla *Actinobacteria* (50.1% and 19.7% of reads in July 2013 bulk and rhizosphere soils, respectively), *Proteobacteria* (23.3% and 63.3%), *Acidobacteria* (6.0% and 2.9%), and *Bacteroidetes* (5.0% and 5.8%). Variation in bacterial community structure among samples was most strongly associated with bulk-rhizosphere differences and sampling locations, with a relatively small portion of variation associated with treatments. OTU composition was significantly affected (p<0.05) by treatment at all locations in May bulk soil and July rhizosphere soil, but treatment effects in July 2013 bulk soil were observed only at Farmington. Principal component analysis and redundancy analysis indicated that pelleted poultry manure was the treatment most strongly differing from other treatments. Of the total variance in bacterial family composition that was explainable by amendment treatments and soil physicochemical test values, only 4.9% was uniquely attributable to treatment differences independent of

physicochemical tests, indicating that the observed differences in bacterial community structure were largely driven by physicochemical parameters, including pH, moisture, OM, and nutrients.

Family and phylum composition of bulk soil bacterial communities, as well as family composition of rhizosphere communities, was significantly predictive (p<0.0001) of soil functional profiles (Table 2.9). Variation in soil function was significantly explained (p=0.0003) by bacterial order abundances in bulk soil samples. The power of order abundances to explain variation in soil function was partially, but not fully, redundant with that of soil physicochemical values: rhizosphere and bulk soil bacterial community composition uniquely explained 37.9% and 39.3% of the total explainable variation in function, respectively.

Families and phyla were identified in bulk and rhizosphere communities whose relative abundances were most predictive of soil functional profiles (Table 2.10). Rhizosphere localized phyla were not included, due to their overall nonsignificance in predicting functional profiles. Strong associations were observed in both bulk and rhizosphere soil between functional profiles and the families *Cytophagaceae,* a member of the *Bacteroidetes* (p=0.001), and *Micrococcaceae,* a member of the *Actinobacteria* (p=0.001 and p=0.044 in bulk and rhizosphere, respectively). Overall, the *Actinobacteria* emerged as the phylum that was most significantly predictive (p=0.001) of functional profiles. Relative abundances of this phylum were also significantly affected by organic amendment treatment in rhizosphere soil, but not bulk soil, as described in Chapter 1. With conditioning on soil physicochemical parameters, the *Actinobacteria* were no longer significantly associated with soil functional profiles (p=0.874), while the *Firmicutes* and *Nitrospirae* became most predictive (p=0.032 and p=0.024, respectively). Those emerging as most significant with conditioning are of particular interest, as they provide predictive value that is not redundant with that already present in routine soil tests. However, we observed little

overlap between this set of families and those that were associated with treatment differences, as described in Chapter 1.

3.6 Correlation of soil function and physicochemical properties with bacterial community composition

Redundancy analysis (Figure 3) indicated that soil physicochemical parameters were highly predictive ($p<0.0001$) of bacterial phylum and family abundances in both bulk and rhizosphere soil. Of the soil parameters tested, moisture, pH, OM and Bray-P were the most strongly predictive of both bulk and rhizosphere community composition, with additional significant ($p<0.05$) prediction by Ca at the phylum level and Na and NO_3-N at the family level.

Correlations were assessed between abundances of individual bacterial families and soil physicochemical and functional parameters (Tables 2.11 and 2.12) in the July 2013 bulk soil samples. Physicochemical parameters most frequently correlated with bacterial family abundances were organic matter, pH, and exchangeable Ca, Mg, and Na (Table 2.11). There were particularly strong correlations between pH and several phylogenetically disparate families, including *Xanthobacteraceae* ($r=0.54$, $p<0.001$), *Pseudonocardiodaceae* ($r=0.63$, $p=0.001$), *Propionibacteraceae* ($r=0.69$, $p<0.001$), and *Sinobacteraceae* ($r=0.69$, $p<0.001$). Bacterial families that were positively or negatively correlated with moisture tended to also have same-sign correlations with OM, a pattern that persisted even when values were recentered to location averages. Soil respiration and net N mineralization were positively correlated with bacterial community OTU richness. Both OTU richness and diversity were negatively correlated with β-glucosidase activity ($r=-0.32$ and $r=-0.51$; $p=0.007$ and $p<0.001$ for OTU richness and diversity, respectively). Strong positive and negative associations with

bacterial families were observed for β-glucosidase activity, including *Xanthobacteraceae* (r=0.50, p<0.001)*, Sphingomonadaceae* (r=-0.28, p=0.02)*,* and *Streptomycetaceae* (r=-0.54, p<0.001). Associations with family abundances were not observed for NAGase activity, and rarely for phosphatase. Of families showing significant positive or negative correlation with β-glucosidase activity, 63% also had significant opposite-sign correlations with net N mineralization. This is consistent with our observation above that N mineralization was negatively associated with β-glucosidase activity. Many of the observed correlations were no longer significant at α=0.05 when values were recentered to location averages (Table 2.12), including nearly all associations between bacterial families and enzyme activities and net N mineralization.

3.7 Bayesian inference of relationships among taxa and functional and physicochemical

parameters

Bayesian modeling of physicochemical, functional, and community structure variables was used to identify directional relationships among these parameters (Figure 4). None of the measured functions appeared as nodes in the network, indicating that there were no inferred relationships between specific functional activities and particular bacterial families or physicochemical parameters. However, there were inferred relationships between bacterial families, as well as overall OTU richness, and organic matter and nutrient levels. Concentrations of NO_3-N, SO_4-S, and OM were identified as parent nodes affecting the abundance of at least one family, while not themselves having child relationships to other measured parameters. Exchangeable Ca was a child node to three bacterial families and was in turn a parent of exchangeable Mg.

OTU richness (Chao1) had both parent and child relationships to bacterial families. Interestingly, moisture and pH, although identified by RDA and correlation analyses as highly predictive of bacterial community composition, did not emerge as parent nodes to any family abundances.

4. Discussion

4.1 Physicochemical effects of treatments and their relationship to bacterial community composition

Cover crop and fertilizer treatments produced changes in soil function; however, community structure and function were much more strongly affected by site (soil type) than by amendment treatments. This is consistent with the findings of previous farming-system and landscape-scale studies elucidating the physicochemical determinants of soil microbial communities. (Bossio et al., 1998; Kuramae et al., 2012; Bisset et al., 2011). Soil pH and moisture have been repeatedly confirmed to be major drivers of community composition (Fierer et al. 2006; Lauber et al., 2009). Associations of soil microbial community structure with salinity, exchangeable cations, soluble P, and OM have also been documented (Lozupone and Knight, 2007; Kuramae et al., 2012).

The effects of pelleted poultry manure and Sustane treatments, which provided large boosts in soluble N levels, were somewhat similar to those that would occur with mineral N addition, including lowered pH (Barak et al., 1997). As was noted in Chapter 1, a decrease in bacterial diversity was also observed in these treatments. Beef manure, which did not increase NO_3-N, did not produce similar effects. This suggests that N immobilization may have

overwhelmed N release in the beef manure treatment, which has been previously observed with solid beef manure (Beauchamp, 1986).

Synchronizing N availability with plant demand is a complex task, and relatively little is known about the relationship between soil microbial community structure and timing of N release (Grandy et al., 2012). The Farmington site was the only location where NO_3-N levels were higher in the cover crop treatments than in the no-amendment control. This suggests that cover crops at this site may have been providing an N credit by scavenging, or, in the case of hairy vetch, fixing N during their growth periods that was then released during the corn growing season. Characteristics distinguishing the Farmington site from those at Lamberton and Madison included a sandier and lower-OM soil, (Tables 2.1 and 2.3), higher rainfall in both the 2012 and 2013 growing seasons (MN DNR 2015), and differences in initial bacterial communities present before treatment application, including greater prevalence of members of phyla *Proteobacteria* and *Bacteroidetes* and lower prevalence of *Actinobacteria* (see Chapter 1). It is not clear, however, which of these, if any, might be predictive of the observed synchrony in timing of N release.

4.2 Treatment effects on enzyme activity

We measured large disparities (>300%) in enzyme activity among subsamples within plots, illustrating the difficulty of measuring parameters that may vary at sub-plot scales in the highly heterogeneous soil environment (Ruamps et al., 2013). While differences in phosphatase activity would generally be expected with the addition of amendments containing differing quantities of phosphorus in differing states of availability (Olander and Vitousek, 2000; Garg and Ball, 2008), we observed few significant changes in soluble phosphate levels with treatment, and

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treatments affecting Bray-P did not correspond to those affecting phosphatase activity (data not shown).

Our study environments were not strongly N-limited, with NO_3-N values that would be considered low for conventional, but were appropriate for organic production in these relatively high-OM soils. While addition of N can repress NAGase activity under N-limited conditions, we observed the opposite effect under the relatively high-N conditions of this experiment; both instances in which NAGase activity increased corresponded to increased NO_3-N levels (Olander and Vitousek, 2000). Because NAGase is a chitin-degrading enzyme, these differences may reflect increases in fungal biomass, which would be active in degrading rye residue and taking advantage of the growth medium provided by the chicken pellets. This is also consistent with previous observation that activity of N-cycling enzymes tends to increase with C input (Bowles et al., 2014). Although treatment clearly affected N cycling processes, net N mineralization did not respond to amendment treatment.

Our results do not confirm Bandick and Dick's (1999) finding that β-glucosidase is a particularly sensitive indicator of management effects; however, this may be due to the situation of this experiment wholly within organic systems. Activity of this enzyme is generally higher under organic management, and may therefore be less sensitive to management under established organic conditions (Garcia-Ruiz et al., 2008). However, β-glucosidase activity was well correlated with individual family abundances, suggesting that it may be more closely associated with changes in bacterial community composition. Net N mineralization showed a similar pattern, with no significant effect of treatment, but was involved in several correlations with bacterial families. For these functions, soil microbial community profiles may be useful for predicting activity levels, even if associations with specific practices are not yet clear.

4.3 Differences among methods of assessing relationships between bacterial community

composition and soil physicochemical and functional parameters

Although soil bacterial community composition was associated with soil nutrient levels

and other physicochemical properties, different analyses produced different assessments of

which variables were most significantly related to community structure (Table 2.13).

Redundancy analysis, correlation analysis, and Bayesian inference identified distinct

relationships among bacterial taxa and soil functions. Bayesian inference, in fact, did not identify

any directional relationships between taxa and functions.

In many cases, correlations that were detected between soil physicochemical

parameters and soil functions (Tables 2.7 and 2.8), or between these variables and bacterial

community structure parameters (Tables 2.11 and 2.12), did not appear when variables were

recentered to location means. This may indicate confounding of these correlations with soil

properties that differed among locations; however, in some cases, it may simply indicate that

within-location variability was likely not sufficient for detecting valid correlations.

These disparities underscore the need for caution in attributing causation to

correlations between taxa and functions; in many cases they likely represent co-occurring

responses to soil conditions. However, taxa may be useful as indicators of functions, even if they

are not the agents of those functions (Rames et al., 2013; Phillipot et al, 2010).

4.4 Implications for use of 16s profiling in agronomic decision-making

Microbial diversity is generally considered to be advantageous to soil health and

function, and is often implied to be a part of the "package" of soil quality benefits promoted by

organic practices (Sullivan, 1999). While greater bacterial OTU richness was both positively and

negatively associated with specific nutrient-cycling functions (Table 2.11), Simpson diversity of

bacterial OTUs was not positively associated with any function. As was noted in Chapter 1,

diversity did not increase in our organic amendment treatments, compared to the no-

amendment control. It is important to avoid simplistic assumptions about both the causes and

the benefits of microbial diversity. While 16S sequencing does offer a more detailed assessment

of bacterial phylogenetic diversity than previous community profiling methods, the relative

abundances of specific taxa were more useful parameters for predicting soil function.

While our findings do not contradict previous studies indicating that soil

physicochemical properties are key drivers of soil function (Bowles et al., 2014), differences in

soil function were better explained using conventional soil physicochemical tests and

community structure data together, rather than using soil tests alone. This indicates that

pursuing a better understanding of bacterial community composition and how it is affected by

farming practices is a promising avenue for increasing our ability to predict the effects of

management on important soil functions. It is also worth bearing in mind that 16S sequence-

based profiling captures only the bacterial members of the soil community, and does not

provide information about fungi, which are also key participants in decomposition and nutrient

cycling (McGuire and Treseder, 2010). Although agricultural soils are generally considered to be

bacterial-dominated, this may be less true in organically managed systems (Ngosong et al.,

2010; Six et al., 2006). As sequencing procedures and analysis pipelines for fungal community

structure profiling become better established, future research will need to include fungal

communities in investigations of soil community structure.

Conclusions

Application of cover crops and organic fertilizers produced changes in soil physicochemical parameters and soil respiration and nutrient cycling activities, which were associated with differences in soil bacterial community structure and diversity. These effects were observed in the cropping season following treatment application, including in the corn crop rhizosphere, indicating that changes to microbial communities produced by incorporation of organic materials can affect the functional microbial environment encountered by growing crops. Our observations included increases in β-glucosidase activity following winter rye, oilseed radish, and pelleted poultry manure; and decreases in phosphatase activity following beef manure, pelleted poultry manure, and Sustane fertilizer; however, patterns were not were not consistent across sites and sampling timepoints.

Datasets including both soil physicochemical test values and bacterial family and phylum abundances enabled greater explanation of variation in soil functional profiles than physicochemical test values alone. This suggests that sequencing-derived soil bacterial community structure profiles can be a useful tool for making site-specific predictions of the effects of fertility management practices on important and crop-relevant soil functions. As sequencing costs continue to drop, future studies will be increasingly able to incorporate the extensive replication needed to overcome the enormous variability in measures of soil function and community structure. Our work shows many correlations between bacterial community structure features and important soil nutrient-cycling activities. Future directions for research in this area may include the development of models to produce explicit predictions of function from soil community phylogenetic profiles, including factors crucial to healthy and sustainable crop production that have previously been difficult to predict, such as crop response to applied fertilizers, nutrient release from incorporated organic material, and effects of inoculation.

Acknowledgements

Tables

Table 1.1: Locations and soil descriptions of experimental sites

Site	Type	Coordinates	Soil Type
Lamberton, MN	Elwell Agroecology Farm (U of MN Southwest Research and Outreach Center)	44.23, -95.31	Webster clay loam (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls)
Madison, MN	Collaborating farm	45.01, -96.15	Normania loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls)
Farmington, MN	Collaborating farm	44.65, -93.04	Wadena loam (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls)

Table 1.2: Soil physicochemical parameters measured in 2012-2013 at experimental sites in Farmington, Lamberton, and Madison, MN

Variable	Notes
Bray-P	
K	Water-soluble + exchangeable
NO_3-N	
SO_4-S	
Organic matter (OM)	Loss on ignition method
Mg	Exchangeable
Na	Exchangeable
Ca	Exchangeable
pH	Water method

Table 1.3: Number and phylum composition of OTUs and sequence reads from bulk and rhizosphere soil samples collected in 2012 and 2013 at Farmington, Lamberton, and Madison, MN

	OTUs per site	OTUs per sample	Actinobacteria		Proteobacteria		Bacteroidetes		Acidobacteria		Firmicutes		Planctomycetes		Other	
									Percent							
Timepoint[a]			OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads
B812	9749 ±3152	1971 ± 1394	10.9	35	31.6	34	8	9.7	7.5	6.3	5.8	2.4	12.6	3	23.6	9.6
B513	16,948±2726	4258 ± 1248	8.8	48.6	22.8	23.7	5.6	7.2	5.1	6.5	6.2	4.7	8.5	2.4	43	7
B713	15,678 ±2796	3945 ± 1157	10.6	50.1	32.3	23.3	7.1	5	6.4	6	9	6.2	12.4	2.6	22.3	6.8
R713	7660 ±1991	1549 ± 866	11.8	19.7	31.8	63.3	9.3	5.8	7	2.9	6.4	2.1	12	1.3	21.7	4.9
Site																
Farmington			9.8	39.7	32.7	31.3	7.8	7.2	6.6	6.8	7.3	5.1	11.6	2.5	24.2	7.3
Lamberton			13.1	47.2	31	29.6	7.7	5.4	7.5	4.7	8.3	4.4	12.5	2.1	19.9	6.6
Madison			7.6	43.9	21.3	29.6	5.5	6.8	4.5	5.8	5.2	4.2	8.9	2.7	47.1	7

[a]B812, August 2012 bulk soil; B513, May 2013 bulk soil; B713, July 2013 bulk soil; R713, July 2013 rhizosphere soil.

Table 1.4: Bacterial families most significantly associated with dispersion of organic amendment treatments in discriminant analysis of July 2013 bulk and rhizosphere soil samples.

Rhizosphere			Bulk		
Variable	Lambda[a]	p-value	Variable	Lambda	p-value
Bacillaceae	0.731	0.001	Sanguibacteraceae	0.565	< 0.001
Sphingobacteriaceae	0.757	0.002	Microbacteriaceae	0.599	< 0.001
Pseudonocardiaceae	0.759	0.002	Sphingobacteriaceae	0.691	< 0.001
Acidimicrobiaceae	0.767	0.003	Micrococcaceae	0.769	< 0.001
Intrasporangiaceae	0.795	0.009	Nocardiaceae	0.785	< 0.001
Chloroflexi.KD4-96.unclassified	0.813	0.016	Pseudomonadaceae	0.796	< 0.001
Xanthobacteraceae	0.816	0.017	Erysipelotrichaceae	0.813	< 0.001
Pseudomonadaceae	0.823	0.022	Geobacteraceae	0.83	< 0.001
Geodermatophilaceae	0.823	0.022	Alcaligenaceae	0.832	< 0.001
Actinobacteria.AKIW543.unclassified	0.824	0.023	Flavobacteriaceae	0.832	< 0.001
Actinosynnemataceae	0.826	0.024	Methylophilaceae	0.836	< 0.001
Conexibacteraceae	0.84	0.038	Verrucomicrobia.OPB35.unclassified	0.84	< 0.001
Micromonosporaceae	0.84	0.038	mle1-27	0.841	< 0.001
Solirubrobacteriaceae	0.845	0.044	Caulobacteraceae	0.843	< 0.001
Planctomycetaceae	0.846	0.046	Acidobacteriaceae	0.861	0.001

[a]Unidimensional test of equality of the means of the classes (treatments)

Table 1.5: Effects of amendment treatments on bacterial community composition in May and July 2013 bulk and July 2013 rhizosphere soil samples at Farmington, Lamberton, and Madison, MN.

	May bulk			July bulk			July rhizosphere		
	F[a]	L	M	F	L	M	F	L	M
amova p-value[b]	**<.001**	**0.047**	**0.02**	**0.005**	0.164	0.98	0.118	**0.044**	**0.048**
Individually significant treatment differences	HV-BW[c]	WR-SU		HV-CP				HV-WR	WR-CP
	HV-CP			WR-CP				WR-NC	WR-SU
	HV-SU			BM-CP				BM-NC	BM-NC
	WR-BW			CP-NC				CP-NC	CP-NC
	WR-CP								SU-NC
	OR-CP								
	BW-CP								
	BM-CP								
	CP-SU								
	CP-NC								

[a]F, Farmington; L, Lamberton; M, Madison

[b]P-value represents the probability that bacterial community composition was similar for all treatments. Values in boldface represent significant effects of treatment at α=0.05.

[c]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

Table 1.6: OTU richness (Chao1) and diversity (Inverse Simpson) of May and July 2013 samples following organic amendment treatments at Farmington, Lamberton, and Madison, MN.

	Farmington				Lamberton				Madison			
	Chao1		Inv. Simpson		Chao1		Inv. Simpson		Chao1		Inv. Simpson	
May bulk												
HV[b]	6018	a[a]	165	b								
WR	6192	a	197	bc	6384	a	209	bc	5271	a	161	b
OR	6060	a	200	bc	6114	a	210	bc	4981	a	172	b
BW	6178	a	276	d	6597	a	159	a				
BM	5970	a	238	cd	6056	a	221	c	5002	a	169	b
CP	5441	a	119	a	5832	a	180	ab	4491	a	103	a
SU	5662	a	166	b	6272	a	200	bc	4853	a	156	b
NC	5519	a	263	d	5727	a	206	bc	4930	a	167	b
July bulk												
HV	6141	ab	201	ab								
WR	6773	c	211	abc	4501	a	147	b	5368	a	142	a
OR	6548	bc	209	abc	4305	a	137	ab	5322	a	147	a
BW	6410	bc	234	c	4743	a	117	a				
BM	6570	bc	289	d	4829	a	142	ab	5385	a	149	a
CP	5719	a	184	a	4790	a	171	c	5158	a	158	a
SU	6024	ab	206	ab	4751	a	153	bc	5575	a	148	a
NC	5631	a	232	bc	4348	a	150	b	5090	a	153	a
July rhizosphere												
HV	2139	a	16	a								
WR	2508	a	18	a	1273	a	5	a	2362	d	19	a
OR	2227	a	17	a	1672	a	21	a	2027	bc	12	a
BW	2462	a	15	a	952	a	7	a				
BM	2426	a	11	a	1326	a	7	a	1921	abc	7	a
CP	2281	a	16	a	1358	a	5	a	1623	a	6	a
SU	2236	a	7	a	1596	a	10	a	1735	ab	7	a
NC	2531	a	69	b	1538	a	9	a	2072	cd	23	a

[a]Mean separations are within location, sampling timepoints, and bulk/rhizosphere soil.

[b]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

Table 1.7: Abundance of key bacterial taxa as a percentage of total sequence reads by treatment at Farmington, Lamberton, and Madison, MN in bulk and corn rhizosphere soil

	Nitrosomonadaceae			Rhizobiales			Actinobacteria		
	F[a]	L	M	F	L	M	F	L	M
	Percent of total reads								
B513[b]									
HV[c]	1.4 c[d]			5.0 b			46.4 a		
WR	1.4 c	0.8 bc	1.0 c	5.4 bc	5.2 a	5.6 b	46.7 a	51.7 a	50.4 a
OR	1.4 c	0.8 bc	1.0 c	5.4 bc	5.8 a	5.6 b	47.9 a	52.3 a	48.5 a
BW	1.5 cd	0.8 abc		5.8 c	5.3 a		41.7 a	52.6 a	
BM	1.4 c	0.9 c	0.9 bc	5.3 bc	5.3 a	5.4 b	44.3 a	47.9 a	49.8 a
CP	0.9 a	0.6 a	0.7 a	4.4 a	4.7 a	4.3 a	47.1 a	53.2 a	52.4 a
SU	1.1 b	0.7 ab	0.8 ab	4.9 ab	4.7 a	5.2 ab	46.4 a	52.2 a	49.1 a
NC	1.6 d	0.8 abc	1.0 c	5.1 bc	6.0 a	6.0 b	42.2 a	49.3 a	49.3 a
B713									
HV	1.7 bc			5.1 a			45.6 a		
WR	1.8 c	1.0 a	1.1 a	5.4 a	5.7 a	6.0 a	45.0 a	55.1 a	49.5 a
OR	1.6 abc	0.8 a	1.2 a	5.5 a	5.1 a	5.8 a	43.9 a	60.4 a	49.2 a
BW	1.8 c	1.0 a		5.6 a	5.7 a		43.4 a	56.4 a	
BM	1.8 c	0.9 a	1.1 a	5.0 a	5.5 a	6.2 a	41.1 a	56.2 a	48.1 a
CP	1.4 a	0.9 a	1.0 a	4.7 a	5.9 a	5.8 a	47.9 a	54.0 a	49.7 a
SU	1.5 ab	0.9 a	1.2 a	5.3 a	5.5 a	5.3 a	45.9 a	55.8 a	51.4 a
NC	1.7 bc	0.9 a	1.1 a	5.0 a	5.3 a	6.0 a	43.7 a	57.6 a	48.4 a
R713									
HV	0.7 bc			2.4 ab			24.8 a		
WR	0.7 bc	0.2 a	0.5 c	3.3 bc	1.6 a	2.2 b	24.2 a	17.2 a	17.1 bc
OR	0.5 ab	0.3 a	0.4 bc	2.3 ab	2.2 a	2.0 ab	24.2 a	29.6 b	14.0 ab
BW	0.5 ab	0.2 a		2.6 ab	1.6 a		19.3 a	15.1 a	
BM	0.5 ab	0.3 a	0.2 ab	2.4 ab	1.6 a	1.4 a	22.9 a	16.6 a	9.8 a
CP	0.5 ab	0.2 a	0.2 a	2.2 ab	2.3 a	1.0 a	21.9 a	21.1 a	11.0 a
SU	0.3 a	0.2 a	0.2 a	1.9 a	2.0 a	1.4 a	15.4 a	20.8 a	12.3 a
NC	1.0 c	0.2 a	0.5 c	3.9 c	1.6 a	2.6 b	31.0 a	22.0 a	19.0 c

[a]F, Farmington; L, Lamberton; M, Madison

[b]B513, May 2013 bulk; B713, July 2013 bulk; R713, July 2013 rhizosphere.

[c]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

[d]Mean separations are within location, sampling timepoints, and bulk/rhizosphere soil.

Table 2.1: Organic cover crop and fertilizer treatments applied at Farmington, Lamberton, and Madison, MN in fall 2012

Treatment	N-P-K Content	Seeding or application rate
Hairy vetch (*Vicia villosa*)	N/A	22 kg ha^{-1b}
Winter rye (*Secale cereale*)	N/A	112 kg ha^{-1}
Oilseed radish (*Raphanus sativus*)	N/A	22 kg ha^{-1}
Buckwheat (*Fagopyrum esculentum*)	N/A	56 kg ha^{-1}
Beef manure	1-1-1	10.5 Mg ha^{-1}
Pelleted poultry manure	5-3-2	7.7 Mg ha^{-1}
Sustane 8-2-4 (OMRI-approved commercial fertilizer[a])	8-2-4	3.9 Mg ha^{-1}
No-amendment control	N/A	N/A

[a]Organic Materials Review Institute, 2015

[b]Seeding rates represent pure live seed

Table 2.2: Data collected on soil physicochemical, functional, and bacterial community parameters

Variable	Determination
Physicochemical parameters	
Moisture	Gravimetric
Bray-P	Bray 1-P extractant
K	Water-soluble + exchangeable
NO3-N	Nitrate and nitrite
SO4-S	Extractable
Organic matter (OM)	Loss on ignition method
Mg	Exchangeable
Na	Exchangeable
Ca	Exchangeable
pH	Water method
Functions	
Net N mineralization (N-min)	KCl extraction
Total respiration	Gas chromatography
Corn yield	Field sample
B-glucosidase	Fluorimetric assay
N-acetyl-β-D-glucosaminidase	Fluorimetric assay
Phosphatase	Fluorimetric assay
Bacterial community structure	
Taxon abundances	16S V5-V6 rDNA sequencing
Diversity	Inverse Simpson index
OTU richness	Chao1 estimator

Table 2.3: Average soil physicochemical test values in May and July 2013 at Farmington, Lamberton, and Madison, MN.

	Farmington		Lamberton		Madison	
	May	July	May	July	May	July
Moisture (percent)	9.7	8.2	17.4	8.5	17.5	17.0
Bray-P (ppm)	35	30	17	16	27	45
OM (percent)	2.57	2.41	5.12	4.58	5.48	4.86
pH	6.40	6.52	6.92	7.06	7.32	7.48
Ca (ppm)	1366	1434	3636	3498	4079	3929
Mg (ppm)	219	226	680	624	759	725
Na (ppm)	1.6	2.0	10.3	8.3	16.4	12.0
K (ppm)	122	125	164	140	298	234
NO3-N (ppm)	30.3	7.0	31.9	13.8	52.1	8.4
SO4-S (ppm)	4.2	3.8	6.1	5.8	6.8	5.1

Table 2.4: Effects of organic amendments on soil pH and NO3-N in May and July 2013 at Farmington, Lamberton, and Madison, MN

	May pH		July pH			May NO3-N		July NO3-N	
Farmington						*ppm*			
HV[a]	6.3	bc[b]	6.4	a		15.1	a	6.8	bc
WR	6.63	d	6.45	a		7.4	a	9	c
OR	6.85	d	6.9	a		9.6	a	5.6	ab
BW	6.62	cd	6.47	a		7.4	a	6.1	abc
BM	6.68	d	6.73	a		11.2	a	7.5	bc
CP	5.78	a	6.38	a		106.1	c	9.5	c
SU	5.93	ab	6.31	a		70.9	b	7.7	bc
NC	6.5	cd	6.53	a		8.5	a	3.4	a
Lamberton									
WR	7.23	c	7.23	a		7.6	a	10	a
OR	7.2	c	7.3	a		14.9	a	7.8	a
BW	6.8	ab	6.9	a		8.6	a	6.2	a
BM	7	bc	7.08	a		11.4	a	9.3	a
CP	6.5	a	6.78	a		97.8	c	35.5	b
SU	6.55	a	6.95	a		64	b	17.3	a
NC	7.1	bc	7.1	a		7.6	a	6.9	a
Madison									
WR	7.5	b	7.6	c		6.9	a	5.7	a
OR	7.36	b	7.45	ab		15.6	a	6	a
BM	7.45	b	7.53	bc		14.9	a	8.5	ab
CP	7.08	a	7.38	ab		185.8	c	15	c
SU	7.08	a	7.3	a		78.5	b	9.3	b
NC	7.48	b	7.6	c		11.1	a	5.9	a

[a]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

[b]Mean separations are within locations and sampling times.

Table 2.5: Activity of nutrient-cycling enzymes in bulk soil samples in May and July 2013 at Farmington, Lamberton, and Madison, MN

	NAGase				B-glucosidase				Phosphatase			
	May		July		May		July		May		July	
	mmol g^{-1} dry soil h^{-1}											
Farmington												
HV[a]	6.77	a[b]	4.57	abc	18.72	a	16.89	a	38.4	a	28.14	a
WR	8.31	a	7.59	e	27.82	a	29.01	c	54.77	a	39.62	a
OR	8.03	a	6.11	cde	27.66	a	25.96	c	30.97	a	33.54	a
BW	26.06	a	5.47	bcde	22.34	a	25.2	c	32.06	a	26.1	a
BM	6.59	a	4.39	ab	22.87	a	16.95	a	14.9	a	27.45	a
CP	22.72	a	6.48	de	12.82	a	22.95	bc	19.72	a	33.38	a
SU	9.44	a	3.92	a	18.26	a	17.44	a	19.22	a	20.86	a
NC	6.71	a	5.17	abcd	21.39	a	17.81	ab	34.34	a	24.83	a
Lamberton												
WR	7.61	a	6.02	a	39.38	a	42.31	a	41.84	b	35.79	a
OR	6.99	a	5.87	a	41.3	a	37.48	a	59.01	bc	26.75	a
BW	7.19	a	4.26	a	39.55	a	31.94	a	75.95	c	33.38	a
BM	6.86	a	6.08	a	40.16	a	33.4	a	61.06	bc	45.57	a
CP	10.8	a	7.13	a	39.24	a	37.62	a	68.16	c	70.14	a
SU	10.78	a	6.21	a	35.82	a	33.78	a	45.02	bc	33.77	a
NC	7.33	a	4.98	a	45.57	a	34.14	a	16.94	a	27.94	a
Madison												
WR	6.32	a	18.27	a	36.09	ab	41.56	a	20.88	bc	25.26	a
OR	7.02	a	7.13	a	43.44	c	35.74	a	19.45	ab	18.38	a
BM	5.21	a	6.26	a	32.83	a	55.43	a	10.72	a	16.66	a
CP	14.36	b	7.43	a	45.51	c	32.78	a	20.98	c	14.54	a
SU	6.94	a	5.26	a	40.11	bc	44.43	a	17.15	ab	25.8	a
NC	6.21	a	4.96	a	36.4	ab	38.8	a	22.84	c	17.8	a

[a]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

[b]Mean separations are within locations, enzymes, and sampling times.

Table 2.6: Yield of corn following cover crop and organic fertilizer incorporation at Farmington, Lamberton, and Madison, MN

Farmington			Lamberton			Madison		
				kg ha^{-1}				
HV[a]	149	b[b]						
WR	127	ab	WR	123	a	WR	91	a
OR	125	ab	OR	130	ab	OR	106	ab
BW	135	ab	BW	142	abc			
BM	143	b	BM	146	bc	BM	125	bc
CP	144	b	CP	145	bc	CP	129	c
SU	153	b	SU	158	c	SU	136	c
NC	108	a	NC	157	c	NC	99	a

[a]HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; NC, no-amendment control

[b]Mean separations are within locations.

Table 2.7: Pearson correlations among soil physicochemical and functional parameters, measured at Farmington, Lamberton, and Madison, MN in May and July 2013

Variables	Moisture	Respiration	Net N-min	Bray-P	OM	pH	Ca	Mg	Na	K	NO3-N	SO4-S	Corn yield	NAGase	β-glucosidase	Phosphatase
Moisture	1	0.49 [a]	-0.1	0.07	0.76	0.43	0.71	0.74	0.64	0.55	0.2	0.41	-0.2	0.19	0.5	0.07
Respiration	0.49	1	0.03	0.09	0.33	-0	0.22	0.24	0.45	0.38	0.61	0.54	-0.1	0.31	0.21	0.19
Net N-min	-0.1	0.03	1	-0	-0.3	-0.2	-0.3	-0.3	-0.2	-0.1	-0	-0.2	-0	-0.1	-0.3	-0.2
Bray-P	0.07	0.09	-0	1	-0.2	-0	-0.1	-0.1	0.1	0.29	0.2	0.02	-0.2	0.12	-0.1	-0.2
OM	0.76	0.33	-0.3	-0.2	1	0.55	0.93	0.93	0.75	0.51	0.15	0.58	-0	0.14	0.65	0.16
pH	0.43	-0	-0.2	-0	0.55	1	0.72	0.68	0.43	0.33	-0.3	0.12	-0.2	-0.2	0.46	-0.3
Ca	0.71	0.22	-0.3	-0.1	0.93	0.72	1	0.98	0.76	0.59	0.13	0.52	-0.1	0.06	0.62	-0
Mg	0.74	0.24	-0.3	-0.1	0.93	0.68	0.98	1	0.79	0.56	0.12	0.53	-0.1	0.02	0.6	0.02
Na	0.64	0.45	-0.2	0.1	0.75	0.43	0.76	0.79	1	0.68	0.47	0.7	-0.1	0.11	0.44	0
K	0.55	0.38	-0.1	0.29	0.51	0.33	0.59	0.56	0.68	1	0.42	0.46	-0.1	0.19	0.31	-0.2
NO3-N	0.2	0.61	-0	0.2	0.15	-0.3	0.13	0.12	0.47	0.42	1	0.71	0.02	0.41	0.03	0.03
SO4-S	0.41	0.54	-0.2	0.02	0.58	0.12	0.52	0.53	0.7	0.46	0.71	1	0.08	0.32	0.35	0.18
Corn yield	-0.2	-0.1	-0	-0.2	-0	-0.2	-0.1	-0.1	-0.1	-0.1	0.02	0.08	1	-0.1	-0.1	0.1
NAGase	0.19	0.31	-0.1	0.12	0.14	-0.2	0.06	0.02	0.11	0.19	0.41	0.32	-0.1	1	0.26	0.28
β-glucosidase	0.5	0.21	-0.3	-0.1	0.65	0.46	0.62	0.6	0.44	0.31	0.03	0.35	-0.1	0.26	1	0.2
Phosphatase	0.07	0.19	-0.2	-0.2	0.16	-0.3	-0	0.02	0	-0.2	0.03	0.18	0.1	0.28	0.2	1

[a] Values represent Pearson correlation coefficients. Significant (α=0.05) positive correlations are highlighted in dark grey; significant negative correlations are highlighted in light grey.

Table 2.8: Pearson correlations among soil physicochemical and functional parameters (parameter values recentered to location averages), measured at Farmington, Lamberton, and Madison, MN in May and July 2013

Variables	Moisture	Respiration	Net N-min	Bray-P	OM	pH	Ca	Mg	Na	K	NO3-N	SO4-S	Corn yield	NAGase	β-glucosidase	Phosphatase
Moisture	1	0.52 ^{a}	-0.1	0.03	0.58	-0.2	0.37	0.48	0.21	0.22	0.17	0.16	0.07	0.27	0.16	0.25
Respiration	0.52	1	-0	0.04	0.47	-0.3	0.24	0.33	0.49	0.33	0.6	0.57	0.02	0.32	0.15	0.25
Net N-min	-0.1	-0	1	-0.1	-0.2	0.06	-0.1	-0.1	0.03	-0	-0	-0	-0	-0.1	-0.2	-0.2
Bray-P	0.03	0.04	-0.1	1	-0.1	-0.1	0.07	-0.1	0.19	0.22	0.22	0.16	-0.1	0.16	0.06	0.07
OM	0.58	0.47	-0.2	-0.1	1	-0.2	0.55	0.61	0.28	0.26	0.14	0.34	0.15	0.27	0.31	0.33
pH	-0.2	-0.3	0.06	-0.1	-0.2	1	0.25	0.03	-0.3	-0.2	-0.5	-0.4	-0.1	-0.3	0.1	-0.4
Ca	0.37	0.24	-0.1	0.07	0.55	0.25	1	0.75	0.24	0.5	0.09	0.18	0.01	0.12	0.19	-0.1
Mg	0.48	0.33	-0.1	-0.1	0.61	0.03	0.75	1	0.34	0.37	0.07	0.21	0.1	0.02	0.1	0.02
Na	0.21	0.49	0.03	0.19	0.28	-0.3	0.24	0.34	1	0.48	0.62	0.62	0.05	0.17	-0	0.12
K	0.22	0.33	-0	0.22	0.26	-0.2	0.5	0.37	0.48	1	0.46	0.37	0.06	0.26	0.02	-0
NO3-N	0.17	0.6	-0	0.22	0.14	-0.5	0.09	0.07	0.62	0.46	1	0.77	0.01	0.41	-0.1	0.02
SO4-S	0.16	0.57	-0	0.16	0.34	-0.4	0.18	0.21	0.62	0.37	0.77	1	0.1	0.36	0.07	0.15
Corn yield	0.07	0.02	-0	-0.1	0.15	-0.1	0.01	0.1	0.05	0.06	0.01	0.1	1	-0.1	-0	0
NAGase	0.27	0.32	-0.1	0.16	0.27	-0.3	0.12	0.02	0.17	0.26	0.41	0.36	-0.1	1	0.31	0.29
β-glucosidase	0.16	0.15	-0.2	0.06	0.31	0.1	0.19	0.1	-0	0.02	-0.1	0.07	-0	0.31	1	0.25
Phosphatase	0.25	0.25	-0.2	0.07	0.33	-0.4	-0.1	0.02	0.12	-0	0.02	0.15	0	0.29	0.25	1

^{a}Values represent Pearson correlation coefficients. Significant (α=0.05) positive correlations are highlighted in dark grey; significant negative correlations are highlighted in light grey.

Table 2.9: Percent of total explainable variance in soil functional profiles attributed to abundances of bacterial taxa and soil physicochemical parameters[a]

Bulk soil	*Family*		*Phylum*	
	soil	18.7	soil	26.2
	taxa	37	taxa	38.7
	soil+taxa	44.2	soil+taxa	35.1
Rhizosphere	*Family*			
	soil	20.3		
	taxa	34.2		
	soil+taxa	45.4		

[a]Soil functions included in profile were net N mineralization, potential activity of N-acetyl-B-D-glucosaminidase, B-glucosidase, and phosphatase enzymes, soil respiration, and corn yield

Table 2.10: Bacterial taxa in bulk field soil and rhizosphere soil identified by redundancy analysis as most predictive of soil functional profiles[a]

Without conditioning	Bulk families	Bulk phyla	Rhizosphere families[b]
	Actinobacteria.AKIW543.unclassified	Actinobacteria	Cytophagaceae
	Cytophagaceae	Firmicutes	Sphingobacteriaceae
	Xanthobacteraceae	Cyanobacteria	Oxalobacteraceae
	Micrococcaceae	Verrucomicrobia	Micromonosporaceae
	MND8	Nitrospirae	Micrococcaceae
Conditioned on soil physicochemical variables[c]	Bulk families	Bulk phyla	Rhizosphere families
	Xanthobacteraceae	Firmicutes	Acidobacteriaceae
	Actinobacteria.AKIW543.unclassified	Nitrospirae	Acidimicrobiaceae
	Chitinophagaceae	Chloroflexi	Moraxellaceae
	Micromonosporaceae	Acidobacteria	Oxalobacteraceae
	Gemmatimonadaceae	Fibrobacteres	Chitinophagaceae

[a]Soil functions included in profile were net N mineralization, potential activity of N-acetyl-B-D-glucosaminidase, B-glucosidase, and phosphatase enzymes, soil respiration, and corn yield

[b]Bacterial family abundances in rhizosphere soils were not significantly predictive of soil functional profiles

[c]Soil physicochemical variables were moisture, Bray-P, K, NO3-N, SO4-S, organic matter (OM), exchangeable cations (Mg, Na, and Ca), and pH.

Table 2.11: Correlations between bacterial 16S sequence diversity and family abundances and soil physicochemical and functional parameters

Variables	Moisture	OM	pH	NO3-N	Bray-P	Ca	Mg	Na	K	SO4-S	Corn yield	Respiration	Net N-min	NAGase	β-glucosidase	Phosphatase
OTU richness (Chao1)	-0.12[a]	-0.66	-0.31	-0.21	0.22	-0.64	-0.62	-0.47	-0.13	-0.51	-0.01	0.37	0.27	0.00	-0.32	-0.16
OTU diversity (inverse Simpson)	-0.36	-0.70	-0.46	-0.12	0.04	-0.70	-0.68	-0.51	-0.36	-0.34	0.07	0.04	0.21	-0.11	-0.51	-0.05
Cytophagaceae	0.48	0.23	0.56	0.12	0.46	0.37	0.37	0.39	0.47	0.25	-0.30	0.21	-0.11	0.23	0.26	-0.28
Acidobacteriaceae	0.10	-0.36	-0.44	-0.23	0.28	-0.47	-0.44	-0.31	0.00	-0.38	-0.14	0.28	0.21	0.04	-0.26	0.17
Sphingobacteriaceae	0.05	-0.08	-0.08	0.42	0.12	-0.06	-0.03	0.22	0.06	0.26	0.04	0.15	0.09	0.06	-0.13	0.13
Xanthobacteraceae	0.47	0.82	0.54	0.14	0.01	0.78	0.80	0.62	0.39	0.45	-0.15	0.03	-0.33	0.02	0.50	0.07
Chitinophagaceae	-0.07	-0.56	-0.33	-0.17	0.28	-0.57	-0.55	-0.40	-0.10	-0.35	-0.17	0.28	0.25	0.17	-0.28	0.01
Microbacteriaceae	-0.24	-0.40	-0.37	0.33	0.10	-0.40	-0.42	-0.12	-0.07	0.00	0.17	-0.01	0.29	0.05	-0.22	0.06
Streptomycetaceae	-0.49	-0.72	-0.65	-0.25	-0.21	-0.79	-0.76	-0.67	-0.51	-0.55	0.19	-0.06	0.26	-0.23	-0.54	0.08
Micrococcaceae	-0.63	-0.68	-0.49	0.16	-0.16	-0.63	-0.65	-0.42	-0.49	-0.20	0.23	-0.26	0.31	-0.20	-0.54	0.00
Intrasporangiaceae	-0.28	-0.42	-0.69	-0.09	-0.08	-0.59	-0.58	-0.47	-0.33	-0.33	0.15	0.15	0.14	-0.06	-0.33	0.32
Micromonosporaceae	-0.18	-0.16	-0.40	-0.04	-0.16	-0.30	-0.25	-0.12	-0.23	-0.05	-0.12	0.05	0.01	-0.08	-0.10	0.23
Solirubrobacteriaceae	0.07	0.62	0.35	0.11	-0.28	0.59	0.59	0.40	0.10	0.34	-0.03	-0.22	-0.34	-0.09	0.38	0.10
Nocardiaceae	0.02	-0.17	-0.17	0.20	0.13	-0.18	-0.18	-0.01	0.14	0.02	0.08	0.19	0.13	0.16	0.03	-0.03
Actinobacteria.AKIW543.unclass.	0.20	0.79	0.56	0.13	-0.24	0.81	0.79	0.49	0.19	0.52	0.01	-0.32	-0.44	0.05	0.51	0.08
Bacillaceae	0.03	0.08	0.18	-0.02	0.03	0.13	0.12	0.15	0.08	-0.09	0.05	0.03	0.17	-0.10	0.03	-0.22
Nocardioidaceae	-0.21	-0.22	-0.07	0.11	-0.07	-0.17	-0.14	-0.14	-0.15	0.02	0.26	-0.09	-0.07	-0.13	-0.15	-0.07
Gemmatimonadaceae	-0.03	-0.39	-0.58	-0.22	0.13	-0.53	-0.50	-0.42	-0.05	-0.35	-0.04	0.14	0.17	-0.06	-0.34	0.21
Pseudonocardiaceae	0.42	0.72	0.63	0.07	-0.01	0.78	0.80	0.62	0.37	0.39	-0.21	-0.08	-0.32	0.10	0.56	-0.11
Sinobacteraceae	0.67	0.57	0.69	0.01	0.33	0.66	0.65	0.53	0.55	0.27	-0.29	0.25	-0.17	0.15	0.50	-0.31
Sphingomonadaceae	-0.17	-0.56	-0.37	0.05	0.20	-0.54	-0.53	-0.30	-0.05	-0.35	-0.05	0.18	0.27	0.12	-0.28	0.00
Nakamurellaceae	-0.40	-0.78	-0.71	-0.19	-0.03	-0.84	-0.86	-0.68	-0.41	-0.51	0.13	0.14	0.40	-0.09	-0.50	0.03
Propionibacteriaceae	0.43	0.80	0.69	0.15	-0.03	0.85	0.86	0.61	0.41	0.49	-0.17	-0.10	-0.43	0.15	0.58	0.02
Sanguibacteraceae	-0.17	-0.18	-0.24	0.38	0.09	-0.19	-0.20	-0.01	0.02	0.14	0.18	-0.07	0.12	0.10	0.00	0.17
MND8	0.87	0.60	0.69	-0.05	0.54	0.67	0.70	0.65	0.77	0.21	-0.35	0.41	-0.12	0.19	0.40	-0.26

[a]Values represent Pearson correlation coefficients. Significant (α=0.05) positive correlations are highlighted in dark grey; significant negative correlations are highlighted in light grey.

Table 2.12: Correlations between bacterial 16S sequence diversity and family abundances and soil physicochemical and functional parameters, values recentered to location averages

Variables	Moisture	OM	pH	NO3-N	Bray-P	Ca	Mg	Na	K	SO4-S	Corn yield	Respiration	Net N-min	NAGase	β-glucosidase	Phosphatase
OTU richness (Chao1)	-0.05[a]	-0.07	**0.29**	0.09	-0.07	0.06	-0.03	-0.17	0.09	0.07	0.07	**0.24**	0.00	0.05	0.01	0.00
OTU diversity (inverse Simpson)	-0.05	-0.08	**0.22**	0.12	0.00	0.05	0.09	0.01	0.02	**0.25**	0.02	-0.01	-0.09	0.11	0.07	0.07
Cytophagaceae	-0.09	-0.16	**0.38**	0.21	0.17	0.13	0.03	0.02	0.10	**0.27**	-0.09	-0.05	0.00	0.14	0.08	0.06
Acidobacteriaceae	**0.30**	**0.29**	**-0.23**	0.00	0.05	0.05	0.06	-0.06	0.16	0.07	-0.07	0.09	-0.01	-0.04	0.00	0.05
Sphingobacteriaceae	-0.13	-0.13	-0.16	**0.50**	0.01	-0.15	-0.11	**0.30**	-0.01	**0.41**	0.11	0.08	0.08	0.08	0.04	0.09
Xanthobacteraceae	0.15	**0.26**	-0.05	-0.05	-0.05	0.08	0.03	-0.10	0.05	0.02	-0.07	**0.27**	0.10	-0.09	-0.10	-0.07
Chitinophagaceae	0.03	-0.02	0.12	0.11	0.10	-0.03	-0.03	-0.07	0.08	0.19	-0.16	0.10	-0.03	0.07	0.03	0.04
Microbacteriaceae	**-0.24**	-0.15	-0.18	**0.51**	0.08	-0.13	**-0.24**	**0.23**	0.15	**0.35**	0.18	-0.09	0.16	0.06	0.01	0.02
Streptomycetaceae	-0.08	-0.17	-0.11	-0.14	**-0.22**	**-0.38**	**-0.30**	**-0.29**	-0.11	-0.20	0.09	0.03	0.03	-0.04	-0.01	0.02
Micrococcaceae	**-0.44**	**-0.41**	0.04	**0.32**	0.01	-0.20	**-0.34**	0.11	-0.09	0.17	0.10	-0.20	0.16	-0.02	-0.05	-0.04
Intrasporangiaceae	**0.42**	**0.59**	**-0.32**	0.08	-0.02	**0.26**	**0.32**	0.06	0.16	0.11	0.05	**0.33**	-0.15	-0.08	-0.02	0.03
Micromonosporaceae	0.02	0.12	**-0.23**	0.00	-0.15	-0.18	-0.11	0.02	-0.03	0.14	-0.17	0.20	-0.01	-0.16	-0.15	-0.12
Solirubrobacteriaceae	-0.20	0.02	-0.11	-0.19	-0.19	-0.18	**-0.24**	-0.20	-0.10	-0.20	-0.09	0.15	0.05	**-0.24**	**-0.23**	-0.21
Nocardiaceae	-0.16	-0.10	-0.19	**0.33**	-0.03	-0.18	**-0.24**	0.05	0.16	**0.22**	0.17	0.08	0.07	0.06	0.01	0.01
Actinobacteria.AKIW543.unclass.	0.17	0.17	0.20	**-0.37**	-0.06	**0.24**	0.03	**-0.35**	-0.01	-0.11	-0.06	0.10	0.02	0.04	0.07	0.06
Bacillaceae	**-0.32**	**-0.26**	0.03	-0.05	-0.06	**-0.26**	**-0.39**	-0.11	-0.08	-0.21	0.12	0.04	**0.31**	-0.20	-0.19	-0.20
Nocardioidaceae	**-0.22**	**-0.22**	0.09	0.13	-0.08	-0.17	-0.20	-0.18	-0.03	0.13	0.21	0.03	-0.01	0.09	0.09	0.09
Gemmatimonadaceae	**0.23**	**0.27**	**-0.31**	-0.01	-0.04	-0.02	-0.02	-0.17	**0.22**	0.10	-0.02	0.03	-0.03	-0.12	-0.11	-0.06
Pseudonocardiaceae	-0.18	-0.17	0.03	-0.17	-0.16	-0.17	**-0.25**	**-0.22**	-0.12	-0.13	-0.13	0.04	0.15	**-0.22**	**-0.22**	**-0.23**
Sinobacteraceae	0.20	0.11	**0.33**	-0.01	0.04	**0.33**	0.16	-0.09	0.11	0.08	-0.08	0.13	0.09	0.01	-0.02	-0.06
Sphingomonadaceae	-0.17	-0.21	-0.03	**0.28**	0.05	-0.17	**-0.28**	-0.01	0.18	0.07	-0.02	0.07	0.08	0.00	-0.06	-0.04
Nakamurellaceae	**0.35**	**0.27**	-0.16	0.09	-0.08	0.18	0.12	0.02	0.17	0.11	0.07	0.20	0.05	0.09	0.10	0.09
Propionibacteriaceae	-0.19	-0.16	0.10	-0.14	-0.16	**-0.22**	**-0.30**	**-0.41**	-0.11	-0.10	-0.14	0.06	0.00	-0.08	-0.08	-0.07
Sanguibacteraceae	-0.17	-0.09	-0.20	**0.44**	0.14	-0.13	-0.17	0.17	0.18	**0.29**	0.17	-0.08	0.07	0.03	0.00	0.02
MND8	0.05	0.05	0.09	-0.01	0.10	0.07	-0.02	-0.10	0.18	0.04	0.01	0.05	0.21	-0.05	-0.09	-0.06

[a]Values represent Pearson correlation coefficients. Significant (α=0.05) positive correlations are highlighted in dark grey; significant negative correlations are highlighted in light grey.

Table 2.13: Soil physicochemical parameters identified as associated with relative abundance of bacterial families by redundancy analysis, correlation analysis, and Bayesian inference

	RDA	Correlation	Bayesian
Variables	Moisture	Ca	OM
	pH	Mg	Ca
	OM	Na	NO3-N
	Bray-P	OM	SO4-S
	Na	SO4-S	
	NO3-N	K	

Figures
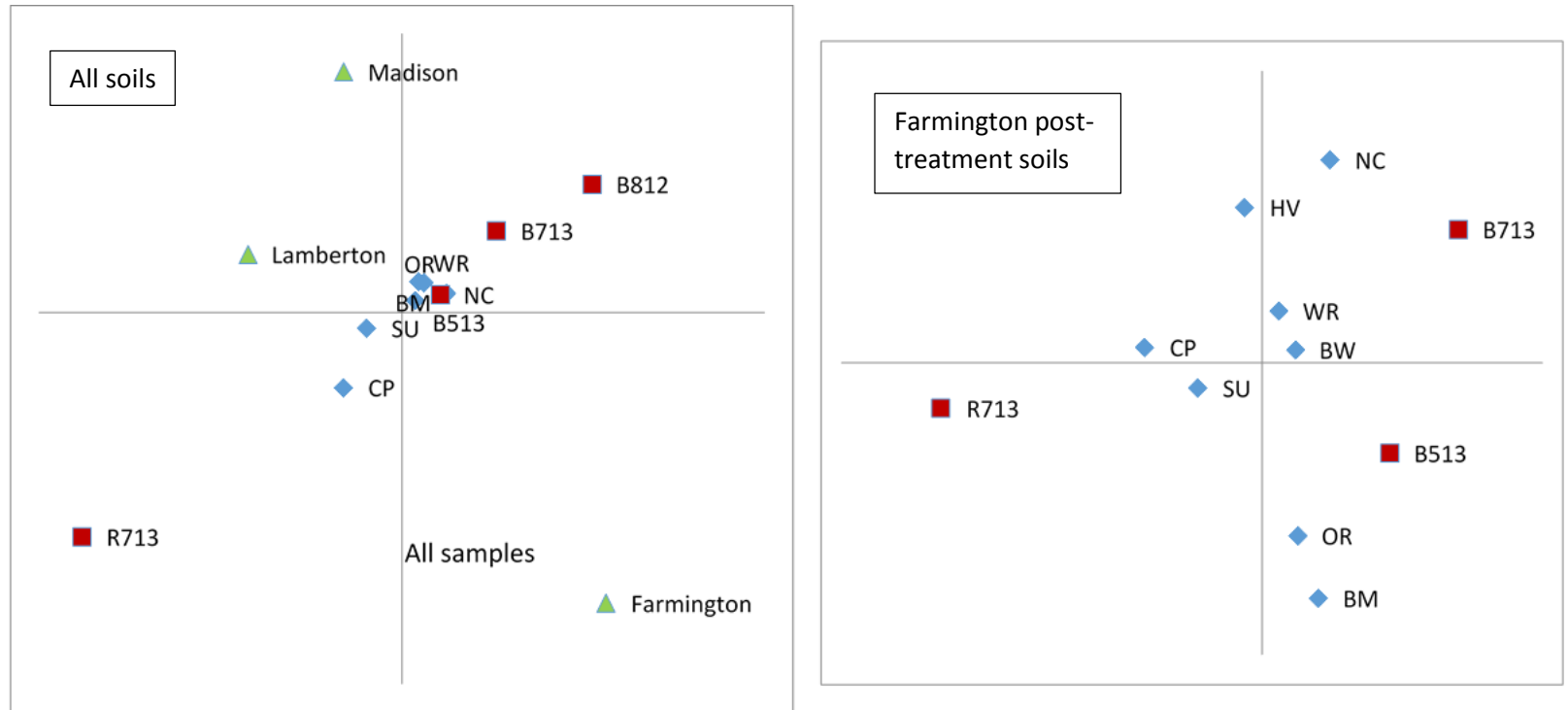
Figure 1.1: Principal component analysis of bacterial family abundances in soils sampled at Farmington, Lamberton, and Madison, MN preceding and following cover crop and organic fertilizer application. Triangles represent location centroids, diamonds represent treatment centroids, and squares represent sampling timepoint centroids. B812, B513, B713, and R713 indicate August 2012 bulk soil, May 2013 bulk soil, July 2013 bulk soil, and July 2013 rhizosphere soil samples, respectively. Treatments are: HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; and NC, no-amendment control.
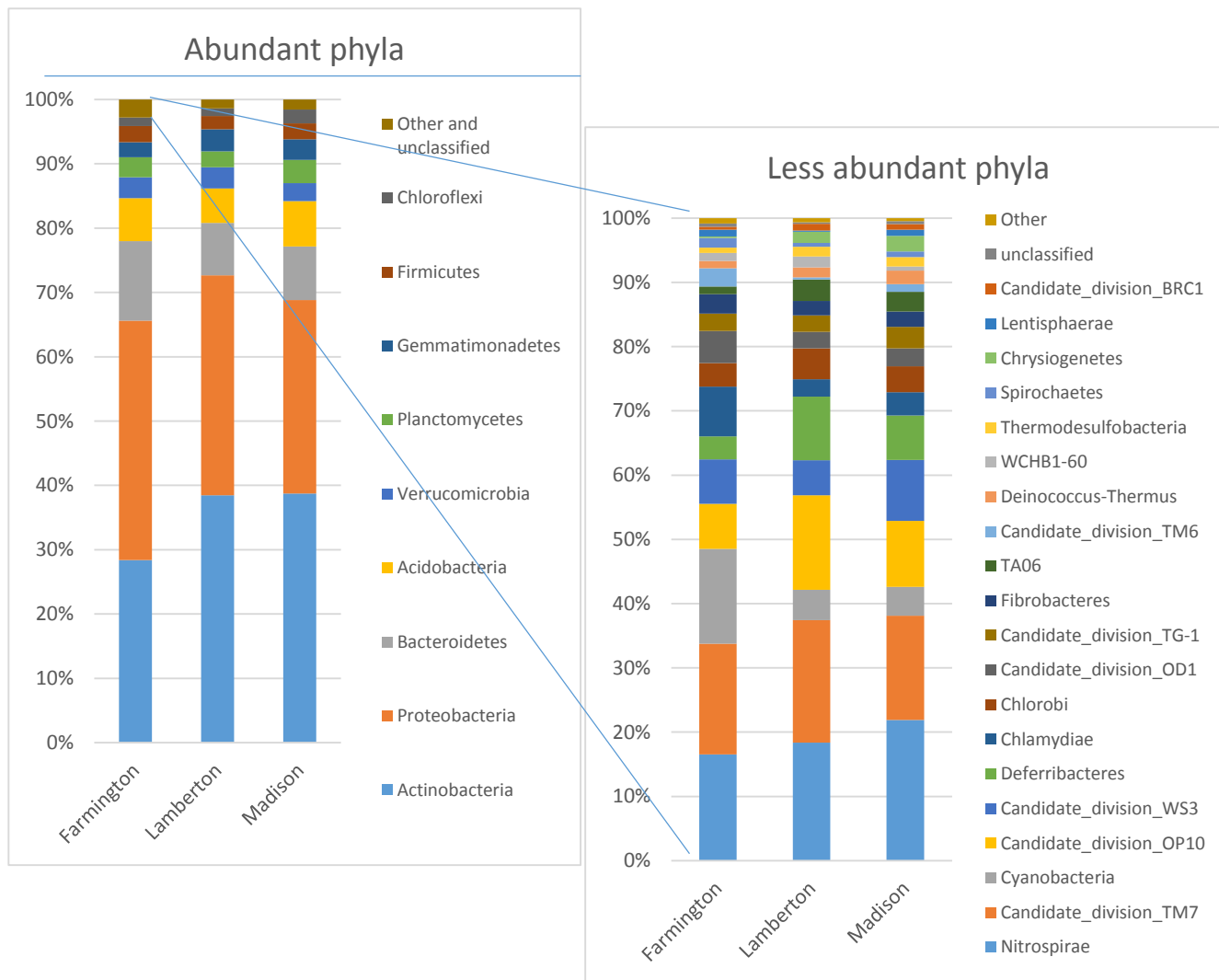
Figure 1.2: Phylum composition of bacterial communities sampled at Farmington, Lamberton, and Madison, MN in August 2012
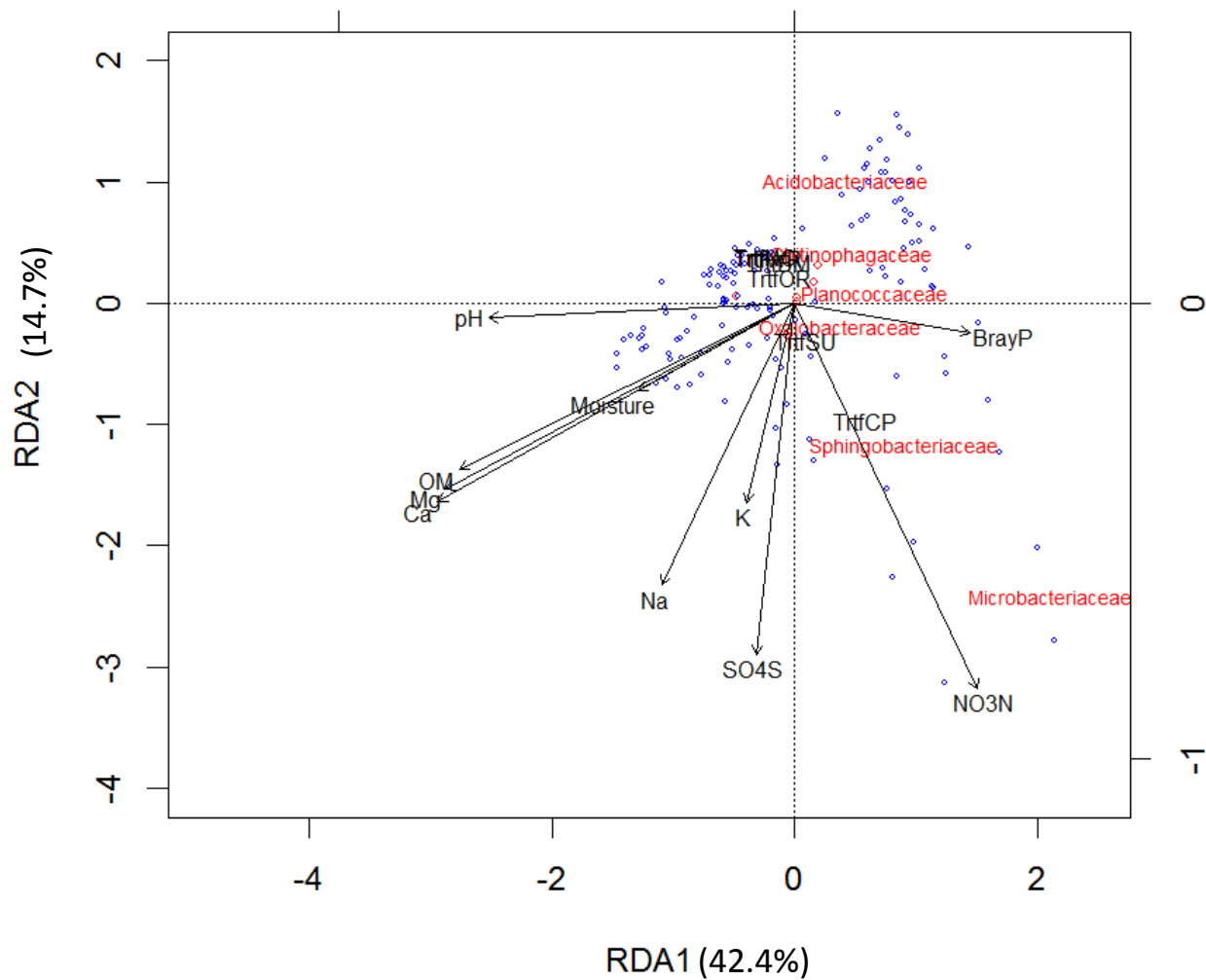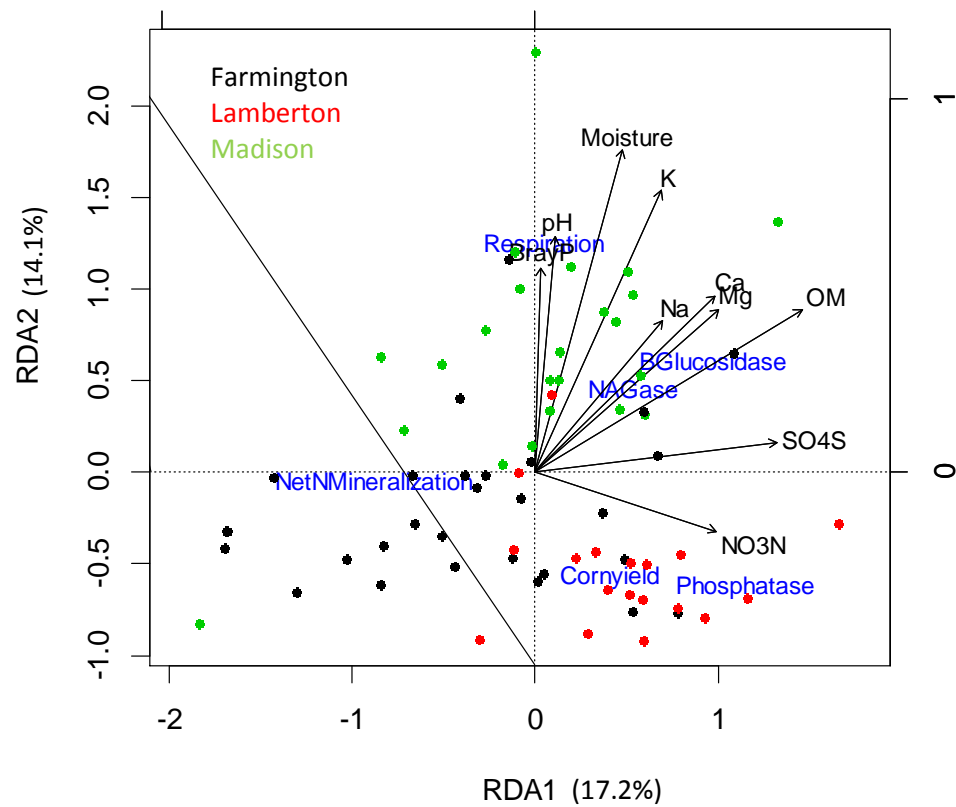
Figure 1.3: Redundancy analysis showing variation in relative abundances of bacterial families explained by soil physicochemical test values and cover crop and organic fertilizer treatments. Treatment centroids are shown as: TrtfHV, hairy vetch; TrtfWR, winter rye; TrtfOR, oilseed radish; TrtfBW, buckwheat; TrtfBM, beef manure; TrtfCP, pelleted poultry manure; TrtfSU, Sustane 8-2-4; and TrtfNC, no-amendment control.

Figure 2.1: Redundancy analysis showing variation in soil functions explained by soil physicochemical parameters at Farmington, Lamberton, and Madison, MN in July 2013.
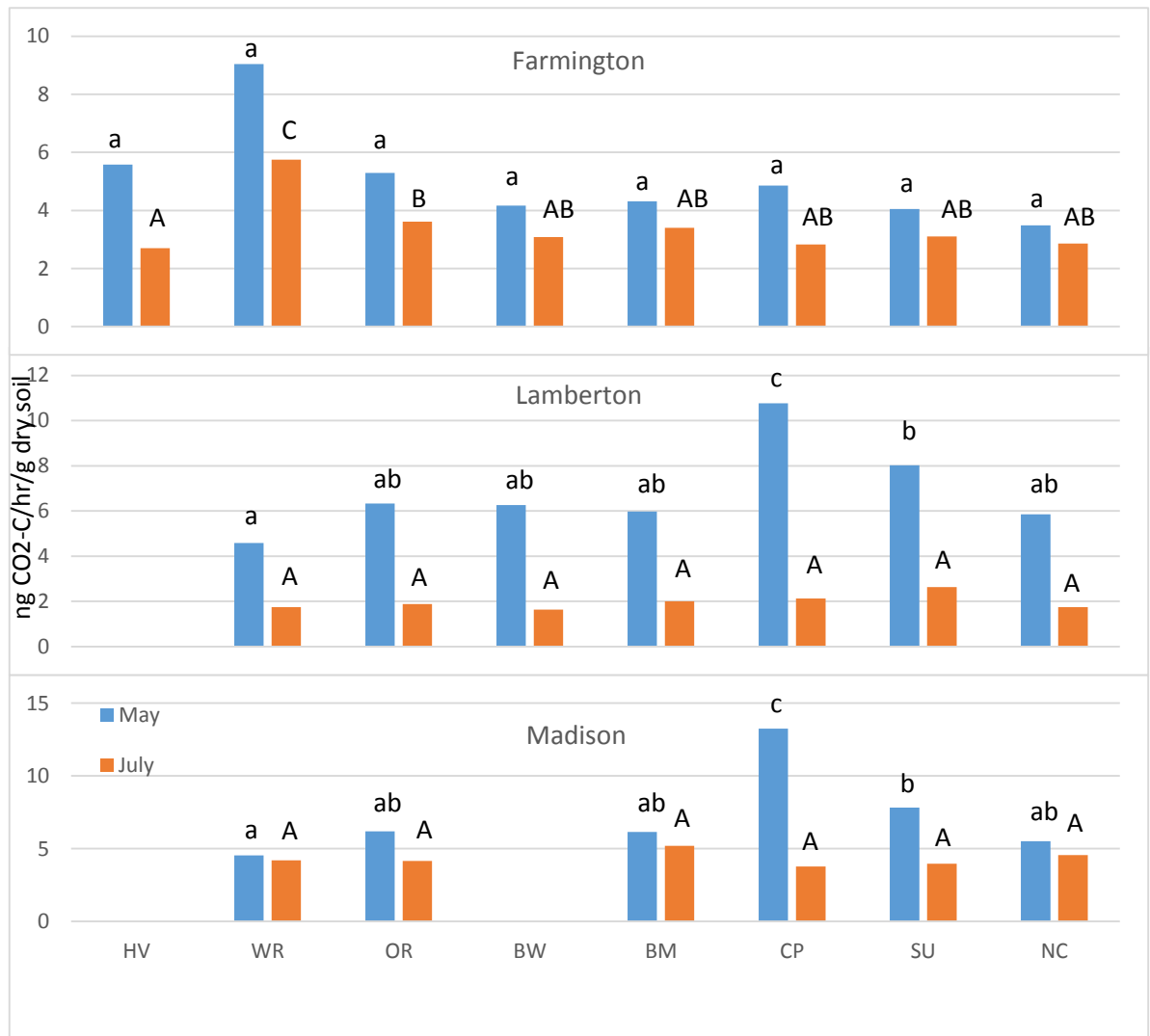
Figure 2.2: Soil respiration in May and July 2013 at Farmington, Lamberton, and Madison, MN following cover crop and organic fertilizer treatment. Treatments are: HV, hairy vetch; WR, winter rye; OR, oilseed radish; BW, buckwheat; BM, beef manure; CP, pelleted poultry manure; SU, Sustane 8-2-4; and NC, no-amendment control. Mean separations are within locations and within sampling months.
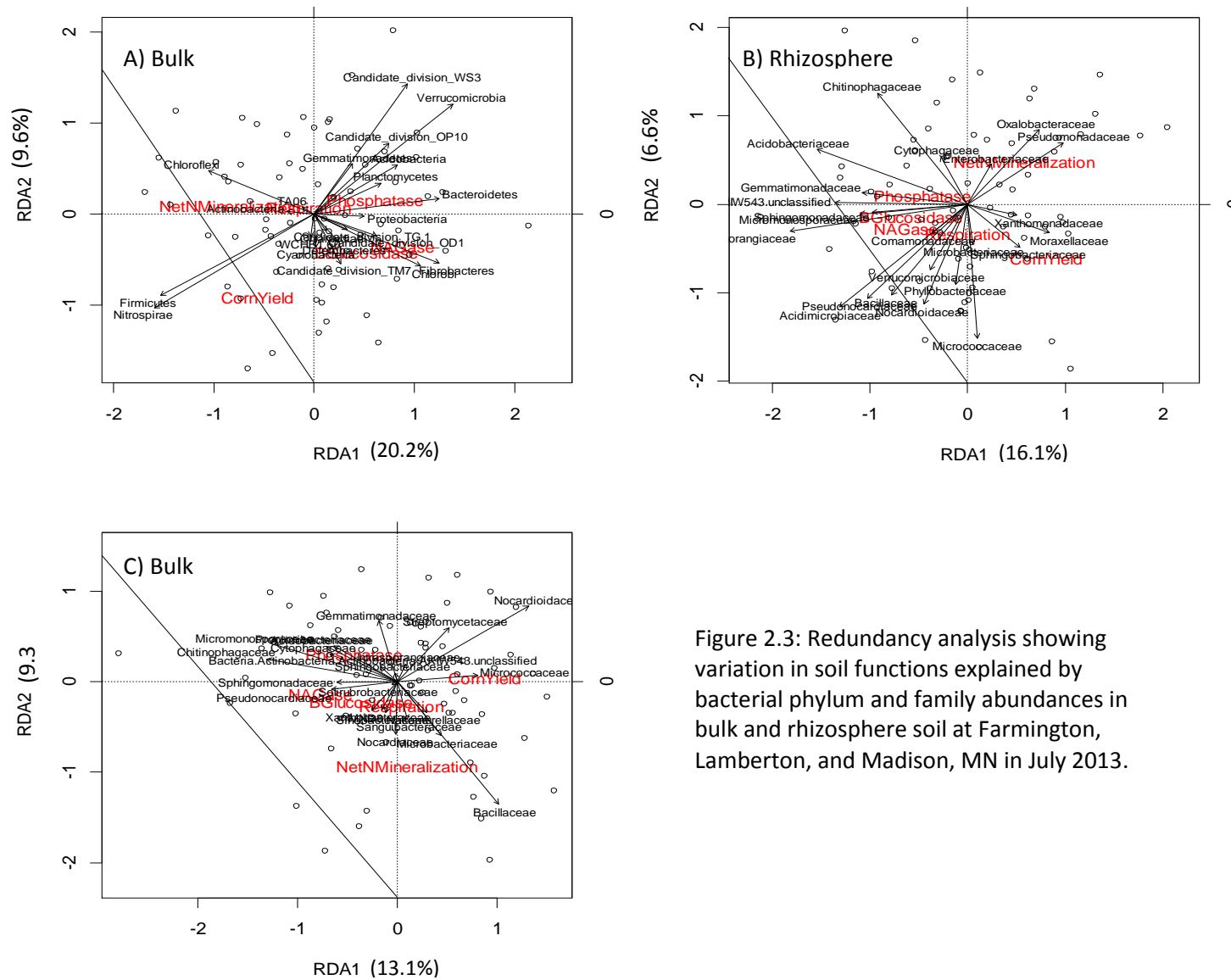
Figure 2.3: Redundancy analysis showing variation in soil functions explained by bacterial phylum and family abundances in bulk and rhizosphere soil at Farmington, Lamberton, and Madison, MN in July 2013.
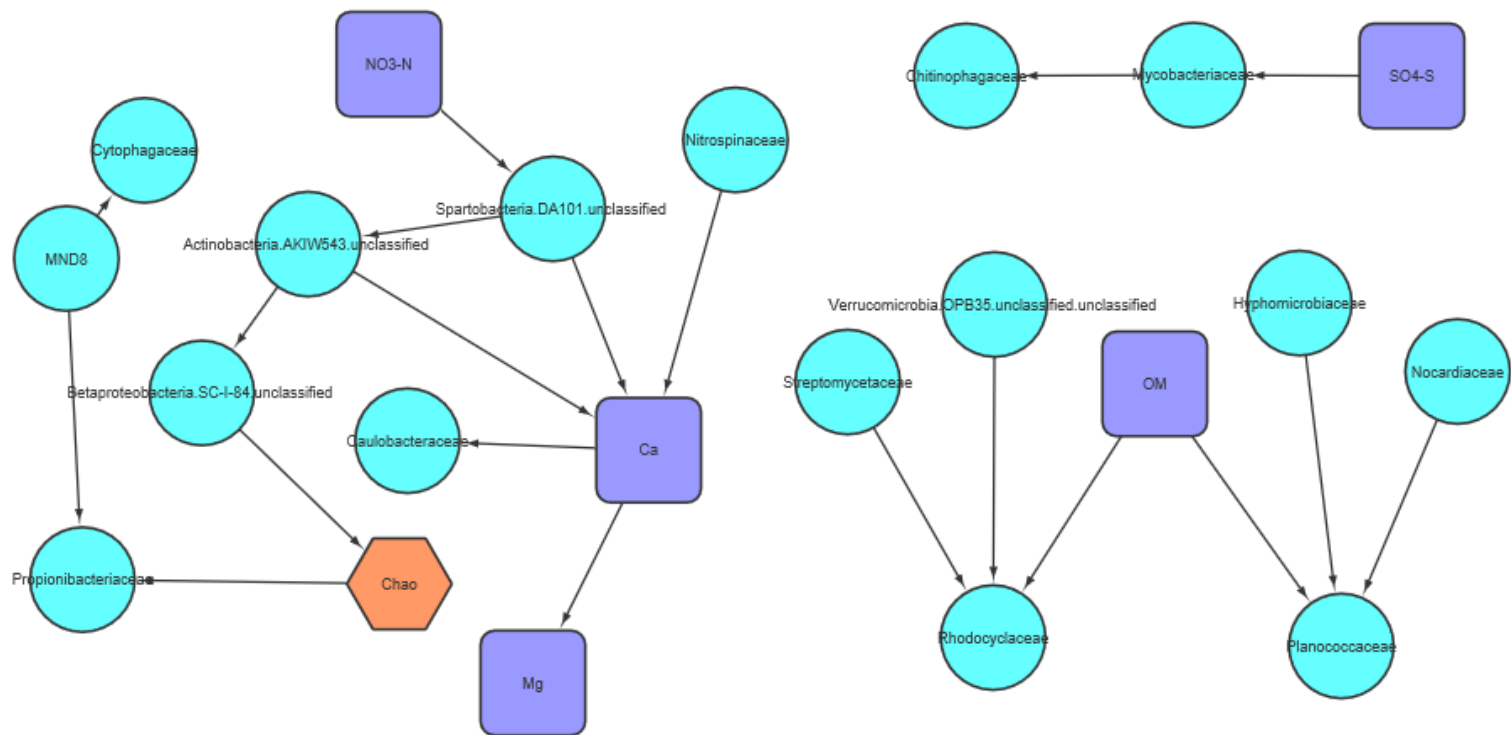
Figure 2.4: Bayesian network showing inferred directional relationships among bacterial families, bacterial diversity parameters, and soil physicochemical properties. Circles represent bacterial families, hexagon represents OTU richness (Chao1 index), and squares represent physicochemical values.

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