Final Report: Metagenomic Exploration of Cover Crop and Amendment Effects on Functional Bacterial Communities in Organic Soil

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Summary

Agricultural soils are home to an enormous diversity of microbes, which carry out many functions essential to soil and crop health, including nutrient cycling, disease suppression, and building soil organic matter and aggregate structure. Organic farmers recognize the soil as a living system whose health depends upon this diversity of microbial functions and knowing how their management practices such as fertilizers and cover crops affect the soil would be valuable. Previous research has documented that additions of organic matter can affect the structure ("who's there") and function ("what are they doing") of soil microbial communities. However, the detailed effects of particular cover crops and organic amendments on particular soil microbes and their functions, and the relationships between effects on soil community structure and changes in soil function are not well described. The development of high-throughput next-generation sequencing technology now allows a uniquely detailed look at the makeup of soil microbial communities. This project used sequencing of bacterial 16S rDNA, which serves as a "fingerprint" for identifying bacteria, to investigate the effects of cover crops and organic fertility amendments on soil bacterial communities. This information was combined with assays of soil respiration, N mineralization, and nutrient-cycling enzyme activity, as well as routine soil tests and corn yield data, to determine whether changes in crop-relevant soil functions were correlated with changes in bacterial community structure. Treatments used were hairy vetch (*Vicia villosa*), winter rye (*Secale cereale*), oilseed radish (*Raphanus sativus*), buckwheat (*Fagopyrum esculentum*), beef manure, pelleted chicken manure, and Sustane 8-2-4, plus a no-amendment control. Cover crops were planted and fertilizers applied in fall 2012 and incorporated in spring 2013. Corn was planted in May 2013. Research was conducted on three organic farms in diverse environments within Minnesota.

This project found that cover crops and fertilizer treatments produced changes in soil properties, nutrient levels, nutrient cycling functions and bacterial community composition. Highlights of our results include:

- Fertilizer and cover crop effects on bacterial communities were stronger in May 2013 than in July 2013, indicating that soil community differences produced by organic matter incorporation are partly short-lived. Bacterial diversity was affected by treatment, with fertilizer and cover crops tending to decrease diversity in May, but increase it by July, when decomposition of the incorporated material was presumably further advanced.
- Fertilizers and cover crops had larger effects on bacterial community composition than on nutrient-cycling enzyme activity by the soil microbes. Changes in the makeup of the bacterial community did not always affect the enzyme activity of the soil.
- Enzyme activity, total bacterial diversity, soil respiration, and net N mineralization did not affect corn yield.

- Fertilizers and cover crops affected soil respiration, which is an indicator of total microbial activity. In particular, pelleted poultry manure produced an early-season spike in activity, while rye provided a later-season boost.
- Members of the phylum *Actinobacteria*, a group associated with disease suppression, were increased near corn roots in oilseed radish at Lamberton and in winter rye at Madison. This bacteria group was more sensitive to fertilizers and cover crops than other bacteria.
- Effects of amendments on bacterial community structure varied widely among sites, possibly due to differences in initial bacterial communities and their responsiveness to changes in soil conditions. Further research will be necessary to examine how initial physical and microbial conditions affect the impacts produced by fertilizers and cover crops.
- Many of the observed associations of the fertilizer and cover crop treatments are not immediately translatable into recommendations for growers. However, this research indicates that pursuing a better understanding of bacterial community composition and how it is affected by organic farming practices is a promising avenue for increasing our ability to predict the effects of management on important soil functions.

Introduction

Designing management systems to produce desired effects on soil microbial communities and their functions is a new frontier in agronomic research and one that is particularly relevant to organic growers, who rely on biological sources of fertility and plant protection (Bowles et al., 2014). Organic field crop growers face the complex task of designing diverse management systems that build soil health and also support strong crop yields. Because soil microbes are responsible for many of the functions that are considered to constitute soil health, there is a need for better understanding of the ways that agricultural practices can affect soil microbial community structure ("who's there") and function ("what are they doing").

The structure and function of soil communities are highly relevant to agricultural production. Microbially-mediated transformations are key to the availability and uptake of most macro- and micronutrients important to crop growth (Miransari, 2013). Microbial communities are responsible for plant disease prevalence and suppression (Bezemer and van Dam, 2005; Hayat et al., 2010). Additionally, microbes are essential to 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). Although bacterial community structure is largely determined by abiotic soil characteristics, particularly pH (Drenovsky et al., 2010; Fierer et al., 2012; de Vries et al., 2012), microbial communities are not merely the "mechanism" carrying out the effects of soil physicochemical properties, but may also be independent drivers of soil function (Strickland et al. 2009).

Crop-relevant soil microbial functions are affected by agricultural management. Management factors with demonstrated effects on community structure include tillage, cover cropping, and fertilizer and organic amendment addition (Buyer et al., 2010). The work of Caporaso et al. (2012) indicates that a

vast “seedbank” of bacterial taxa exists in environmental samples, from which particular taxa may rise to abundance as conditions permit. This suggests that the microbial community of a given soil, and its associated functions, will depend largely on conditions prevailing in that soil, although the availability of inoculum likely also plays a role in the presence of certain taxa. Next-generation DNA sequencing technologies allow microbial community structure to be investigated at unprecedented levels of detail. In this project, we used Illumina sequencing of a hypervariable region of bacterial/archaeal 16S DNA that serves as a molecular “fingerprint” for identifying the presence, abundance, and detailed phylogenetic identity of bacterial and archaeal community members.

To date, next-generation sequencing has been little used in studies of agricultural soils. Other studies, often using culture-independent but not sequencing-based profiling techniques such as fatty-acid or DNA restriction fragment signatures, have investigated the impacts of organic material incorporation on soil communities. These studies have differed in their findings on whether different cover crops produce distinct effects on soil communities (Bowles et al., 2014; Buyer et al., 2010; Pieta and Kesik, 2008; Marschner et al., 2003). In this experiment, we combined bacterial 16S sequencing with assays of enzyme activity, N mineralization, and soil respiration to correlate the observed differences in the bacterial communities with measures of soil community function. Enzymes used were β-Glucosidase, which is involved in soil C cycling, N-acetyl-β-D-glucosaminidase (NAGase), which is a chitinolytic enzyme contributing to N and C cycling, and phosphatase, which releases plant-available inorganic phosphate from organic molecules.

We investigated the effects of four cover crops with very different growth habits and biomass composition – hairy vetch (*Vicia villosa*), winter rye (*Secale cereale*), buckwheat (*Fagopyrum esculentum*), and oilseed radish (*Raphanus sativus*)-- and three organic fertilizers with contrasting chemical and microbial compositions – beef manure, pelleted poultry manure, and bagged commercial organic fertilizer. These treatments were designed to realistically represent practices available to organic farmers in the Upper Midwest. Our objectives were to determine the effects of these practices on the structure and function of soil microbial communities, and to investigate the relationship between bacterial community structure and functional parameters of the whole microbial community. Nutrient cycling functions including net N mineralization, soil respiration, and activity of C-, N- and P-cycling enzymes, were assayed. Bacterial community composition was characterized by classifying 16S rDNA sequences from soil extracts. Assay values were correlated with observed properties of our 16S community inventories, as well as overall sample diversity. This two-pronged approach was designed to assess the validity of bacterial community composition as a predictor of soil function, guiding future research using sequence datasets.

Methods and Materials

Field experiment

Study fields were established at three Minnesota locations: the Southwest Research and Outreach Center in Lamberton, Carmen Fernholz's A-Frame Farm in Madison, and Scott Johnson's Spruce Valley Organics in Farmington. These sites have 5+ years under organic certification and at least 10 years in organic management.

The experimental design was a randomized complete block with eight treatments: four cover crops, three fertilizers, and a no-amendment control. Plots were 6.1 x 9.1 m with 6.1 m alleys. Tillage operations were conducted perpendicular to wide 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. Fields were cultivated to remove volunteer barley immediately before cover crop planting. Eight organic amendment treatments (Table 1) were applied. Cover crops were planted August 13-15, 2012. Cover crop treatments were hairy vetch, winter rye, oilseed radish, and buckwheat, seeded at rates of 22, 112, 22, and 56 kg ha^{-1}, respectively. Fertilizer treatments were pelleted chicken manure, beef manure, and Sustane 8-2-4, a fertilizer approved for certified organic production. 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. Study fields were tilled in April 2013 to incorporate cover crop residue and terminate overwintered rye and vetch, and were planted to corn in May 2013.

Table 1: Organic amendment treatments

Hairy vetch (*Vicia villosa*)
Winter rye (*Secale cereale*)
Oilseed radish (*Raphanus sativus*)
Buckwheat (*Fagopyrum esculentum*)
Beef manure
Pelleted poultry manure
Sustane 8-2-4 (OMRI-approved commercial fertilizer†)
No-amendment control

† Organic Materials Review Institute, 2015

Due to very dry conditions in the study year, cover crop establishment in fall of 2012 was inconsistent. For all tests of treatment effects, cover crop plots that produced less than 20% ground cover in fall 2012 were excluded.

Soil sampling

Samples of bulk field soil were collected in August 2013 immediately following cultivation and prior to planting of cover crop treatments, in April 2014 immediately following incorporation of cover crop residues, and in July 2013 when corn was at the V13 stage. Ten random cores per plot at a depth of 10 cm were collected using a hand probe, 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. Additional samples were taken and used for N mineralization and soil respiration assays and for chemical analysis. Corn rhizosphere samples were collected at the July 2013 sampling date by washing the adhering soil from root pieces from three corn plants in a gelatin buffer and centrifuging to collect the soil.

Soil chemical analysis

Data collected are summarized in Table 2. Samples were submitted for analysis to the University of Minnesota Research Analytical laboratory. Parameters analyzed were: Bray-P, K (water-soluble + exchangeable), NO_3-N, SO_4-S, organic matter (loss on ignition method), exchangeable cations (Mg, Na, and Ca), and pH (water method).

Nitrogen mineralization assay

50 g of soil were placed in specimen cups and covered with polyethylene film. Cups were incubated at 20° C for 28 days. Soil was sprayed weekly with deionized H_2O to restore to initial sample moisture level. At 0, 7, and 28 days , 10g of soil was removed from incubations and shaken in 1.0M KCl solution for 30 min to extract soluble N. Solution was filtered to remove suspended soil. N content of KCl extracts was quantified using colorimetric salicylate (ammonium) and vanadium (nitrate/nitrite) assays.

Soil respiration assay

50 g 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 hours. Triplicate 5-mL samples of headspace gas were drawn from the jars immediately after closing and following 24 and 48 hours of incubation. Headspace samples were analyzed for CO_2 content using a gas chromatograph. Standards at 0, 645, 1025, and 10,000 ppm CO_2 were used to construct a standard curve, which was used to quantify CO_2 content of headspace samples, from which CO_2 evolution was calculated.

Enzyme activity assays

Methylumbelliferone (MUB)-linked substrates were used to measure activity of phosphatase, N-acetyl-β-D-glucosaminidase (NAGase), and β-glucosidase in each subsample from the May 2013 and July 2013 timepoints. 0.5 g of soil was suspended in 50 mL 100 mM maleic acid buffer (pH 6.8). 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. 25 µM MUB was used as

a reference standard. 0.01 µmol substrate per assay were used. Plates were incubated for 1 hour at 25 C for all substrates. Reactions were stopped by adding 10 µL 0.5 M NaOH, and were read 10 minutes after NaOH addition at 365 nm emission and 450 nm fluorescence.

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. Corn rhizosphere soil in July 2013 was isolated as described above. DNA was extracted from homogenized bulk soil samples and from pelleted rhizosphere soil using MoBio PowerSoil kits. Amplicon preparation and sequencing were performed by the University of Minnesota Genomics Center. 16S rDNA from the V5-V6 hypervariable region was PCR amplified using Nextera primers and barcode indexed. Amplicons were paired-end sequenced on Illumina MiSeq at a read length of 2x300 bp.

Sequence data processing was carried out using mothur software for microbial ecology (Schloss et al., 2012). Sequences were paired-end aligned and screened for quality. Sequence read number was normalized by random subsampling to 35,724 reads per sample. Sequences were aligned to the SILVA database (Quast et al., 2013), and sequences corresponding to chloroplast lineages were removed. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity and classified to the SILVA database. Due to dataset size, clustering and analysis were carried out within sites and sampling time points. Site and treatment effects were evaluated using analysis of molecular variance (AMOVA), which detects differences in community membership, based on Yue and Clayton distance matrices.

Table 2: Data collected on soil properties, function, and bacterial community

Variable	**Determination**
Soil 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-B-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

Statistical analysis

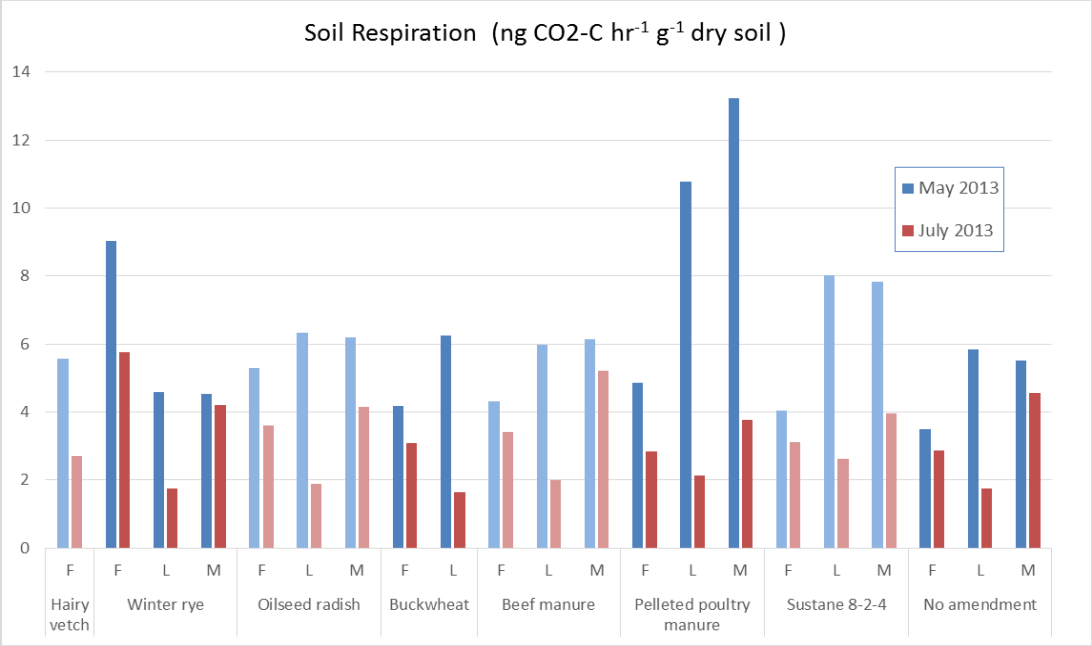
Principal component analysis, principal coordinate analysis, and discriminant function analysis (DFA) were performed using XLStat software. Redundancy analysis and analysis of variance were performed in R using packages vegan, nlme, and lmertest (R core team, 2014). All statistics were evaluated at $\alpha = 0.05$.

Results and Discussion

Cover crop and fertilizer treatments affected soil test values and measures of soil function. Pelleted poultry manure and to a lesser degree, Sustane, produced large increases in NO_3-N in May, which were mostly depleted but still visible in July. This increase was accompanied by decreases in pH, an effect that would also be expected with inorganic N application. At Farmington, though not at the other sites, N was also higher in July in cover crop treatments than in control, suggesting that covers 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. Organic matter was not affected by treatment.

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 studies (Bossio et al., 1998; Kuramae et al., 2012; Bisset et al., 2011). Soil respiration is an indicator of total microbial activity. Pelleted poultry manure produced large increases in soil respiration in May samples at Lamberton and Madison, but this spike was short-lived, and was no longer apparent by the July sampling date (Figure 1). Soil respiration consistently decreased between May and July sampling times, including in the no-amendment control. This is attributable to increasingly dry conditions as the growing season progressed. However, despite the overall decline, we observed higher July respiration values in the winter rye compared to the no-amendment control at Farmington, suggesting a somewhat delayed or sustained boost in soil activity prompted by the slower breakdown of incorporated rye biomass.

Figure 1: Soil respiration as in May and July 2013 as affected by amendments.



High variance made it difficult to detect differences in nutrient-cycling enzyme activity associated with treatment; however, some treatment effects were observed. In almost all cases where treatments differed from the no-amendment control, enzyme activity was higher in the treatments. Both cover crops and fertilizers were observed to raise activity levels of at least one enzyme.

Treatments changed in bacterial community composition and diversity in May bulk field soil, July bulk field soil, and July corn rhizosphere soil. Effects were strongest in the May samples, which showed differences in overall community membership and diversity at all three locations. Treatments affected taxanomic unit (OUT) richness and diversity of both bulk and rhizosphere soils (Table 3). OTUs may be considered roughly equivalent to species, although the concept of species is difficult to apply to bacteria. OTU richness indicates the number of OTUs present in each soil, while diversity measures account for both the total number of OTUs and the evenness of the community (the degree to which a small number of groups dominate the community). As expected, much higher OTU richness and diversity were found in bulk soil than in rhizosphere soil. In most cases, where treatments significantly differed from control, they actually lowered species richness and/or diversity—particularly pelleted poultry manure, although pelleted poultry manure did increase 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), which are microbes that are able to respond to large nutrient inputs with rapid growth. This is also consistent with our observation that the pelleted poultry manure and Sustane treatments produced some effects that were similar to other forms of highly available N fertilizers, including inorganic fertilizers. 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. 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). However, our findings underscore the fact that microbial diversity is a complex and dynamic property that is not easily summarized.

Table 3: 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	6018	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

†Mean separations are within locations, bulk or rhizosphere soil, and sampling times. Letters highlighted in green represent significant increases compared to the no-amendment control; letters highlighted in yellow represent significant decreases.

Treatments were associated with differences in abundance of key bacterial groups associated with important soil functions. 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 pelleted poultry manure, tended to decrease relative abundance of the ammonia-oxidizing family *Nitrosomonadaceae*. At Farmington, this family tended to be reduced in all cover crop and fertilizer treatments.

Relative abundance of members of the class *Rhizobiales*, which includes symbiotic nitrogen fixers *Rhizobium* and *Bradyrhizobium*, was indistinguishable from the control in cover crop treatments, including, surprisingly, hairy vetch; however, it was reduced in both bulk (at Farmington and Madison) and rhizosphere (at Madison) soils in pelleted poultry manure, suggesting that large soluble N additions, in addition to their well-documented tendency to decrease the effectiveness of symbiotic N fixation (Salvagiotti et al., 2008), may be directly detrimental to populations of potential microbial symbionts when a legume host is not present.

The phylum *Actinobacteria,* whose overall 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), was not affected by treatment in bulk soils; however, it was increased in the corn rhizosphere community in oilseed radish at Lamberton and in winter rye 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). Prevalence of members of the phylum *Firmicutes*, also associated with disease suppression, was not affected by treatment.

OTU composition of the full 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". The composition of these subsets was observed to differ at various taxonomic levels from the overall dataset. In both bulk and rhizosphere soil, individual OTUs belonging to the *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. This may reflect the ecological and metabolic significance of these groups, which 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. Alternatively, groups overrepresented in core or treatment-affected OTUs may be those for whom similarity in the 16S rDNA region that we sequenced tends to be better aligned with metabolic similarity, which would result in OTU clustering being a better identifier of ecologically coherent units.

As expected, conventional soil tests were highly predictive ($p<0.0001$) of bacterial community composition 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 significant prediction by Ca as well at the phylum level and Na and NO3-N at the family level. Soil tests were also somewhat predictive of soil functions (enzyme activity, respiration, net N mineralization, and corn yield), but, interestingly, incorporation of bacterial community composition data significantly

improved our ability to predict function over soil test data alone: when both soil test values and bacterial community composition were used as predictors of measured functions, rhizosphere community composition uniquely explained (this does not imply *caused*) 37.9% of the total explainable variation in function, while bulk soil community composition uniquely explained 39.3% of explainable variation in function. A wide variety of individual correlations was observed between specific soil functions and bacterial groups. β-glucosidase activity was negatively correlated with net N mineralization, and several families associated with higher β-glucosidase activity were also associated with lower net N mineralization, and vice versa. Correlations between taxa and functions should not be assumed to imply causation; in most cases they probably represent co-occurring responses to soil conditions.

Conclusions

Cover crop and organic fertilizer applications affected soil properties, nutrient levels, nutrient cycling functions, and bacterial community composition. Pelleted poultry manure produced large boosts in soluble N levels, which were associated with large short-term increases in soil respiration, as well as effects that would generally be expected from large additions of highly available N, including reductions in pH, bacterial diversity, and abundance of symbiotic N-fixing bacteria.

At Farmington, winter rye caused an increase in soil respiration that became more prominent later in the season. Due to high variance in measurements of net N mineralization and nutrient-cycling enzyme activity, differences associated with treatments were difficult to detect. However, winter rye at Farmington increased N- and C-cycling enzyme activity compared to the no-amendment control, and all treatments were associated with increased enzyme activity at a minimum of one location and sampling time.

Cover crops and fertilizers tended to be associated with decreased bacterial diversity in May following application, but with increased diversity in July, when decomposition of the incorporated organic material was presumably more advanced. Fertilizer treatments, particularly pelleted poultry manure, tended to decrease relative abundance of the ammonia-oxidizing family *Nitrosomonadaceae*, as did cover crops in some cases at Farmington. Relative abundance of members of the phylum *Actinobacteria*, which has been associated with disease suppressiveness, was increased in the corn rhizosphere community by oilseed radish at Lamberton and by winter rye at Madison.

Bacterial community structure data improved the predictability of soil functional profiles compared to conventional soil data alone. The relative abundances of specific bacterial groups may be useful as indicators of functions, even if they are not the agents of those functions. Although many of the observed associations between amendment treatments, the abundance of specific bacterial groups, and measured soil functions are not immediately translatable into recommendations for growers, 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.

Outreach

This research was presented at the Organic Field Day in Lamberton in 2013. Experimental design and preliminary results from this research were presented in poster form to a primarily grower audience at the MOSES Organic Farming Conference in February 2014, and to an academic audience at the Soil Science Society of America meeting in November 2014. Three papers for academic publication are under development, and will be submitted for peer review in mid-2015. Summary sheets for grower audiences are being prepared, as well as an article for farmers and interested members of the public, with insight and feedback from our farmer-collaborators, Carmen Fernholz and Scott Johnson. Final results will also be presented at the MOSES conference in February 2016.

This project has also developed closer ties between collaborating farmers and organic researchers at the University of Minnesota, partnerships which have resulted in the development of further on-farm research projects.

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