Effects of organic fertilization on soil bacterial community structure in incubated microcosms

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

Addressing the growing demand for food from a burgeoning population requires agricultural methods that sustainably support increases in crop production while maintaining environmental health. Agricultural practices that include the use of compost, in lieu of mineral fertilizers, have been shown to reduce environmental impacts and improve soil health. Producers seeking to improve sustainability through compost use are challenged by the chemical form and availability of nutrients in organic amendments, limiting their ability to predict when nutrients will be available to crops. To better understand nutrient dynamics in soils amended with organic fertilizers, we compared the soil microbial community response to amendments with differing carbon and nitrogen content. Composted horse manure was chosen to represent an organic high carbon amendment, and alfalfa hay was chosen to represent a high nitrogen amendment. Amended soils were incubated for 97 days and destructively sampled on seven progressively longer incubation intervals. DNA was extracted for microbial community characterization, and measurements of nitrogen, carbon, and biomass were also compared for all samples. Our results showed significant shifts in soil microbial communities due to both time and amendment. Nutrient release was highly associated with amendment composition, with alfalfa showing the greatest release of plant available nutrients. We observed complex interactions between soils and amendments, with specific bacteria associated with nitrogen and carbon metabolism. These bacteria are targets for further characterization and better knowledge of their role in decomposition may contribute to the increased use of high organic matter fertilizer by producers and lead to a better understanding of sustainable crop production techniques.

CHAPTER 1. INTRODUCTION

Agricultural output of crops with high nutrient demand (e.g., maize) requires soils to be amended with nitrogen fertilizers for economical production[1]. Following the green revolution and development of the ability to synthesize mineral fertilizers, agricultural output has increased significantly to feed the growth of the human population[2]. This increase in mineral fertilizer usage and agricultural output has had environmental side effects. Soluble mineral fertilizers are prone to leaching and losses to water ways, where they contribute to eutrophication and degraded water quality[3]. Additionally, fossil fuel use in the production of fertilizers contributes to increases in atmospheric greenhouse gases[4]. To cope with the demands of and increasing human population, agricultural production must become both more sustainable and more efficient to minimize impacts on the environment.

An alternative method to provide nutrients for agriculture is fertilization of soils with inputs approved for organic management. These organic managements techniques exclude the use of synthetic mineral fertilizers and instead rely on addition of nitrogen via amendments with compost, manure, or green (plant-based) manures. To use the nitrogen from these amendments, crops rely on biological transformation nitrogen in organic forms into plant available nitrogen. Currently, little is known about the microbial community during these processes in organic agriculture, especially how to optimize the amounts of nitrogen available to plants post amendment with organic fertilizers. Further, nutrient availability varies by the type and nutrient ratios of organic fertilizers. For example, the carbon to nitrogen ratio of organic amendments impacts the decomposition rate and whether N is mineralized into plant available forms or immobilized by the soil microbial biomass [5,6]. A better understanding of the microbial communities and their responses to nutrients

available from organic amendments will help improve its effective adoption and usage.

Previously, the use of organic management has been observed to reduce nitrogen losses to sub-surface waters. For example, in a study comparing an organic corn soybean production system utilizing extended crop rotations and animal manures for nitrogen fertilization, to a conventional corn-soybean system fertilized with synthetic urea, reduced losses of nitrates were observed in the tile drainage water from the organic system compared to the conventional system[7]. This is evidence to the potential of organic agriculture to ameliorate environmental degradation associated with intensive chemical agriculture. Within this study, reduced nutrient losses into the environment was associated with high variability and reduction in yields, which may be linked to the ability of the soil microbiome to liberate nitrogen bound in complex organic compounds, transforming it into mineral forms and ultimately making it available for plant uptake and growth. This biologically mediated process where plant available nitrogen is released from organic sources is known as mineralization and has been associated with litter/amendment C: N ratios below 25:1. When C: N ratios of more than 25:1 are observed in litters and amendments, immobilization has been observed, which is the utilization of nitrogen compounds for microbial growth at the expense of plant available mineral nitrogen [5]. Thus C: N ratios are a critical factor affecting the impact of organic fertilizers on levels of plant available nitrogen in the soil.

Improving our understanding of the biological players involved in nutrient release could help improve organic production while still leveraging its environmental benefits. A key knowledge gap for varying organic management strategies is understanding the soil nitrogen (N) pool and the role of associated microbial communities as drivers of N cycling. In this study, we study the impacts of amendments of both alfalfa and compost, provided at

equal rates of total nitrogen, to a soil but with differing C: N ratios chosen to result in immobilization (compost) or mineralization (alfalfa). We characterize both the chemical and microbial response to these amendments and hypothesize that specific microbial communities will respond to initial nitrogen and carbon availability and that this membership will be specific to varying amendments. We expect that these distinct early microbial responders will dominate soil microbial communities in response to organic amendments and will decrease in abundance through time. Our objective was to characterize these early responding microbial communities for various organic amendments and to identify potential microbial membership within organic amendments that may be involved in plant nutrient availability.

CHAPTER 2. MATERIALS AND METHODS

Four organic nitrogen amendments were chosen based on current use in organic agriculture and predicted effects on soil nitrogen cycling: (1) an amendment of alfalfa residue with low C: N ratio, simulating plow down of alfalfa hay as a nitrogen source before maize production; (2) stable composted horse manure with a high C: N ratio used as an amendment replicating the use of composted manure on many organic farms; (3) an amendment consisting of a mixture of alfalfa residue and compost was constructed to represent a neutral C: N ratio; and (4) a control treatment receiving no amendment included in the study as a reference. Alfalfa hay samples were collected following hay harvest and processed by passing fresh hay through a grinder and then through a 2mm mesh screened cyclone mill. Dry alfalfa was then stored in an air-tight vessel prior to use as amendment. Compost samples were collected on September 23rd 2015 from a large windrow of composted horse manure and saw dust bedding, the mixture was composted by the ISU Compost Facility, located at 52274 260th St. Ames, Iowa. Compost was dried, processed, and stored in the same manner as the alfalfa amendment. Soil originated from the USDA-ARS Organic Water Quality (OWQ) research site [7], situated near Boone, Iowa; on the ISU Agronomy Research Farm. Surface soil (0-15 cm) was taken from alfalfa plots that were in a four-year corn-soybean-oat/alfalfa/alfalfa rotation under organic management. The site was located on the Clarion-Nicollet-Webster soil association with fine-loamy texture soils. A total of 25 kg of soil was taken and processed through a 2 mm sieve, allowed to air-dry and stored in air-tight vessels before use.

Microcosms were constructed by mixing soil and each amendment treatment. Each microcosm consisted of 50g of air dried 2mm soil plus amendment, applied at a rate of 134.5

kg/ha of total nitrogen (Table 1). The amended and control soil samples were incubated for 97 days under aerobic conditions at 30°C in 3.79 l glass jars. During the incubation, samples were aerated every 24 hours by removing the lid and kept moist via addition of deionized water to the bottom of the 1-gallon jar. Soil samples were wetted to 60% water-filled pore capacity and placed into the incubator for a pre-incubation at 30° C before analysis of the first samples on day seven. Samples were subsequently analyzed on day 14, 21, 35, 45, and 97 yielding 336 incubated samples for the four treatments with n = 12 for replicates. Samples representative of Day 0 conditions were constructed by extracting DNA from un-wetted soils mixed in the same ratios as incubated samples.

Figure 1. *Organic inputs for soil microcosms.*

2 mm air dried soil, the receiving soil used to construct all microcosms (left); alfalfa hay ground to 2mm after air drying, the alfalfa amendment (center); composted horse manure and sawdust ground to 2mm, the compost amendment (right).

Laboratory Methods

The bacterial community was characterized by phylotyping using DNA sequencing. Microcosm soils were homogenized during destructive sampling and a sub sample of soil was frozen immediately using dry ice, for preservation until extraction could be performed. DNA extraction was performed using the HTP 96 well power soil kit from Qiagen using 0.25 g of soil. Following extraction, 16S rRNA genes were sequenced on an Illumina MiSeq using 16S V4 primers at Argonne National Lab in Lemont, Illinois. Mothur version 1.41.0 pipeline was used for sequence processing of the 151 bp paired end reads and operational taxonomic units (OTUs) were defined based on 97% genomic similarity [8]. Taxonomic assignment of OTUs was completed by alignment to the most similar representative gene in the Silva 16S ribosomal database version 123.

Microbial biomass was quantified using moist microcosm soil subsampled during destruction of microcosms on sampling days. Microbial biomass carbon was calculated and measured using standard soil fumigation-extraction methods modified for a 20 g sample. Briefly, two sub-samples of 20 g of moist soil from each microcosm were weighed into 50 ml beakers and 125 ml bottles. The 125 ml bottles with 20 g of soil were extracted with 0.5 M K_2SO_4 and carbon was quantified. This soil fraction represents the non-fumigated portion of the microbial biomass. The 50 ml beakers with 20 g of soil were placed into a fumigation chamber and fumigated with chloroform overnight and extracted after 24 hours with 0.5 M K_2SO_4. This soil fraction represents the fumigated portion of microbial biomass carbon. Dissolved organic carbon in the filtrate was determined using flow injection technology using a Torch TOC Combustion analyzer (Teledyne Tekmar, Mason, Ohio) and carbon associated with microbial biomass was calculated using the correction factor (k=0.33) [9].

Inorganic nitrogen was determined by extraction with 2.0 M potassium chloride from homogenized moist microcosm soil. Concentrations of NO_3 and NH_4 were quantified in the filtrate using Lachat Instruments flow injection analyzer (Lachat Instruments, Milwaukee, WI).

Total carbon and nitrogen in microcosm soils was determined using dry combustion analysis of 2 g of air-dry, soil ground with mortar and pestle. Dry homogenized soil was combusted using Thermo Scientific FLASH Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA). The pH of soils was measured potentiometric ally in a 2:1 soil-to-water slurry using a dual electrode pH meter [10]. Soil water content was determined gravimetrically with overnight drying at 105°C.

Statistical Methods

Differences in microbial community composition

Dissimilarities in the composition of sample bacterial communities were visualized by NMDS ordination of the Bray-Curtis distances between samples. Samples included both the microcosm inputs (i.e., the organic amendments) and the incubated microcosms (i.e., amended soils). NMDS ordinations were performed using the metaMDS() function from the Vegan package in R and were visualized with the plot_ordination() function from the Phyloseq package. To test if the composition of OTUs was different in samples, we used the adonis() function from the vegan package in R. Adonis() uses a non-parametric multivariate analysis of variance method to test the null hypothesis that there are no differences in microbial communities.

To characterize the impact that environmental variables had on the dissimilarities of communities in each treatment we performed CAP (Canonical Analysis of Principal coordinates) analysis of the environmental variables of inorganic nitrogen, microbial biomass

and C: N ratio in conjunction with the Bray-Curtis distance matrix. CAP seeks to display ordinations along with explanatory variables to reduce dimensionality and can reveal patterns in multivariate data with reference to a priori hypotheses.

Differences in soil chemistry

All environmental variables were fit to a linear mixed effects model using the function lme() from the nlme package in R. Treatment and day were set as fixed effects in the linear model and means were estimated using the emmeans() function from the emmeans package in R. Comparisons were performed between each treatment by each day (i.e., treatment x day). ANOVA was performed on the results from the linear model. A post-hoc test for the ANOVA analysis was performed Tukeys HSD and are reported in a table found in the supplementary data.

CHAPTER 3. RESULTS

The response of soil microbial communities to the varying amendments were characterized throughout the incubation. First, the characteristics (microbial community structure and nutrient composition) were measured for all three amendment inputs. The microbial community of each amendment was characterized through sequencing of 16S rRNA genes, a phylogenetic marker conserved among bacteria. The resulting phylogenetic profile was identified for each amendment, showing distinct communities for alfalfa and compost amendments (Figure 2). The distribution of phyla from OTUs with abundance greater than 2% in the alfalfa amendment is dominated by unclassified bacteria (sharing no homology to any known phyla) and Proteobacteria, while the compost amendment is dominated by unclassified bacteria, Actinobacteria, and Firmicutes. These amendments are also distinct from the incubation soil used in this study, which is primarily dominated by Acidobacteria, Actinobacteria, and Proteobacteria.

In addition to differences in the microbial communities of the inputs, we also observed differences in the amounts of inorganic N and C: N ratio in the inputs (Table 1). The alfalfa amendment was highest in inorganic nitrogen concentration at 25.53 ppm and lowest in carbon to nitrogen ratio with 20.14 parts carbon to one-part nitrogen. The compost amendment was lowest in inorganic nitrogen at 13.91 ppm and had the highest C: N ratio at 28.97. It has been previously reported that a C: N ratio below 25:1 in an input will result in net mineralization while greater than 25:1 will result in net immobilization [11]. Consequently, the alfalfa amendment is expected to result in nitrogen mineralization, and a net increase in inorganic nitrogen compared to reference soils, while the compost amendment is expected to

result in nitrogen immobilization and a net decrease in inorganic nitrogen compared to reference soils.

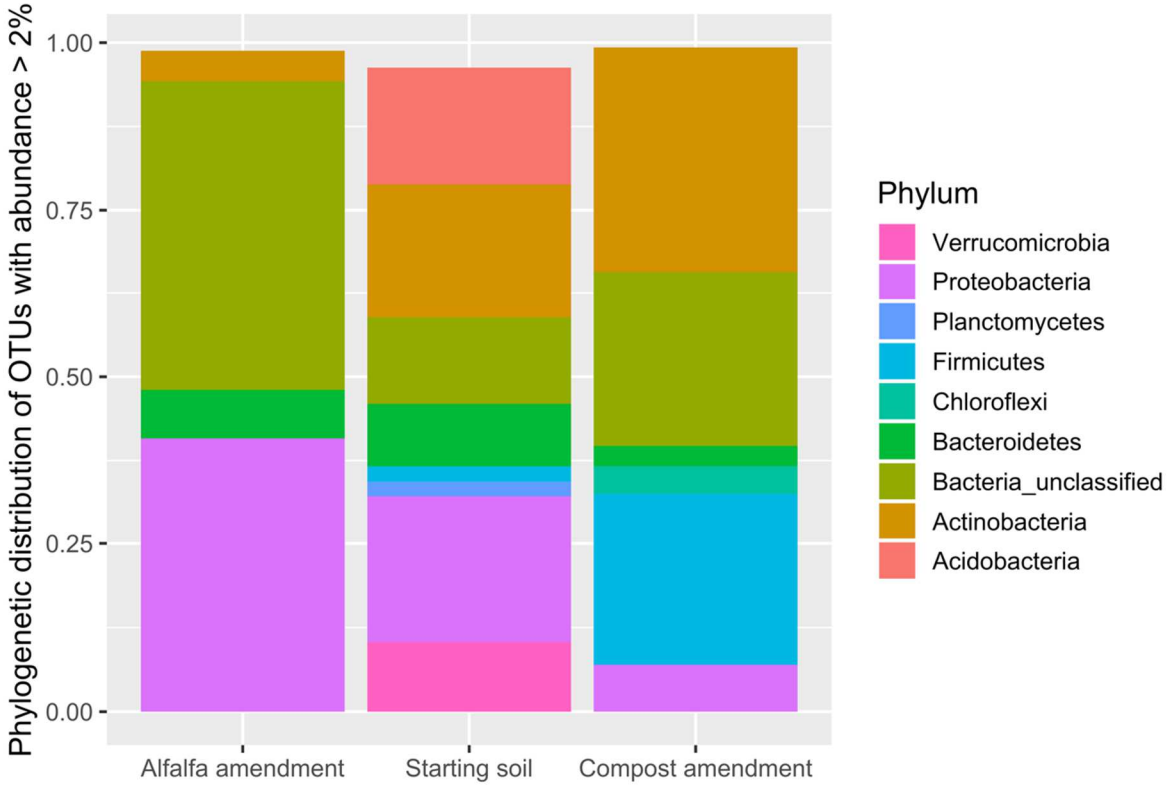

Figure 2. Distribution of phyla in inputs used for constructing microcosms.

The distribution of OTUs across phyla from the input amendments. These OTUs represent those that had abundance greater than 2 % within all sequencing reads.

Table 1. Total carbon, total nitrogen, inorganic nitrogen, and C:N ratio of inputs.

The mass of the inputs used to construct the microcosms. Each microcosm started with 50.0 g of soil and was amended with the indicated mass of either alfalfa, mixed compost + alfalfa (Mix), compost. Reference microcosms were not amended.

Amendment	**Mass Added**	**% C**	**% N**	**C:N**	**Inorganic N**
Alfalfa amendment	1.8 g	41.7	2.1	20.2	25.5
Mix amendment	1.3 g	36.2	1.4	25.1	Not measured
Compos amendment	0.8 g	33.8	1.2	29.0	13.9
Starting soil	50.0 g	3.1	0.3	12.6	3.3

Inorganic nitrogen

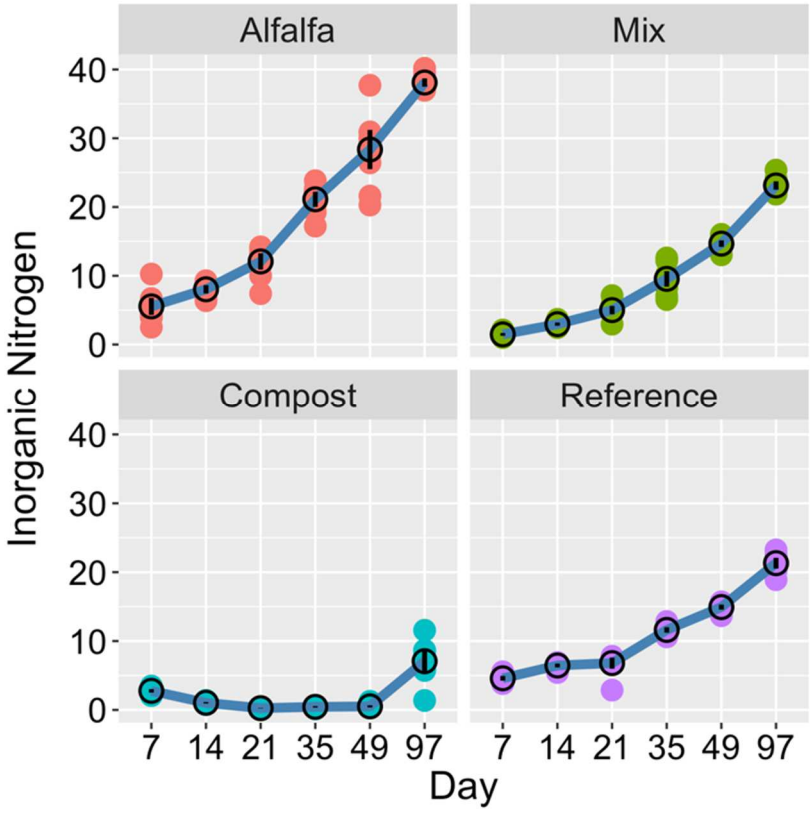

Figure 3. Inorganic nitrogen concentrations in microcosms over the course of the incubation.

Plot of the linear mixed effect model for inorganic nitrogen with treatment and day as fixed effects. The blue line denotes the empirical mean of the data. The black points with the vertical lines are the predicted means and corresponding 95% confidence intervals.

Concentrations of inorganic nitrogen estimate the amount of potential plant available nitrogen (N). Generally, inorganic N is considered to be the sum of NO_3 and NH_3 concentrations in soils. In our experiment, the level of inorganic N in the starting soils were similar and increased over time, with the exception of the compost amendment (Figure 3). Reference soils had a 4.6-fold increase in inorganic N over the course of the incubation. Alfalfa soils had significantly higher inorganic N concentrations on days 14 to 97 than other treatments, and by day 97 had 2-fold greater inorganic N compared to reference soils. Mixed soils initially were observed with lower concentrations of inorganic N but increased to levels comparable to reference soils by day 97. Compost amendments resulted in a significantly lower concentration on all days of the incubation compared to the reference soil and had inorganic N concentrations less than 1 ppm on days 21, 35, and 45 (Figure 3).

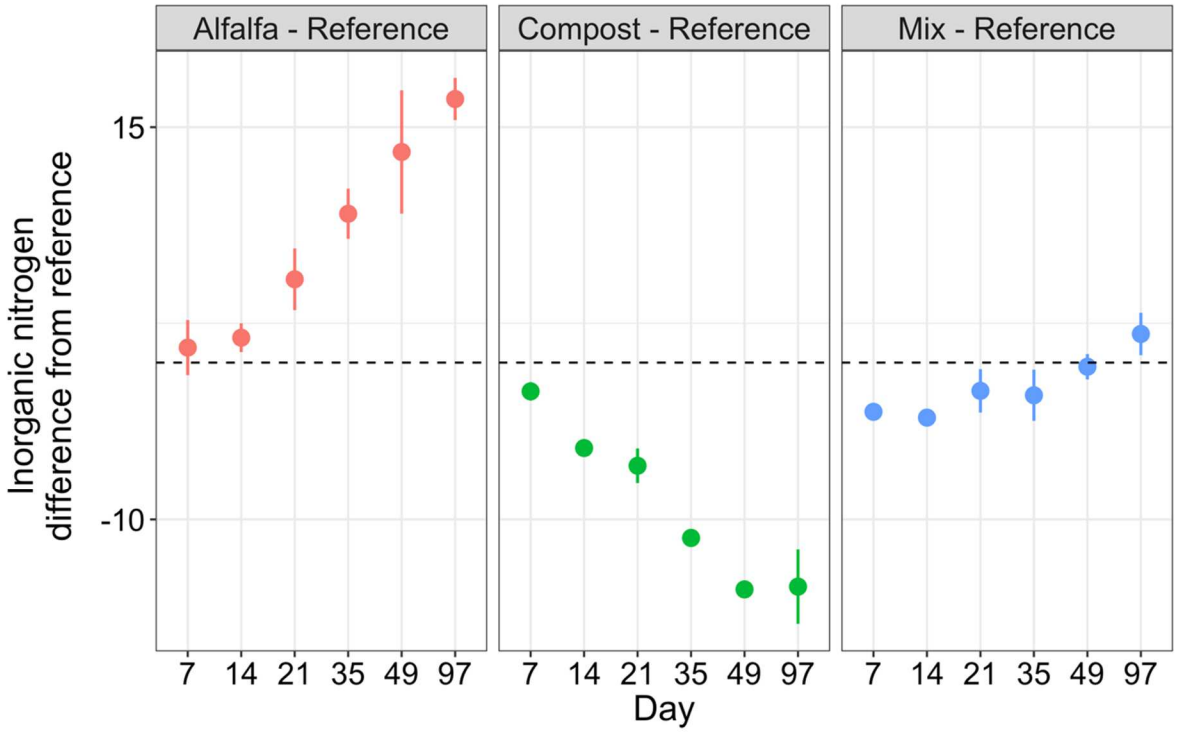

Figure 4. Difference in inorganic nitrogen relative to measurements in reference soils.

The y-axis denotes the difference between the inorganic nitrogen measurements in the treatment and the reference soils. Positive values imply that the treatment was higher in inorganic N compared to the reference, whereas negative values imply the opposite. The vertical lines are 95% confidence intervals (adjusted for multiple comparisons).

Microbial biomass

Microbial biomass provides an indication of the size of the active pool of soil organic matter and increases in microbial biomass indicate microbial utilization of nutrients for metabolic growth and is correlated with nutrient availability. All amended microcosms yielded significantly higher MBC levels than reference control soils (ANOVA linear model with Tukey's HSD, Figure 6). Alfalfa had the most significant positive difference, with a two-three fold higher MBC than reference and achieving the most MBC within the experiment 780 mg C/kg dry wt. soil on day 14 (Figure 5). Reference soils nearly doubled in microbial biomass on day 21 before declining to baseline concentrations on day 97. Mix amended soils had consistently decreasing MBC levels throughout the experiment but

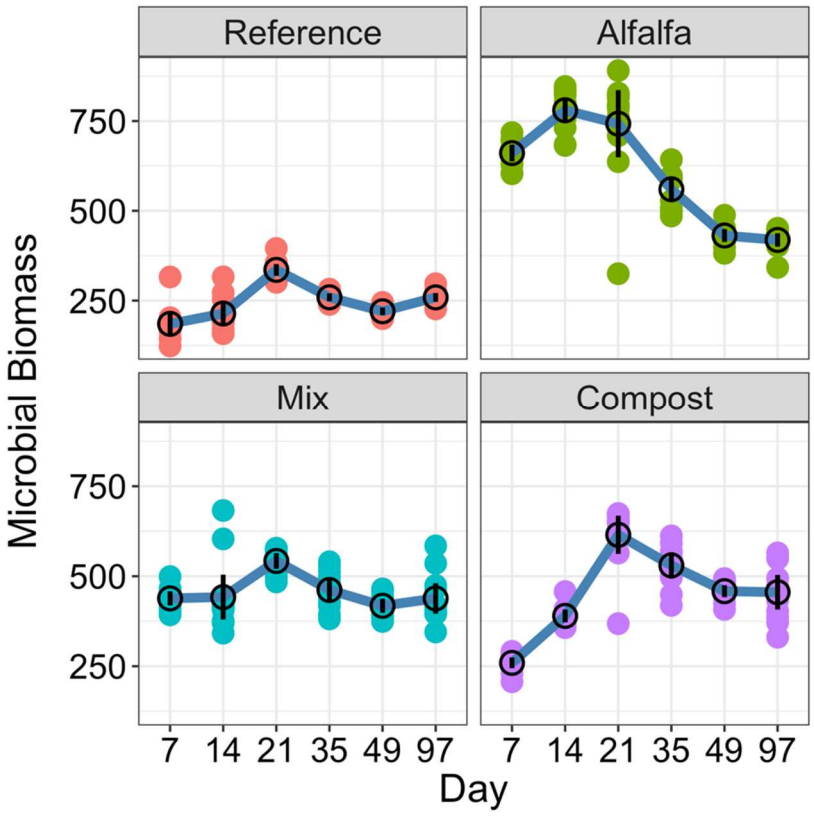

Figure 5. Microbial biomass carbon in microcosms over the course of the incubation.

Plot of linear mixed effect model for microbial biomass carbon with treatment and day as fixed effects. The blue line denotes the empirical means of the data. The black points with the vertical lines are the predicted means and corresponding 95% confidence intervals.

maintained levels significantly higher than reference soils. MBC in compost-amended soils were higher than reference and declined near the end of the incubation.

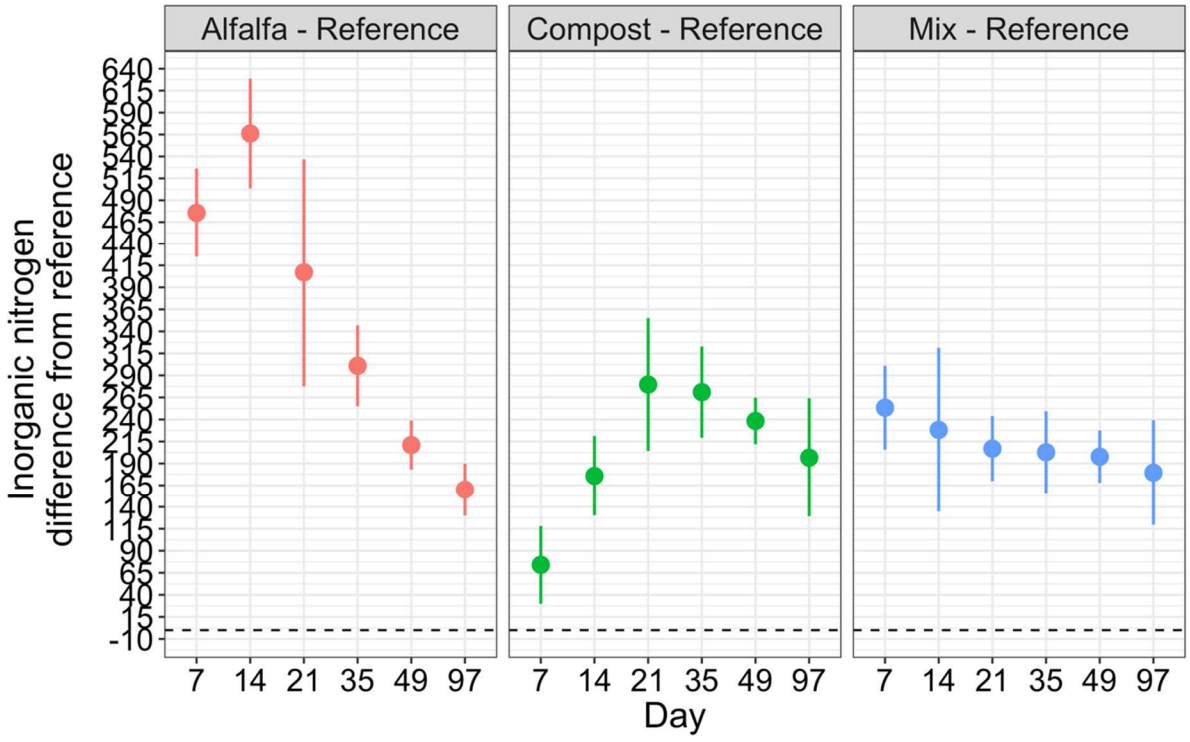

Figure 6. Difference in microbial biomass carbon relative to measurements in reference soils.

The y-axis denotes the difference between the treatment and the reference. Positive values imply that the treatment was higher compared to the reference, whereas negative values imply the opposite. The vertical lines are 95% confidence intervals (adjusted for multiple comparisons).

Bacterial community composition

To discern the impact of treatment and time on soil bacterial community composition, we compared the Bray-Curtis dissimilarity indices between all bacterial communities associated with each microcosm. Non-metric multidimensional scaling (NMDS) ordinations of the resulting distances are shown for each treatment (Figure 8) and day of soil sampling (Figure 8). In the ordinations, points (representing individual samples) that are close together share similar species composition, while points further apart have different species compositions. These results reveal that the microbial community in reference soils are

dissimilar from amended soils, with compost-amended soils being the most similar in composition to reference soils. The alfalfa and mixed amendments had community

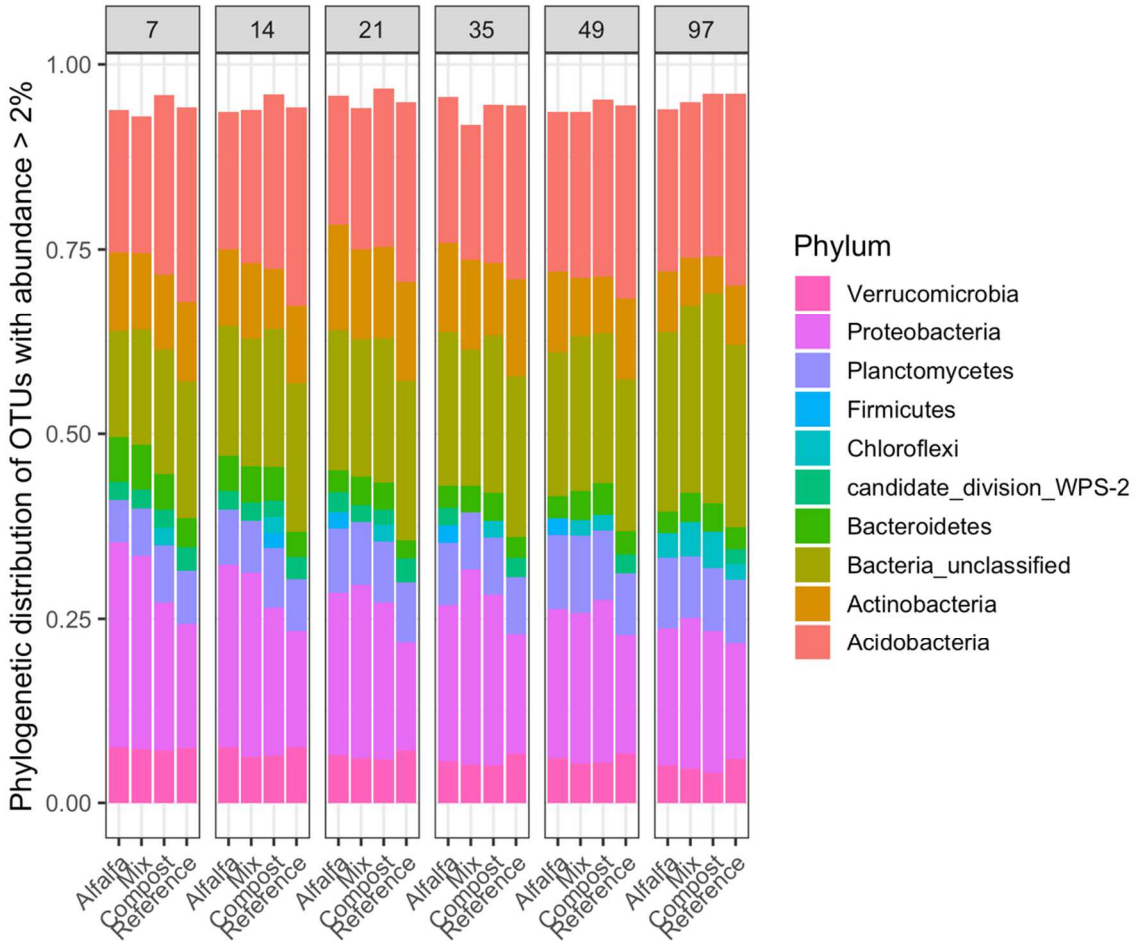

Figure 7. The distribution of phyla in microcosm communities over time.

The distribution of OTUs across phyla from the microcosm bacterial communities. The OTUs that had abundance greater than 2 % after removal of singletons and doubletons were then agglomerated at the phyla level.

compositions that were the most comparable throughout the incubation. The vertical spread of samples along axis 1 suggests that amendment type explains the dissimilarity between treatments, while the clustering of samples by day on the horizontal axis shows that time in incubation also explains variation. Generally, day 7 communities were observed to be the most dissimilar to day 97 communities and are most similar to day 14 and day 21

communities. As the incubation progressed, the dissimilarity between sampling times became more pronounced regardless of treatment. Further, the first three sampling points in time showed the most similar communities, with increasingly different communities after day 21.

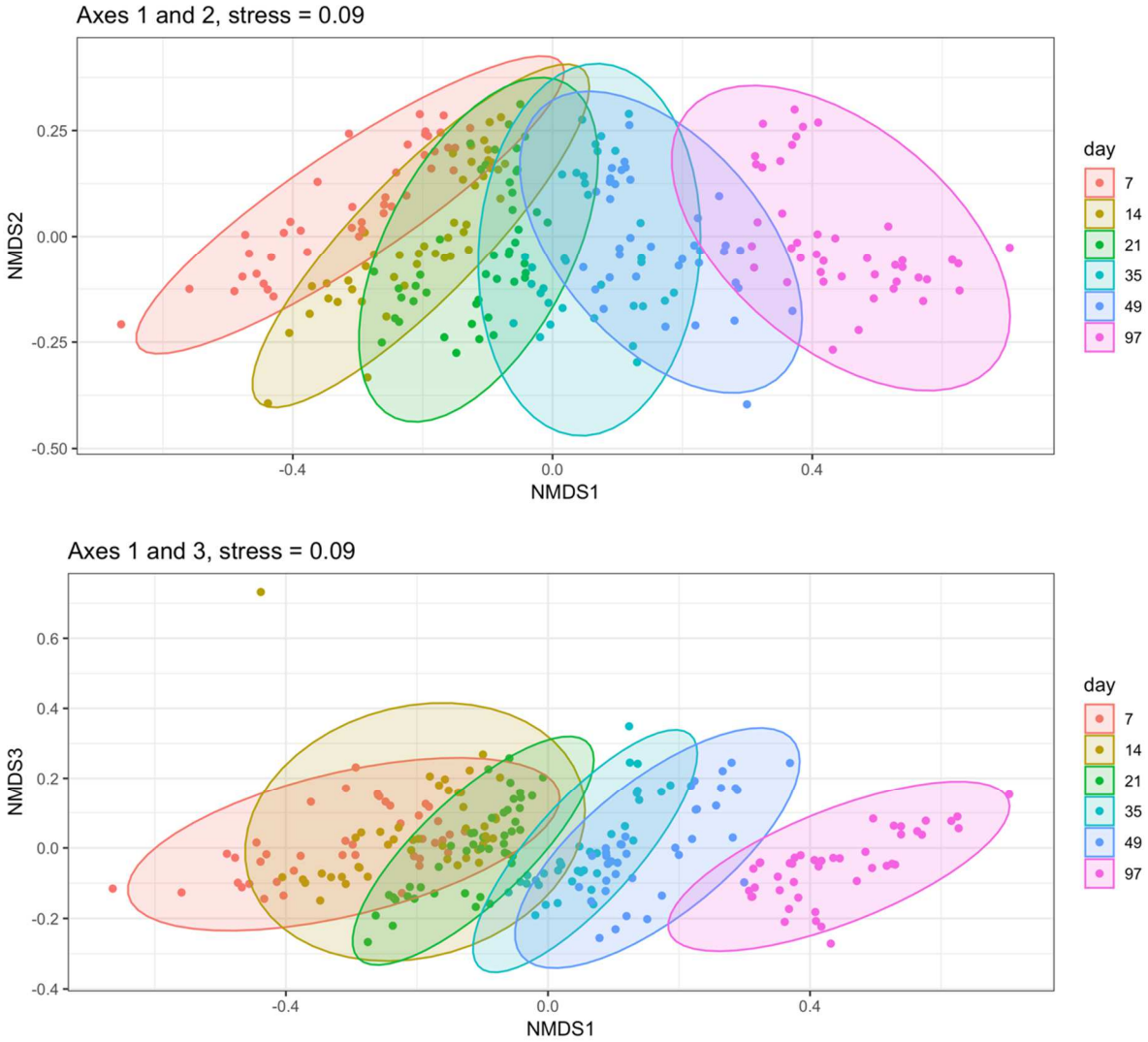

Figure 8. NMDS ordination of Bray-Curtis dissimilarity measure by day.

Ordinations are showing differences across days. Ordinations were generated in 3 dimensions (k=3, stress=0.09) (top) ordination showing axes 1 and 2 while (bottom) ordination showing axes 1 and 3.

To better understand the influence of the various environmental variables that were measured on the microbial community, canonical analysis of principle coordinates was

performed on the Bray-Curtis distance matrix, and environmental variable scores were fitted to this ordination (Figure 10). We observed that measurements of C: N ratios and inorganic

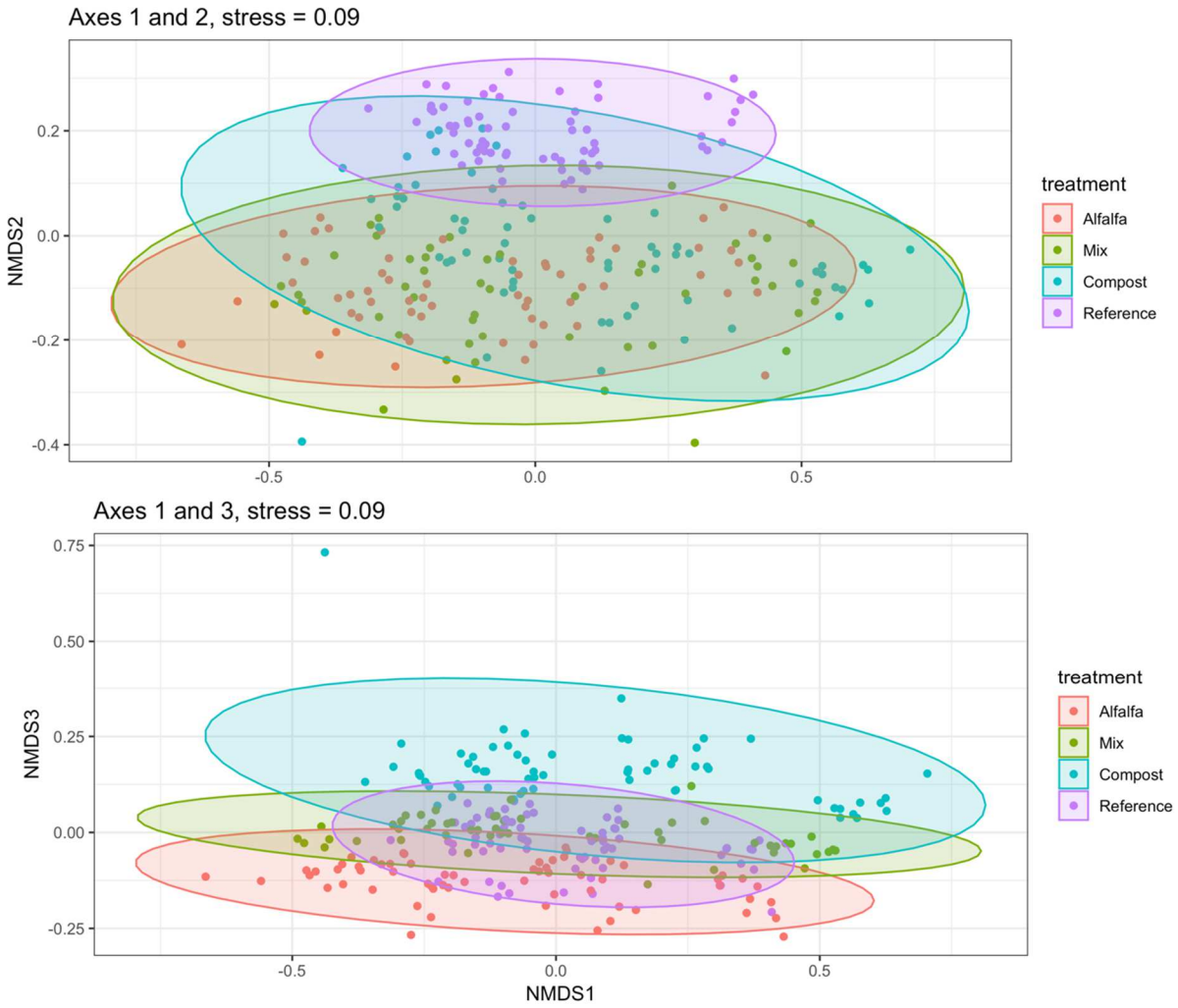

Figure 9. NMDS ordination of Bray-Curtis dissimilarity measure by treatment.

Ordinations are showing differences across treatment. Ordinations were generated in 3 dimensions (k=3, stress=0.09) (top) ordination showing axes 1 and 2 while (bottom) ordination showing axes 1 and 3.

N concentrations were correlated to community variation along an axis, CAP1 (up to 20% variance explained in mixed alfalfa microcosms). In addition, alfalfa-amended soils had MBC also correlated with this axis. The second CAP axis (CAP2) had weak associations

with pH and MBC in all treatments except alfalfa. However, CAP2 was much lower in percentage explained, between 3.1% to 5.1% for all amendments (Figure 10).

Table 2. NPMANOVA results from the Bray-Curtis dissimilarities.

Non-parametric analysis of variance of the effect of treatment and day on community dissimilarities.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
day	5	7.43538682848844	1.48707736569769	15.5766757404099	0.210598634814172	0.001
treatment	3	3.81222149347525	1.27074049782508	13.3106139205755	0.107976714682691	0.001
day:treatment	15	2.76893992888835	0.184595995259223	1.93358599051914	0.0784269846825017	0.001
Residuals	223	21.289411044892	0.0954682109636414	NA	0.602997665820635	NA
Total	246	35.3059592957441	NA	NA	1	NA

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
TreatmentAndDay	23	14.016548250852	0.609415141341393	6.3834352313723	0.397002334179364	0.001
Residuals	223	21.289411044892	0.0954682109636414	NA	0.602997665820635	NA
Total	246	35.3059592957441	NA	NA	1	NA

To understand the microbial communities that are early responders to amendment, we performed hierarchical clustering of the binary Bray-Curtis distance for each treatment. These results were used to guide the definition of "early responders" to amendments. For each amendment, bacterial communities from day 0 samples clustered together, consistent that these samples represent similar initial conditions. These Day 0 communities will henceforth be referred to as the baseline response group. For all amendments, communities from sample days 7, 14, 21 cluster as early responders and 35, 49, 97 cluster as the late responders.

For early and late response groups, the microbial communities that were unique for each amendment were characterized. We identified specific microbial communities that were observed to be significantly different in amendment treatments compared to reference control soils. Significantly different communities were defined as those with a log 2-fold relative abundance increase between amendment versus no amendment control. This

resulted in the identification of 25 and 21 OTUs specific to alfalfa and 43 and 71 specific to compost in the early and late response groups, respectively. The presence of these amendment-specific early responding OTUs was next compared across all treatments (Supplementary data).

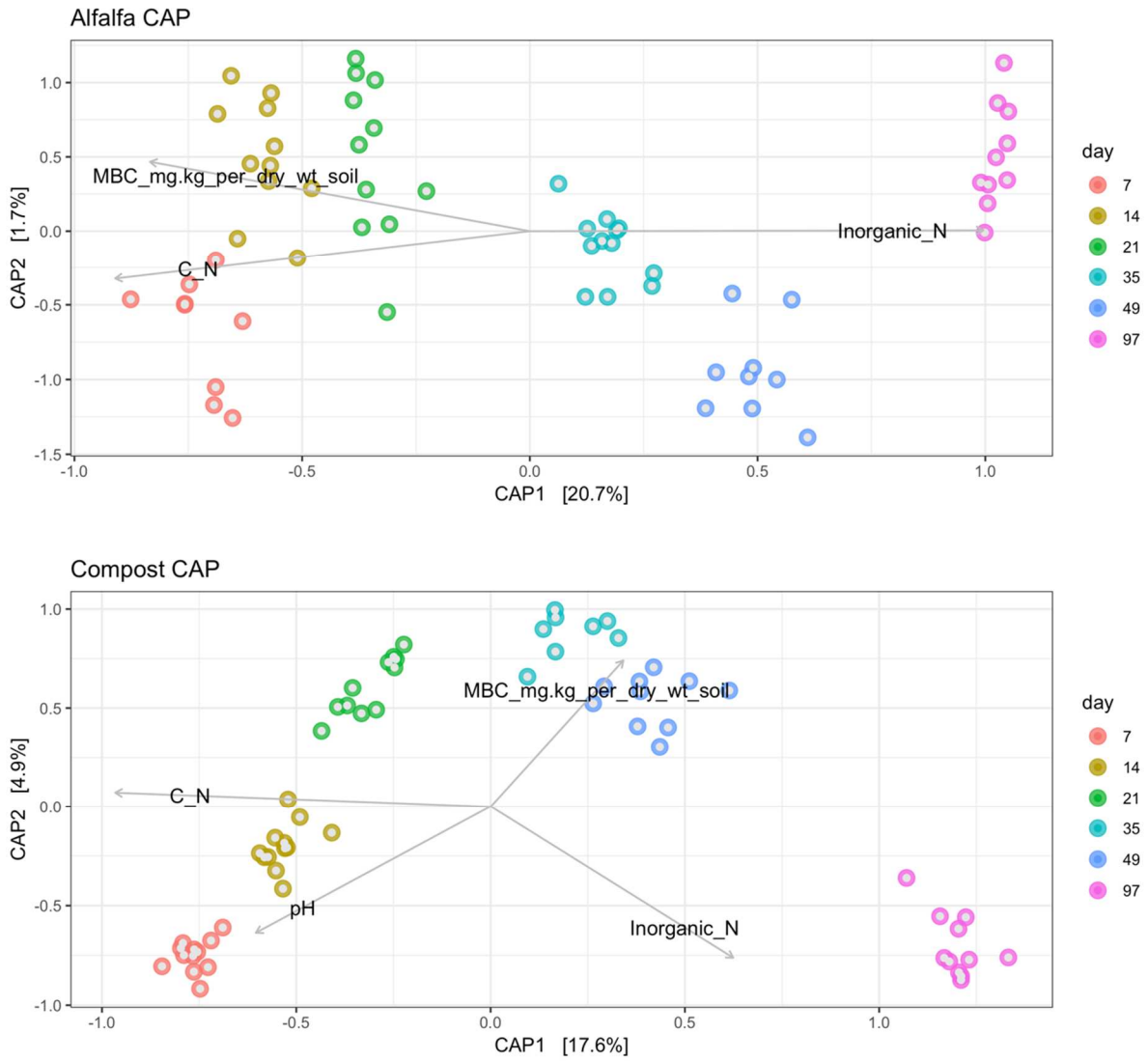

Figure 10 CAP (canonical analysis of principal coordinates) plot

Visualization of the differences in bacterial communities in the alfalfa treated microcosms (above) and the compost treated microcosms (below). The effect of soil parameters underlying the observed variations are visualized with vectors overlaid onto the plot of dissimilarities based on the Bray-Curtis distance.

We observed the phylogenetic distribution of OTU to be different across response group and treatment for both alfalfa and compost amended microcosms. The 25 OTUs unique to alfalfa in the early response group were dominated by OTUs from the Proteobacteria and Firmicutes. The late response group in alfalfa was dominated by OTUs from Proteobacteria and unclassified bacteria, Firmicutes were not observed in the late response group. The compost response groups were also dominated by Proteobacteria in both early and late, while Bacteroidetes made up a greater portion of the early responders than late in the compost. Unclassified bacteria were a large percentage of both response groups in the compost treated soil.

CHAPTER 4. DISCUSSION

Given the value of agricultural products to economies, particularly organic crops, providing solutions that ensure sustainable production coupled with minimal disturbances to natural systems will be crucial. While organic maize production was shown to be more beneficial to water quality [7], predictability of yields can be challenging compared to conventional and are of concern towards the adoption of organic practices. In this study, we conclude that one factor that could contribute to the economic viability of organic production is the C: N ratio of the nitrogen fertilizer source. We observed that similar total nitrogen applications of composted horse manure and alfalfa residue, with a C: N ratio of 28:1 and 20:1, respectively, result in contrasting nitrogen mineralization when applied to organic soils. Specifically, we show that alfalfa amended soils had a significantly greater release of mineral nitrogen due to mineralization than both compost and no amendment reference microcosms. This mineralization response of the alfalfa amendment is due to its low C: N ratio and is in agreement with past studies (Table 1) [12,13].

To better understand the dynamics of soil, plant, and bacterial community interactions in response to amendments, we also characterized the microbial communities present in the soil over time through phylogenetic analysis of the 16S-rRNA gene. This characterization showed unique microbial communities responding to each amendment and that these communities are temporally dynamic. The overall composition of the alfalfa and compost amendments at the phylum level are very similar. However, distinct microbial communities could be identified in alfalfa and compost amendments, both of which were also significantly different than unamended soils. Over the course of the incubation, these communities in all soils also changed but remained distinct by amendment. Importantly, these microbial

communities were found to be correlated to soil nutrients (C: N ratio) and microbial biomass, evidence to the impact of C: N on the microbial community and subsequent release of plant available nutrients (mineralization).

We also observed two distinct temporal groupings of OTUs that were consistent for all amendments. "Early" group days of similar microbial communities were identified as days 7, 14 and 21, while days 35, 42 and 97 formed the second "late" group. Within these groups, we identified unique bacterial OTUs significantly responding to each treatment determined by differential abundance. These OTUs could be of ecological interest and inform our understanding of which bacterial species respond to specific amendments over time. Knowledge of these important bacterial OTUs may facilitate development of technologies that support inorganic nitrogen release from amendments.

In alfalfa amendments, we observed mineralization through the increase in inorganic nitrogen when compared to reference soils (Figure 3). The results highlight the usage of alfalfa as a potential sustainable nitrogen fertilizer source. With alfalfa amendments, we observe inorganic nitrogen increasing rapidly and continually. Further, we expect that the composition of the alfalfa amendment, containing complex molecules like cellulose, hemi-cellulose and proteins, would require initial decomposition and break down before organic nitrogen can be made available. The early group of identified bacterial responders for alfalfa may thus be representative of the microbial community performing initial stages of alfalfa decomposition and whose presence in soils may be of interest for organic farming.

For both alfalfa and compost amended soils, we identified unique early and late bacterial responders specific to each amendment. In general, we expect that early responders represent an ability to facilitate organic amendment usage through initial conversion of

nitrogen and also carbon for plant and microbial growth. The majority of early responders in alfalfa are associated with the phyla Proteobacteria and Firmicutes. We also observed significant increases in sequences associated with the phylum Proteobacteria and the genus *Pseudomonas*. These results are consistent with previous studies showing cellulase enzyme production from *Pseudomonas* [14], and the enrichment of these bacteria may be due to the high cellulose and hemi-cellulose content in alfalfa hay[15]. In addition, the ability of *Pseudomonas* to respond to nutrients from organic materials may be facilitated by the capacity of this genus to also produce anti-microbial compounds that inhibit growth of competing microbes [16]. The second most enriched OTU in the early alfalfa responding group are associated with Firmicutes and the genus *Sporosarcina*, which have been characterized by their urease production potential [17]. The dominance of species associated with long-chain carbon degradation in the early response to alfalfa amendments is consistent with our hypothesis that there are specific communities that may be necessary for optimizing nutrient cycling in organic amendments. We also observed sequences related to legume symbionts from the genus *Rhizobium* and the genus *Burkholderia* in early response groups, and these bacteria are associated with nitrogen cycling, specifically nitrogen fixation[18], suggesting that they can provide plant benefits.

Of the late responding bacteria observed in alfalfa amendments, Proteobacteria, Planctomycetes, and unclassified_bacteria were the three most dominant phyla (Supplementary data). No OTUs associated from the late responder Planctomycetes were observed in the early alfalfa responders, suggesting that this particular species has functions that are needed after initial breakdown of carbon or nitrogen have occurred. Bacteria from the phylum Planctomycetes have been associated with the novel ammonium oxidation

pathway known as anammox [19]. Anammox bacteria are able to utilize ammonium and nitrite or nitrate for the generation of nitrogen gas and may be responding to the high levels of inorganic nitrogen in the late alfalfa microcosms. Since the scope of this experiment cannot confirm that this OTU is associated with this function, this is opportunity for functional characterization of microbial communities in future research. It is also of interest that there are far more bacteria responding to alfalfa in the late group that are unclassified at the phylum level, highlighting the novel diversity and importance of characterizing novel microbial communities in agroecosystems. In late alfalfa responders, Verrucomicrobia and the genus Spartobacteria_unclassified were significantly increased. The latter OTU had a near 7-fold increase and was also observed to increase in the early alfalfa microcosms. Species from the genus *Spartobacteria* have been associated with the transformation of organic compounds [20] and are likely an important consideration when looking at the microbial potential to liberate nitrogen from organic sources.

Our study highlights the importance of considering both carbon and nitrogen for agricultural management decisions pertaining to the use of organic amendments. Further, we observe that responding soil microbial communities are specific to organic amendments and the time of after amendment. We conclude that simply applying amendments on a total nitrogen basis does not guarantee the same amount of inorganic nitrogen available to plants, and this result is associated with specific microbial communities responding to contrasting nutrients available over time. Soil microbial diversity and management practices have previously been shown to be highly associated with one another [21], and our study extends this to organic amendment management. Long-term management practices have been observed to impact microbial diversity, with distinct microbial membership identified in long-term

organic versus conventional farming soils [22]. The key players within soil microbial communities identified within this study highlights opportunities for research for both alfalfa and compost during initial and late stages of nitrogen availability.

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APPENDIX. SUPPLEMENTAL DATA

Table AS1. Mean of all environmental variables, with Tukey's HSD within treatment and day.

Mean, standard deviation and Tukey's significance of environmental variables by day

Treatment	Day	T_C	T_N	C_to_N	Inorganic_N	NO3	NH3	MBC	pH	G_W_C
Compost	7	3.72 (0.21) a	0.26 (0.01) b	14.44 (0.65) a	2.77 (0.40) b	2.39 (0.42) b	0.38 (0.11) b	259.23 (24.15) c	6.93 (0.08) c	0.37 (0.01) b
Mix	7	3.59 (0.14) a	0.27 (0.01) a	13.24 (0.51) b	1.47 (0.32) c	0.79 (0.27) c	0.68 (0.11) b	438.53 (30.27) b	6.96 (0.06) c	0.38 (0.01) ab
Alfalfa	7	3.3 (0.18) b	0.27 (0.02) a	12.12 (0.21) c	5.55 (1.95) a	4.02 (1.40) a	1.53 (0.86) a	660.82 (36.52) a	7.11 (0.06) b	0.39 (0.02) a
Reference	7	2.81 (0.12) c	0.23 (0.01) c	12.21 (0.26) c	4.59 (0.54) a	4.33 (0.46) a	0.26 (0.25) b	185.16 (46.73) d	7.22 (0.10) a	0.39 (0.01) a
Compost	14	3.93 (0.28) a	0.27 (0.02) a	14.34 (0.60) a	1.03 (0.17) d	0.18 (0.09) d	0.86 (0.16) bc	389.81 (25.94) b	6.88 (0.08) bc	0.36 (0.01) b
Mix	14	3.42 (0.17) b	0.26 (0.01) b	13.25 (0.26) b	2.96 (0.37) c	1.95 (0.32) c	1.01 (0.14) ab	442.17 (99.22) b	6.82 (0.08) c	0.37 (0.00) b
Alfalfa	14	3.21 (0.12) c	0.27 (0.01) ab	11.85 (0.22) c	8.03 (0.96) a	6.8 (1.02) a	1.24 (0.24) a	780.13 (56.26) a	6.94 (0.06) b	0.38 (0.01) a
Reference	14	2.89 (0.08) d	0.24 (0.01) c	12.03 (0.19) c	6.45 (0.44) b	5.71 (0.38) b	0.74 (0.30) c	214 (45.51) c	7.04 (0.06) a	0.38 (0.01) a
Compost	21	4.01 (0.87) a	0.29 (0.07) a	14.06 (0.49) a	0.26 (0.05) d	0.04 (0.03) d	0.22 (0.05) c	615.18 (83.72) b	6.6 (0.04) b	0.37 (0.01) ab
Mix	21	3.35 (0.17) b	0.26 (0.01) ab	12.98 (0.44) b	5.01 (0.96) c	4.65 (0.94) c	0.36 (0.07) b	542.4 (35.42) b	6.51 (0.03) c	0.36 (0.05) b
Alfalfa	21	3.1 (0.10) bc	0.26 (0.01) ab	11.81 (0.27) c	12.09 (1.87) a	11.63 (1.89) a	0.45 (0.04) a	742.68 (146.07) a	6.58 (0.04) b	0.39 (0.01) a
Reference	21	2.83 (0.12) c	0.23 (0.02) b	12.2 (0.40) c	6.8 (1.27) b	6.66 (1.23) b	0.14 (0.06) d	335.52 (26.36) c	6.66 (0.05) a	0.39 (0.00) a
Compost	35	3.68 (0.15) a	0.27 (0.01) a	13.46 (0.25) a	0.45 (0.17) d	0.13 (0.10) d	0.32 (0.17) a	530.14 (56.66) a	6.58 (0.09) b	0.36 (0.01) a
Mix	35	3.33 (0.13) b	0.27 (0.01) a	12.32 (0.24) b	9.55 (1.77) c	9.28 (1.76) c	0.27 (0.11) ab	461.81 (52.04) b	6.35 (0.02) c	0.34 (0.06) a
Alfalfa	35	3.02 (0.09) c	0.27 (0.01) a	11.3 (0.24) d	21.12 (1.73) a	20.83 (1.74) a	0.29 (0.06) a	560.07 (51.43) a	6.59 (0.06) b	0.37 (0.02) a
Reference	35	2.79 (0.07) d	0.23 (0.01) b	11.92 (0.36) c	11.62 (0.63) b	11.45 (0.62) b	0.17 (0.07) b	259.11 (16.50) c	6.7 (0.05) a	0.36 (0.01) a
Compost	49	3.54 (0.21) a	0.26 (0.01) a	13.65 (0.50) a	0.52 (0.25) c	0.2 (0.21) c	0.31 (0.05) a	458.52 (26.00) a	6.66 (0.13) b	0.37 (0.01) b
Mix	49	3.18 (0.09) b	0.26 (0.01) a	12.04 (0.19) b	14.67 (0.74) b	14.42 (0.71) b	0.26 (0.08) ab	417.92 (30.02) b	6.48 (0.05) c	0.37 (0.01) b
Alfalfa	49	2.98 (0.12) c	0.26 (0.01) a	11.34 (0.23) c	28.36 (4.49) a	28.14 (4.48) a	0.22 (0.05) b	431.19 (27.67) ab	6.72 (0.07) ab	0.38 (0.01) a
Reference	49	2.76 (0.12) d	0.24 (0.01) b	11.7 (0.27) b	14.93 (0.57) b	14.72 (0.59) b	0.22 (0.08) b	220.19 (17.03) c	6.79 (0.07) a	0.39 (0.01) a
Compost	97	3.32 (0.18) a	0.28 (0.01) a	12.05 (0.31) a	7.07 (2.43) d	6.61 (2.40) c	0.46 (0.58) a	455.58 (75.43) a	6.64 (0.11) a	0.39 (0.02) ab
Mix	97	3.1 (0.09) b	0.28 (0.01) a	11.12 (0.30) b	23.13 (0.96) b	22.29 (0.93) b	0.85 (0.42) a	438.41 (66.55) a	6.63 (0.11) a	0.36 (0.01) b
Alfalfa	97	2.94 (0.07) c	0.28 (0.01) a	10.47 (0.19) c	38.1 (0.95) a	38.1 (0.95) a	0 (0.00) b	418.85 (29.80) a	6.62 (0.10) a	0.38 (0.05) ab
Reference	97	2.73 (0.07) d	0.24 (0.01) b	11.26 (0.23) b	21.31 (1.23) c	21.31 (1.23) b	0 (0.00) b	258.92 (19.54) b	6.72 (0.07) a	0.41 (0.01) a

Table AS2. Table of early alfalfa responders with LFC > 2.

OTUs with log2 fold change > 2 from the early alfalfa responders		
Phylum	**Genus**	**log2FoldChange**
Proteobacteria	Pseudomonas	3.83894495608561
Firmicutes	Sporosarcina	3.33971085034702
Firmicutes	Bacillus	3.3159873545107
Bacteroidetes	Chitinophagaceae_unclassified	3.2146864705628
Acidobacteria	Gp4_unclassified	3.1507765443299
Proteobacteria	Microvirga	3.14083537349104
Firmicutes	Bacillales_unclassified	3.10322922453328
Proteobacteria	Rhizobium	3.07991887869643
Proteobacteria	Corallococcus	2.91357307300425
Proteobacteria	Enterobacteriaceae_unclassified	2.83085020430554
Actinobacteria	Arthrobacter	2.76293036537635
Proteobacteria	Oxalobacteraceae_unclassified	2.64474228238784
Proteobacteria	Byssovorax	2.553678132916
Bacteria_unclassified	Bacteria_unclassified	2.50826819845188
Verrucomicrobia	Spartobacteria_unclassified	2.4610588299815
Bacteroidetes	Dyadobacter	2.44842044565294
candidate_division_WPS-2	candidate_division_WPS-2_unclassified	2.44830122613627
Bacteria_unclassified	Bacteria_unclassified	2.35403370408952
Firmicutes	Planococcaceae_unclassified	2.26936548893042
Proteobacteria	Cystobacter	2.26897982373268
Actinobacteria	Cellulomonas	2.22233665226249
Chloroflexi	Chloroflexi_unclassified	2.12352627902992
Proteobacteria	Burkholderiales_unclassified	2.10383805787642
Verrucomicrobia	Spartobacteria_unclassified	2.07581772220483
Proteobacteria	Sphingomonas	2.06868005705787

Table AS3. Table of late alfalfa responders with LFC > 2.

OTUs with log2 fold change > 2 from the late alfalfa responders		
Phylum	**Genus**	**log2FoldChange**
Verrucomicrobia	Spartobacteria_unclassified	2.81778396170568
Proteobacteria	Vampirovibrio	2.75216882314234
Bacteria_unclassified	Bacteria_unclassified	2.6465518904745
Bacteria_unclassified	Bacteria_unclassified	2.59159082914833
Chloroflexi	Chloroflexi_unclassified	2.57391399558982
Bacteria_unclassified	Bacteria_unclassified	2.44727499370621
Acidobacteria	Gp10_unclassified	2.40219814015098
Planctomycetes	Planctomycetaceae_unclassified	2.33877009652107
Proteobacteria	Proteobacteria_unclassified	2.3325345010495
Bacteria_unclassified	Bacteria_unclassified	2.30734034996285
Bacteria_unclassified	Bacteria_unclassified	2.29357146326056
Bacteroidetes	Bacteroidetes_unclassified	2.28283856645528
Proteobacteria	Myxococcales_unclassified	2.26580239519386
Proteobacteria	Alphaproteobacteria_unclassified	2.24198005201265
Bacteria_unclassified	Bacteria_unclassified	2.23303162825313
Planctomycetes	Planctomycetaceae_unclassified	2.22020631262162
Planctomycetes	Planctomycetaceae_unclassified	2.18510564649652
Actinobacteria	Acidimicrobiales_unclassified	2.07266855492211
Bacteria_unclassified	Bacteria_unclassified	2.03990894368978
Bacteria_unclassified	Bacteria_unclassified	2.00316726319519
Proteobacteria	Rhizobiales_unclassified	2.0026616932494

Table AS4. Table of early compost responders with LFC > 2.

OTUs with log2 fold change > 2 from the early compost responders		
Phylum	**Genus**	**log2FoldChange**
Proteobacteria	Myxococcales_unclassified	5.07520952887054
Proteobacteria	Myxococcales_unclassified	4.88552563991188
Actinobacteria	Actinomycetales_unclassified	3.70206556078148
Proteobacteria	Cellvibrio	3.54257265448929
Proteobacteria	Gammaproteobacteria_unclassified	3.32817822638392
Proteobacteria	Erythrobacteraceae_unclassified	3.24667044189892
Proteobacteria	Sphingobium	3.01691544918704
Bacteria_unclassified	Bacteria_unclassified	2.99144566656003
Firmicutes	Cohnella	2.98072431948256
Bacteria_unclassified	Bacteria_unclassified	2.96621376792312
Bacteroidetes	Bacteroidetes_unclassified	2.92472640941883
Proteobacteria	Gammaproteobacteria_unclassified	2.87341185470758
Acidobacteria	Gp3_unclassified	2.82296334495211
Bacteroidetes	Fluviicola	2.73608352530821
Planctomycetes	Planctomycetaceae_unclassified	2.73017906600275
Proteobacteria	Deltaproteobacteria_unclassified	2.68601043120515
Proteobacteria	Proteobacteria_unclassified	2.66188472317872
Bacteria_unclassified	Bacteria_unclassified	2.66070413982182
Proteobacteria	Devosia	2.65016674681425
Bacteria_unclassified	Bacteria_unclassified	2.60826702437228
Bacteria_unclassified	Bacteria_unclassified	2.6040307078225
Bacteroidetes	Bacteroidetes_unclassified	2.58840500408871
Planctomycetes	Planctomycetaceae_unclassified	2.5727703896618
candidate_division_WPS-2	candidate_division_WPS-2_unclassified	2.57160339562219
Bacteroidetes	Bacteroidetes_unclassified	2.54137910779679
Bacteroidetes	Cryomorphaceae_unclassified	2.53550153598985
Proteobacteria	Sphingomonas	2.53332171128775
Proteobacteria	Xanthomonadaceae_unclassified	2.52757622586906
Bacteroidetes	Pontibacter	2.52208655371945
Bacteroidetes	Ohtaekwangia_unclassified	2.42753315939327
Bacteria_unclassified	Bacteria_unclassified	2.39757206923468
Proteobacteria	Alphaproteobacteria_unclassified	2.39121196984576
Proteobacteria	Gammaproteobacteria_unclassified	2.32105736136392
Proteobacteria	Deltaproteobacteria_unclassified	2.3079568449618
Planctomycetes	Planctomyces	2.30256387152846
Verrucomicrobia	Spartobacteria_unclassified	2.28277989488723
Proteobacteria	Massilia	2.2523706752902
Verrucomicrobia	Subdivision3_unclassified	2.23144939251538
candidate_division_WPS-2	candidate_division_WPS-2_unclassified	2.22918301383375

Table AS4. (continued)

Gemmatimonadetes	Gemmatimonas	2.2212962885836
Acidobacteria	Gp4_unclassified	2.20151055290521
Verrucomicrobia	Verrucomicrobiaceae_unclassified	2.08227804174603
Proteobacteria	Gammaproteobacteria_unclassified	2.03985705996288

Table AS5. Table of late compost responders with LFC > 2.0

OTUs with log2 fold change > 2 from the late compost responders		
Phylum	**Genus**	**log2FoldChange**
candidate_division_WPS-2	candidate_division_WPS-2_unclassified	5.23089012311398
Chloroflexi	Anaerolineaceae_unclassified	5.09759530810164
Bacteria_unclassified	Bacteria_unclassified	4.19338894817396
Proteobacteria	Betaproteobacteria_unclassified	3.82343388550454
Bacteria_unclassified	Bacteria_unclassified	3.74130684437733
Verrucomicrobia	Spartobacteria_unclassified	3.53526592863835
Proteobacteria	Proteobacteria_unclassified	3.49341790060513
Proteobacteria	Byssovorax	3.32622328735892
Parcubacteria	Parcubacteria_unclassified	3.21690110703107
Planctomycetes	Planctomyces	3.18524956138212
candidate_division_WPS-1	candidate_division_WPS-1_unclassified	3.14828847429788
Acidobacteria	Acidobacteria_unclassified	3.10790429102313
Bacteria_unclassified	Bacteria_unclassified	3.10781336952061
Bacteria_unclassified	Bacteria_unclassified	3.10662900366213
Verrucomicrobia	Alterococcus	3.10536982806076
Planctomycetes	Planctomycetaceae_unclassified	3.10100326351415
Bacteria_unclassified	Bacteria_unclassified	3.04779724258072
Bacteria_unclassified	Bacteria_unclassified	2.97584017301545
Parcubacteria	Parcubacteria_unclassified	2.87675147446825
Parcubacteria	Parcubacteria_unclassified	2.86713376054761
Proteobacteria	Myxococcales_unclassified	2.84420338035778
Planctomycetes	Planctomycetaceae_unclassified	2.82488512349183
Proteobacteria	Myxococcales_unclassified	2.8220823120863
Bacteroidetes	Bacteroidetes_unclassified	2.81793538409637
Armatimonadetes	Armatimonadetes_gp5_unclassified	2.77939650260518
Proteobacteria	Rickettsiaceae_unclassified	2.75605156975241
Proteobacteria	Betaproteobacteria_unclassified	2.75245882128495
Planctomycetes	Planctomycetaceae_unclassified	2.73868790173612
Proteobacteria	Proteobacteria_unclassified	2.66854116956062
Proteobacteria	Proteobacteria_unclassified	2.66689494552524
Proteobacteria	Alphaproteobacteria_unclassified	2.66538503428949
Bacteria_unclassified	Bacteria_unclassified	2.62181941158108
Bacteria_unclassified	Bacteria_unclassified	2.61820466202629
Proteobacteria	Proteobacteria_unclassified	2.60132386884938
Bacteria_unclassified	Bacteria_unclassified	2.59609047179899
Bacteria_unclassified	Bacteria_unclassified	2.56137412815193
Bacteria_unclassified	Bacteria_unclassified	2.53818540769731
Proteobacteria	Erythrobacteraceae_unclassified	2.53622104239117
Bacteroidetes	Bacteroidetes_unclassified	2.52206606201714

Table AS5. (continued)

Proteobacteria	Panacagrimonas	2.51181212945434
Actinobacteria	Acidimicrobiales_unclassified	2.50231798451362
candidate_division_WPS-1	candidate_division_WPS-1_unclassified	2.48182305552998
Planctomycetes	Pirellula	2.41695451385335
Bacteria_unclassified	Bacteria_unclassified	2.39687997320606
Gemmatimonadetes	Gemmatimonas	2.36739783007219
Actinobacteria	Micromonosporaceae_unclassified	2.34279505422148
Chloroflexi	Chloroflexi_unclassified	2.31754176786213
Bacteria_unclassified	Bacteria_unclassified	2.30420006876641
Bacteria_unclassified	Bacteria_unclassified	2.28126698238508
Acidobacteria	Gp17_unclassified	2.27786107372821
Planctomycetes	Planctomycetaceae_unclassified	2.27624350749513
Chloroflexi	Chloroflexi_unclassified	2.26347578307667
Proteobacteria	Hyphomicrobiaceae_unclassified	2.25082308907392
Bacteria_unclassified	Bacteria_unclassified	2.22909334064839
Bacteria_unclassified	Bacteria_unclassified	2.17191110740775
Verrucomicrobia	Subdivision3_unclassified	2.17153164733421
Proteobacteria	Rhodospirillaceae_unclassified	2.16900123253392
Bacteria_unclassified	Bacteria_unclassified	2.16860017760717
Nitrospirae	Nitrospira	2.14822135432052
Planctomycetes	Planctomycetaceae_unclassified	2.14521875462349
Bacteria_unclassified	Bacteria_unclassified	2.14036738110087
Proteobacteria	Rhizobiales_unclassified	2.13389208761951
Proteobacteria	Proteobacteria_unclassified	2.11603536917186
Bacteria_unclassified	Bacteria_unclassified	2.11052279562631
Proteobacteria	Betaproteobacteria_unclassified	2.08940529816312
Acidobacteria	Gp17_unclassified	2.06172768262793
Bacteria_unclassified	Bacteria_unclassified	2.05686955649418
Proteobacteria	Rhizobiales_unclassified	2.05619838254561
Proteobacteria	Gammaproteobacteria_unclassified	2.04529667441432
Proteobacteria	Gammaproteobacteria_unclassified	2.03104789581455
Bacteria_unclassified	Bacteria_unclassified	2.01094222795022

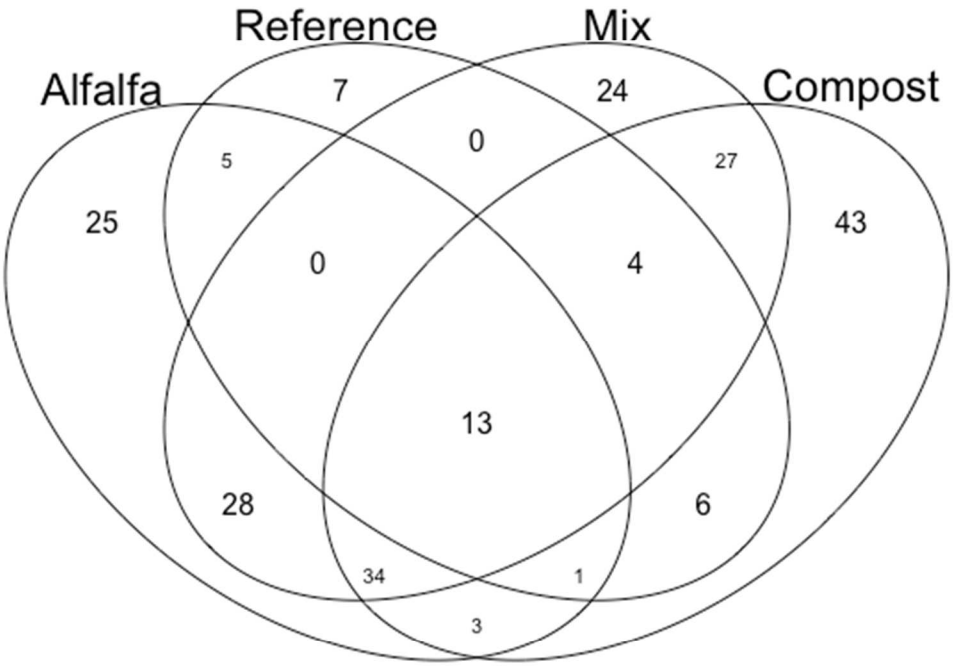

Figure AS1. Early responding OTUs and their distribution across amendments.

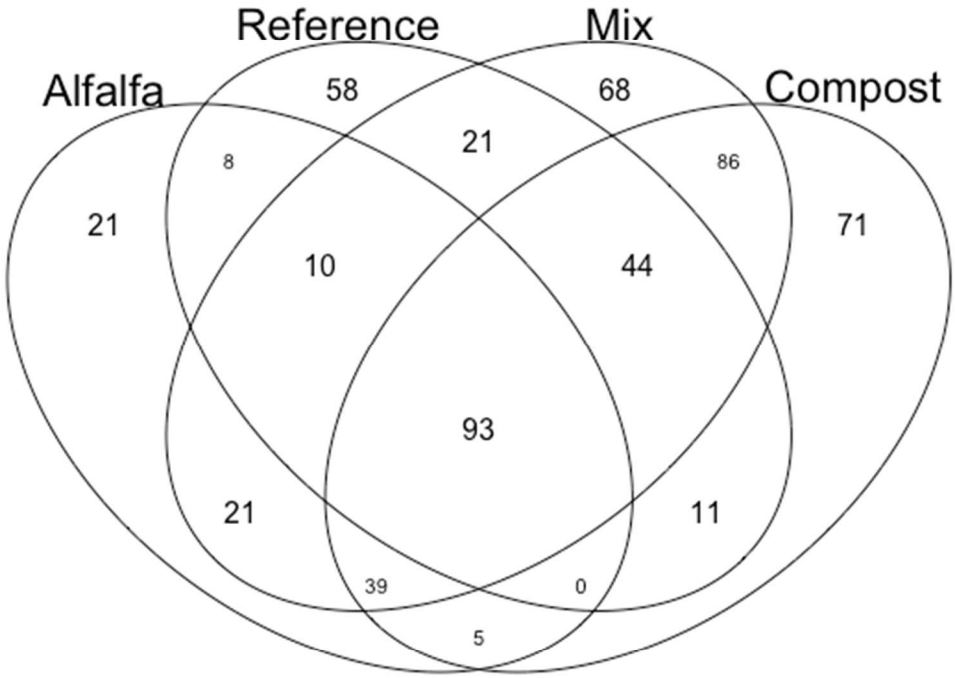

Figure AS2. Late responding OTUs and their distribution across amendments.

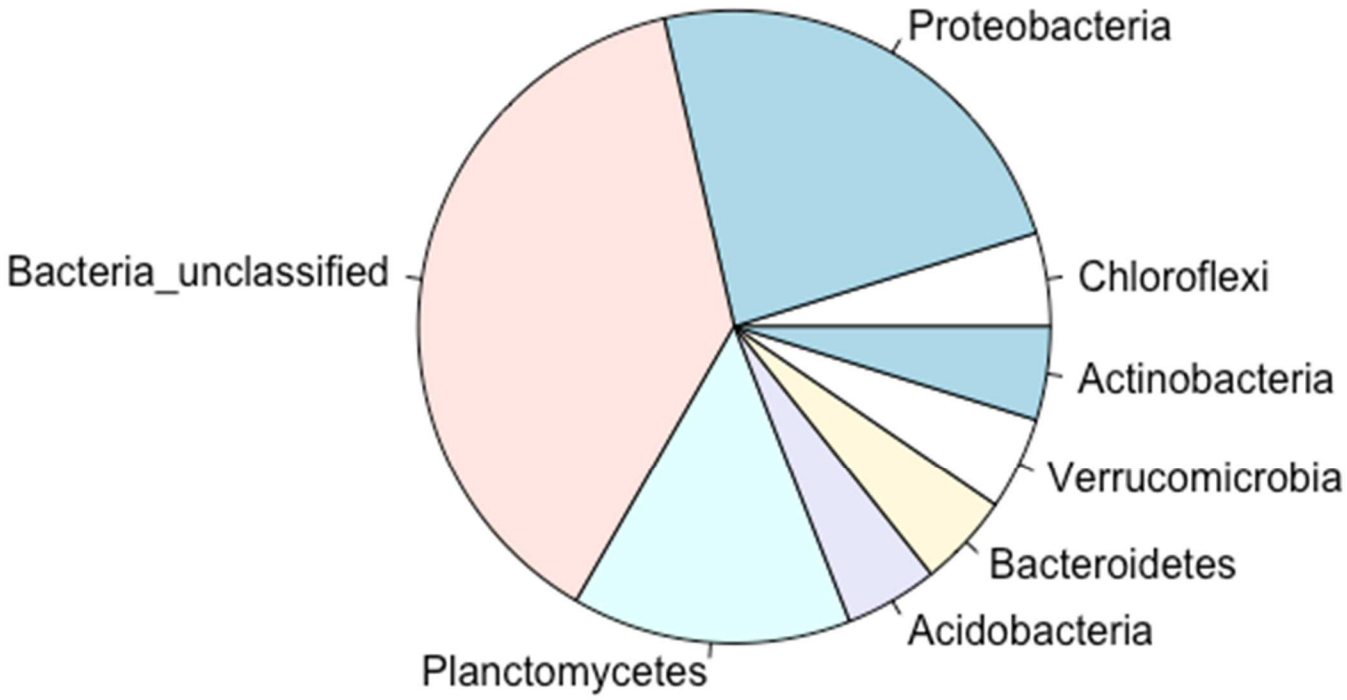

Figure AS3. Portion of each phyla represented in the late alfalfa response group.

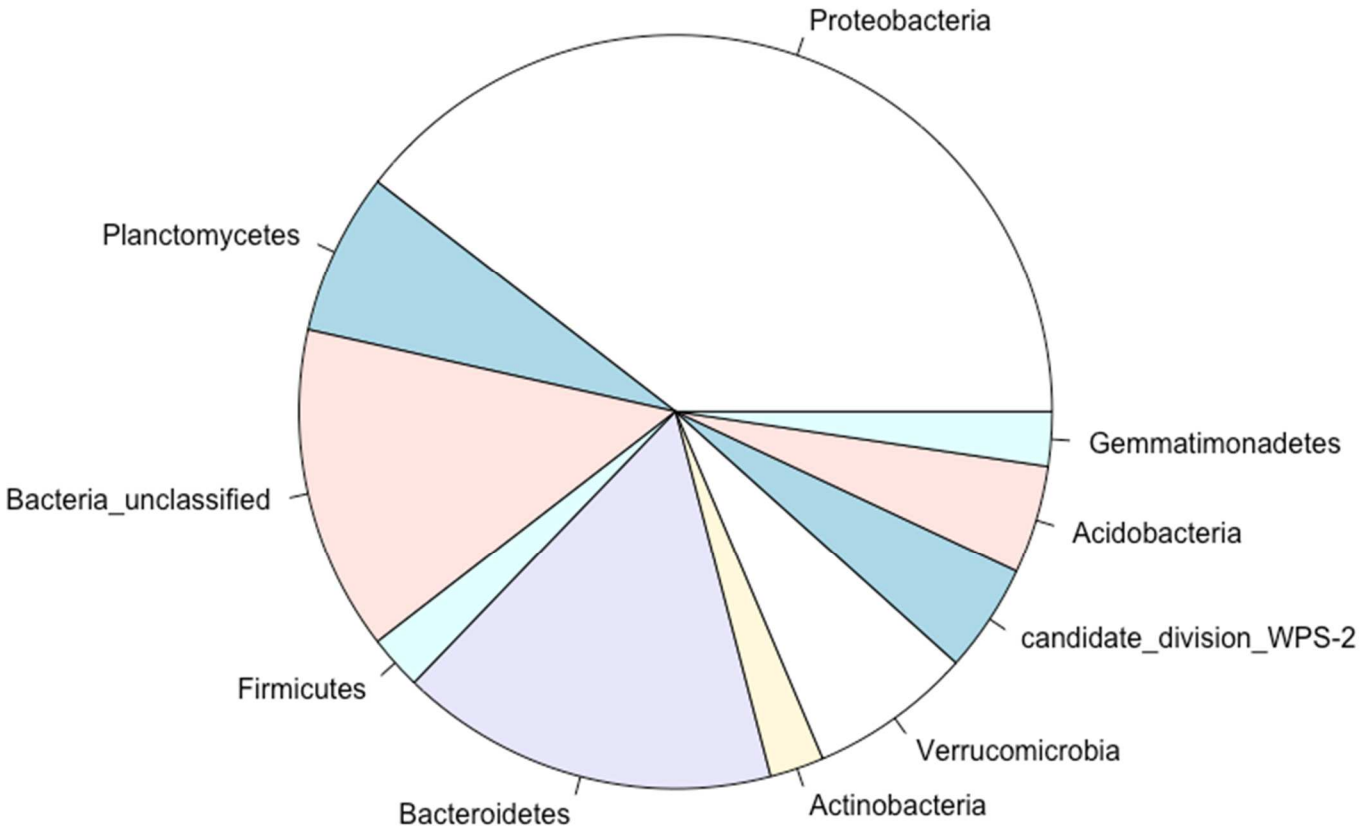

Figure AS4. Proportion of each phyla represented in the early alfalfa response group.

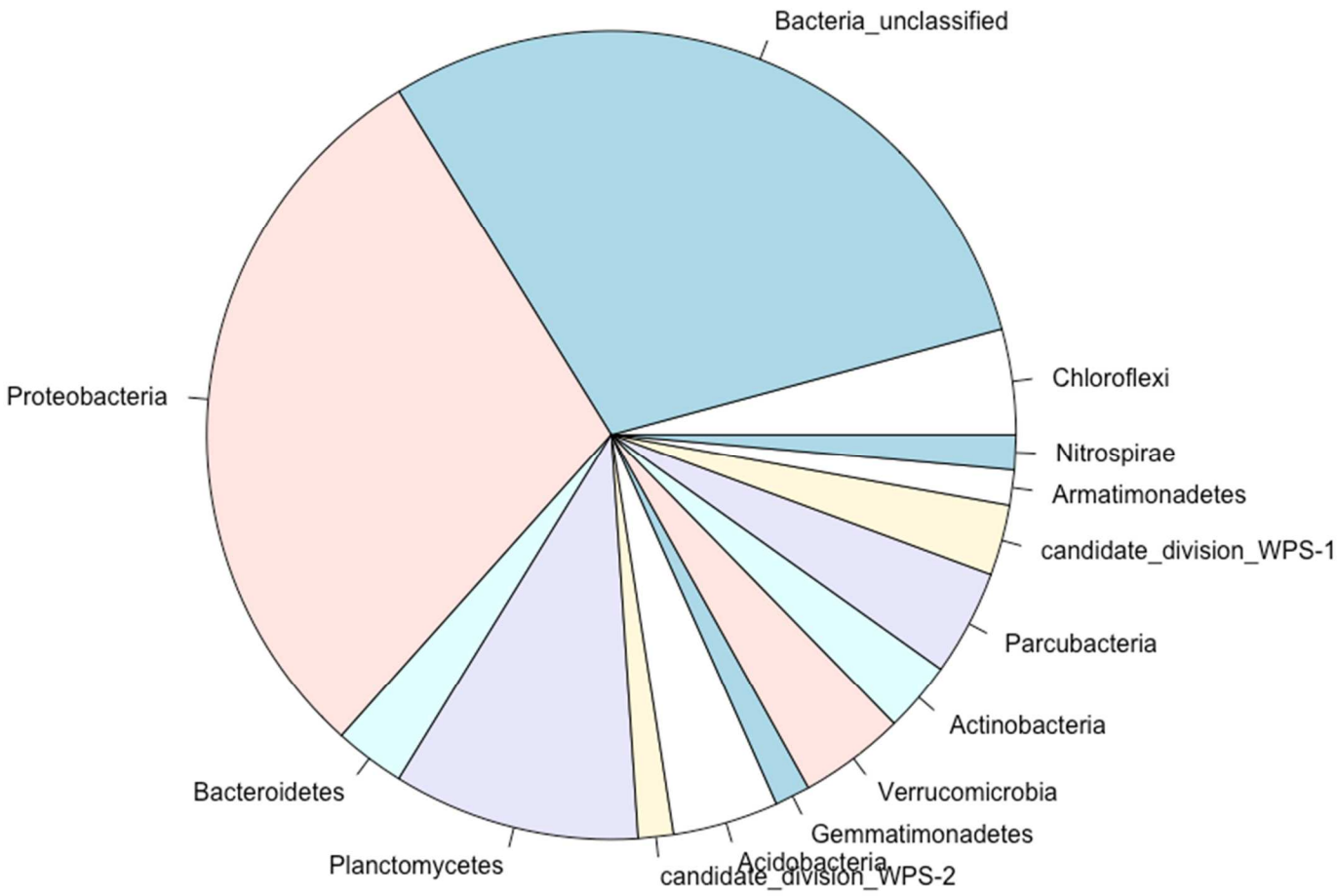

Figure AS5. Proportion of each phyla represented in the late compost response group.

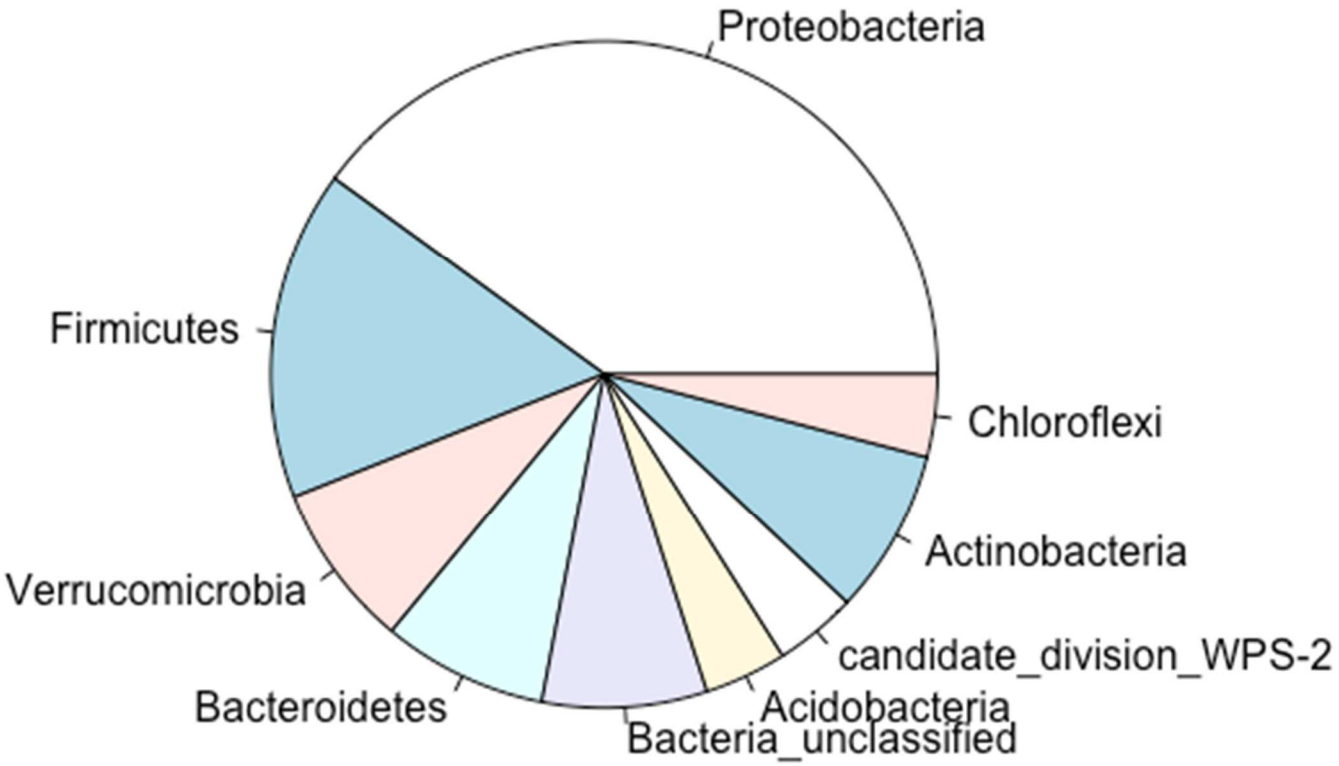

Figure AS6. Proportion of each phyla represented in the early compost response group.