Short-term nitrogen fertilization affects microbial community composition and nitrogen mineralization function in an agricultural soil

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ABSTRACT

Soil extracellular enzymes play a significant role in the N mineralization process. However, few studies have documented the linkage between enzyme activity and the microbial community that performs the function. This study examined the effects of inorganic and organic N fertilization on soil microbial communities and their N mineralization functions over four years. Soils were collected from silage corn field plots with four contrasting N treatments: control (no additional N), ammonium sulfate (AS100 & 200 kg N ha\(^{-1}\)), and compost (200 kg N ha\(^{-1}\)). Illumina amplicon sequencing was used to comprehensively assess overall bacterial community (16S rRNA genes), bacterial ureolytic community (ureC), and bacterial chitinolytic community (chiA). Selected genes involved in N mineralization were also examined using quantitative real-time PCR and metagenomics. Enzymes and marker genes included protease (npr and sub), chitinase (chiA), urease (ureC), and arginase (rocF). Compost significantly increased diversity of overall bacterial communities even after one application, while ammonium fertilizers had no influence on the overall bacterial communities over four seasons. Bacterial ureolytic and chitinolytic communities were significantly changed by N fertilization. Compost treatment strongly elevated soil enzyme activities after four-years of repeated application. Functional gene abundances were not significantly affected by N treatments, and they were not correlated with corresponding enzyme activities. N mineralization genes were recovered from soil metagenomes based on a gene-targeted assembly. Understanding how the structure and function of soil microbial communities involved with N mineralization change in
response to fertilization practices may indicate suitable agricultural management practices that improve ecosystem services while reducing negative environmental consequences.

**IMPORTANCE**

Agricultural N management practices influence the enzymatic activities involved in N mineralization. However, specific enzyme activities do not identify the microbial species directly involved in the measured process, leaving the link between the composition of the microbial community and the production of key enzymes poorly understood. In this study, the application of high-throughput sequencing, real-time PCR and metagenomics shed light on how the abundance and diversity of microorganisms involved in N mineralization respond to N management. We suggest that N fertilization has significantly changed bacterial ureolytic and chitinolytic communities.

**KEYWORDS**

N fertilization, compost, microbial diversity, soil enzyme activity, N transformation rates, protease, urease, chiA, sub, npr, ureC, rocF.

**INTRODUCTION**

Human input of chemical nitrogen (N) fertilizers to agricultural systems has increased more than 10 fold in the past 50 years to increase the yield of crops and prevent food shortage for a growing human population (1). However, excessive and repeated use of chemical N fertilizers may result in water and air pollution, soil degradation including reductions in soil organic matter and soil pH (2), and increases in nitrate leaching and
reactive N gas production (1, 3). Therefore, avoiding the combination of high external
N inputs with low N use efficiency remains a major concern for the sustainability of
agroecosystems (4, 5). Application of organic N fertilizers such as compost and
manure is one effective strategy to improve soil quality and functionality (6) while
maintaining N supply.

Soil microorganisms play a crucial role in the maintenance of soil fertility, and
they are often sensitive to N fertilization and management. Ammonium fertilizers
contribute a large, but transient flush of inorganic N upon application, while organic
N sources show a slow inorganic release pattern due to N mineralization (7).
Therefore, mineral and organic N fertilization may exert different influences on soil
microbial communities (2, 8, 9). Numerous field studies have shown that repeated
mineral N fertilization decreases while organic N fertilization increases bacterial
diversity (10–13). However, these studies were mainly conducted in long-term field
fertilization experiments with only one sampling time and provided limited
information about the temporal response of the soil microbial community in the field.

A wide variety of microbial-derived extracellular enzymes mediate the
depolymerization of the large N-containing polymers to monomers and ammonium
(14). Most previous studies have focused on agricultural N management practices
influencing the enzymatic activities involved in N mineralization (15–19). However,
specific enzyme activities do not identify the microbial species directly involved in
the measured process, leaving the link between the composition of the microbial
community and the production of key enzymes poorly understood. For example,
bacteria are assumed to be the main degraders of urea and chitin (20, 21). It is still
largely unknown how diverse are bacterial ureolytic and chitinolytic communities in
soils and whether they are influenced by agricultural N management (22, 23). The
development of real-time PCR and high-throughput sequencing could provide important information about how the abundance and diversity of microorganisms involved in N mineralization respond to N management (22–26).

Therefore, this study aimed to examine the short-term (<5 years) effects of inorganic and organic N management on soil microbial community composition, the abundance of functional genes involved in N mineralization, N transformation rates, and soil enzyme activities in replicated field plots. High-throughput sequencing of marker genes for bacterial ureolytic (ureC) and chitinolytic (chiA) communities was used to identify specific urea and chitin degraders and examine their response to N fertilization. As a complementary resource, we also assembled several genes involved in N mineralization from soil metagenomes. We asked whether soil microbial community and soil enzyme activities would differentially respond to inorganic and organic N fertilization. In addition, we also asked how N mineralization functions link with soil microbial communities in the context of contrasting N management. Understanding how the structure and function of soil microbial communities change in response to different N fertilization practices is essential information for the selection of suitable agricultural management practices that improve the ecosystem services and reduce negative environmental consequences.

RESULTS

Soil N transformation rates and enzyme activities. In August 2014, N treatments significantly affected most measured soil N transformation rates and enzyme activities, although there was no significant difference for gross mineralization rate and gross
ammonium consumption rate (Table 1). Gross nitrification, net N mineralization, and net nitrification had higher rates in AS and compost treatments compared to the control, but they showed no difference among AS and compost treatments. Control and compost treatments had higher gross nitrate consumption rates than AS treatments. Compared to other treatments, compost was significant higher in soil respiration rate, dehydrogenase activity, acid phosphomonoesterase activity, and alkaline phosphomonooesterase activity.

Both N treatments and sampling time showed significant effects on activity of urease, arginase, protease and β-glucosaminidase (Fig.1 & Table S1). Generally, those four enzymes had much higher activities in compost treated soils than in the other treatments (Fig. 1). However, enzyme activities showed different patterns in temporal variation over sampling time. Urease and arginase activities were lowest in July. Protease activity increased through the growing season and had higher activities in September and October. In contrast, β-glucosaminidase activity was relative constant throughout the season.

**Abundance of genes involved in N mineralization.** N treatment and sampling time showed no significant effect on the abundances of the four functional genes related to N mineralization in 2014 (Fig.S1 and Table S2). The mean values of the abundances of sub, npr, chiA, and ureC were $3.8 \times 10^7$, $2.7 \times 10^5$, $2.2 \times 10^8$, $9.7 \times 10^7$ copies per gram of dry soil, respectively. Pearson correlation analysis indicated that there was no significant correlation between the abundance of these functional genes and the corresponding enzyme activity (Table S3).
The abundance of ureC was repeatedly measured in Aug from 2011 to 2014. Repeated measures ANOVA indicated that year (p<0.01), but not N treatment (p=0.43), significantly changed the abundance of ureC, with an increase from 3.2 × 10^7 per gram of dry soils in 2011 to 1.08 × 10^8 per gram of dry soils in 2014 (Fig. S2).

**Bacterial community composition.** In total, 1,944,732 high-quality 16S rRNA gene reads were obtained for 32 samples in 2011 and 2014, with 17,152 OTUs. Across all soil samples, we detected 45 distinct prokaryotic phyla, although only twelve bacterial and one archaeal phyla were the most prevalent (>1%) (Fig. S3). *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Gemmatimonadetes* were the five most abundant phyla, which comprised more than 75% of the relative abundance of the bacterial community. Two-way ANOVA indicated that the relative abundance of *Proteobacteria, Actinobacteria,* and *Acidobacteria* were significantly changed by N treatments (Fig. 2a and Table S4). *Actinobacteria* and *Acidobacteria* abundances were decreased by compost in 2011, while *Proteobacteria* abundance was increased by compost in 2014. The abundances of those dominant phyla of the prokaryotic community were significantly influenced by sampling time (Table S4). For example, *Acidobacteria, Bacteroidetes,* and *Verrucomicrobia* abundances increased, while *Actinobacteria* and *Fimicutes* abundances decreased from 2011 to 2014.

N treatment but not year significantly influenced the alpha diversity of the prokaryotic community (Fig. 3). Compost treatment increased Chao1, observed OTUs, and Shannon diversity in both 2011 and 2014. Bacterial community structure as revealed by Weighted UniFrac distance grouped differently in 2011 versus 2014, and
compost treatment was distinct from AS and control treatments in both years (Fig. 2b).

Two-way PerMANOVA further confirmed that bacterial community structure was significantly affected by year (p=0.001) and N treatment (p=0.008).

Based on log2-fold change of relative abundance of OTUs, many OTUs responded significantly to inorganic and organic N fertilization (Fig. 4). Most of these responsive OTUs, mainly from *Proteobacteria* and *Bacteroidetes*, were enriched by N fertilization. In 2011, there were only a few OTUs that were responsive to inorganic N addition, while 41 OTUs were significantly changed by compost treatment. In 2014, there were 33, 51, and 78 responsive OTUs in AS100, AS200, and compost, respectively. AS100 and AS200 shared half of their responsive OTUs, while less than 4% responsive OTUs were shared between AS and compost treatment (Fig S4a). In the compost treatment, only seven responsive OTUs were shared between 2011 and 2014 (Fig S4b).

**Ureolytic community composition.** In total, 875,995 high-quality *ureC* reads were obtained for 16 soil samples in Jun 2014, with 8550 OTUs (95% amino acid identity cut off). Based on the nearest match to reference taxonomy, we detected 10 distinct prokaryotic phyla, although only seven bacterial phyla were the most prevalent (>1%) (Fig. 5a). The majority of sequences were assigned to *Proteobacteria* (72%) and *Actinobacteria* (12%). *Thaumarchaeota* were also detected but their relative abundance was very low (0.4%). There was no significant difference in the relative abundance of phyla among N treatments. The ureolytic community composition as revealed by weighted UniFrac distance matrices was significantly changed by N treatments.
Pairwise comparison demonstrated that compost treatment was significantly different from AS100 (p=0.026) and AS200 (p=0.031) treatments.

Top 50 ureC OTUs were used for detailed phylogenetic analysis (Fig. 6), which accounted for 24% of the total sequences. Most of these top OTUs were assigned to *Proteobacteria* with families of *Burkholderiales*, *Rhizobiales*, and *Myxococcales*.

OTU 455 was closely affiliated with *Rhizobiales* was not grouped together with other *Proteobacteria* families. OTU 88 and OTU 436 were affiliated with family of *Nitrospiraceae* and *Myxococcales*, respectively. Interestingly, several most abundant OTUs such as OTU 54, OTU 100, and OTU 456 had no very closest match in the current reference database. Among the top 50 OTUs, 15 OTUs were significantly changed by N treatments. For example, OTU 313 and OTU 668 were increased by compost treatment. However, several OTUs, such as OTU 483 and OTU 233 were significantly reduced by AS200 treatment.

**Chitinolytic community composition.** In total, 53,709 high-quality *chiA* reads were obtained for 16 soil samples in Jun 2014, with 3572 OTUs (95% amino acid identity cut off). Based on the nearest match to the reference taxonomy, most of OTUs were assigned to *Actinobacteria* and *Proteobacteria* (Fig. 5c). The relative abundance of *Actinobacteria* was lowest, but *Proteobacteria* was highest in compost treatment. The chitinolytic community composition as revealed by weighted UniFrac distance matrices was significantly changed by N treatments (p=0.01) (Fig. 5d). Pairwise comparison demonstrated that the control treatment was significantly different from AS100 (p=0.029) and AS200 (p=0.034) treatments.
Top 50 chiA OTUs were used for detailed phylogenetic analysis (Fig. 7), which accounted for 26% of the total sequences. Most of these top OTUs were assigned to *Streptomyces* (e.g., OTU 127 and OTU 265), *Lentzea* (e.g., OTU 70 and OTU 200), and *Actinoplanes* (e.g., OTU 642) family in the *Actinobacteria*. OTU 155, the most abundant OTU, was closest to *Xanthomonadaceae*. Among the top 50 OTUs, 13 OTUs were significantly changed by N treatments. For example, OTU 642, OTU 52 and OTU 42 were increased by compost treatment, while OTU 155 and OTU 433 were significantly increased by AS treatments.

**Gene-targeted assembly for N mineralization genes.** Four of the selected N mineralization genes (*npr, chiA, rocF, and ureC*) were recovered in our four soil metagenomes based on the gene-targeted assembly (Table S5), the *sub* gene was not recovered by the same method. The number of OTUs recovered at 95% aa identity for these four selected N mineralization genes ranged from 4 to 280, and their *rplB* normalized abundances ranged from 0.01 to 0.44 in four soil metagenomes. The *rocF* and *ureC* had higher OTU numbers and abundances than the other N mineralization genes. Top 10 OTUs of these N mineralization genes and their best matches to reference databases are also summarized (Table S6). Top *npr* and *chiA* OTU representatives often had relative lower similarity to reference sequences from FunGene with the average of 61%. Most of top *rocF* OTUs were assigned to *Acidobacteria* and *Proteobacteria*. Interestingly, most top *ureC* OTUs were assigned to *Thaumarchaeota*.

**Microbial community composition in steer-waste compost.** The bacterial
community composition from steer-waste compost in 2011, which was added to the
compost treatment, was also analyzed together with soils samples. There were six
prevalent phyla (>1%) in the steer-waste compost (Fig. S5a) and Proteobacteria,
Bacteroidetes, and Actinobacteria were the three most abundant phyla. Three months
after compost application, about 42% of OTUs from steer-waste compost were
detected in the compost-treated soils. There were 20% of OTUs from steer-waste
compost that were only detected in compost-treated soil rather than in the control
soils, this accounts for 6.6% of OTUs in compost-treated soils (Fig. S5b). For these
OTUs only shared between steer-waste compost and compost-treated soils, we found
that 18 OTUs were also present in the group of 41 responsive OTUs.

Ureolytic and chitinolytic community composition from steer-waste compost
used in 2013 were also measured. Around 90% of ureC sequences were assigned to
Proteobacteria (Fig. S5). One year later, more than 50% of ureC OTUs from steer-
waste compost were recovered in compost treated soils, 6.5% of ureC OTUs from
compost-treated soils were presented in both the steer-waste compost and compost-
treated soils. Except unclassified phyla, most of chiA sequences in steer-waste
compost were assigned into Actinobacteria. One year later, only 1.5% of chiA OTUs
from compost-treated soils were present in both steer-waste compost and compost-
treated soils (Fig. S6).

**DISCUSSION**

In this study, the application of the organic N fertilizer (steer-waste compost)
significantly changed the structure of the bacterial community. This change was
detected three months after the application. Compost strongly increased the richness
and diversity of the bacterial community. This observation is consistent with most
previous studies (8, 9, 27–29). The elevated diversity of bacterial community in
compost treated soils was partly due to the stimulation in growth of native soil bacteria
by high available nutrients and diverse organic carbon fractions (6). In addition, we
found that more than half of OTUs from the steer-waste compost were recovered in
compost treated soils three months after application. The direct introduction of
exogenous species to the soils may contributed to the increased microbial diversity (30),
although those microbes originating from compost might be less competitive with
native soil microbial community over the long-term (8, 28).

Although previous studies reported that mineral N fertilization decreased the
diversity of bacterial community in agricultural soils (29, 31, 32), our results showed
that mineral N fertilization had no effect on the diversity and structure of overall
bacterial community. This observed stability may be related to the agricultural
management history. Our field plots were repeatedly planted with wheat and received
urea as N sources for the decade before 2011. This cultivation with crop monoculture
and repeated urea fertilization may homogenize the microbial community favoring
those that are resistant to change due to mineral N fertilization (33).

We found that the overall bacterial community significantly varied between 2011
and 2014. Soil microbial communities often show strong seasonal and inter-annual
variability (34). Duncan et al. (35) reported the considerable changes in bacterial
community between two years in fields planted with corn. Lauber et al. (36) found that soil community composition was variable over time in agricultural soils, and the changes in communities were positively correlated with soil moisture and temperature. More interestingly, we found that the numbers of responsive OTUs increased significantly from 2011 to 2014. Most of these responsive OTUs were from *Proteobacteria* and *Bacteroides*, which are generally considered copiotrophs (37). Furthermore, only a very small proportion of the OTUs was shared between 2011 and 2014. These results suggest temporal variability of bacterial community were very high in the short-term after N fertilization.

The ureolytic community composition was significantly changed by N treatments in our soil. More specifically, inorganic N and organic N treatments harbored distinct ureolytic community compositions. Other studies in agricultural soils have shown that organic matter from compost or manure may affect the soil ureolytic microbial community (23). We also observed a significant difference in soil organic C between AS and compost treatments in 2014 (38) In our study we found that more than 50% of OTUs from steer-waste compost were present in compost-treated soils even three months after application. These microorganisms inhabiting compost may also play an important role in shaping the bacterial ureolytic community composition in compost-treated soils. We also found that 15 out of the top 50 OTUs were significantly changed by the N treatments. Interestingly, five of those affected OTUs were enriched by compost application, while most of the affected OTUs were decreased by AS treatments. These results indicate that some ureolytic microorganisms may be repressed under
ammonium-based fertilizers.

In this study, amplicon sequencing indicated that the ureolytic communities were mainly affiliated with *Proteobacteria*. This is consistent with Collier et al. (21), which summarized that bacterial urease was most commonly found in *Proteobacteria*. It is important to note that many *ureC* OTUs, even some top OTUs, had no identified matches (>85% identity) to current references. This suggests that the primer-based amplicon sequencing provides some information on previously uncultured ureolytic organisms. Interestingly, our gene-targeted assembly of soil metagenomes showed that many of these recovered top *ureC* OTUs were affiliated with *Thaumarchaeota*. We did detect *ureC* sequences closely related to *Thaumarchaeota* based on amplicon sequencing, although their relative abundances were very low. Our previous studies found that AOA, which often contain *ureC*, were abundant in our soils (38, 39). The difference between metagenome and amplicon sequencing of *ureC* suggests a potential primer bias. However, both amplicon sequencing and metagenome confirmed that *Nitrospira* were important potential urease producers, since several top *ureC* OTUs were from *Nitrospira*, some of them even close matches to the comammox organism *N. inopinata* (41).

Chitin is one of the most abundant organic N polymers in soil environments (42). Previous studies have found that bacterial chitinolytic communities were significantly changed by chitin amendment (22). Since compost contains multiple organic N polymers, we hypothesized that compost application would also shape the chitinolytic community. We did observe that compost treatment significantly increased several top
However, there was no significant difference in the chitinolytic community between control and compost treatment after four-years repeated compost application. This is partly due to the high variability of chitinolytic community composition in compost treatment. Three of the replicates in the compost treatment were highly separated from the control treatment, but one of the compost-treated plots was closely clustered with controls. In addition, we found that only 1.5% chiA OTUs in steer-waste compost were recovered in compost-treated soil, indicating chitinolytic microorganisms in compost are less competitive than the indigenous chitinolytic community and weakly survive in soil. Interestingly, AS treatments significantly changed the chitinolytic community. Several top chiA OTUs were strongly enriched by ammonium-based fertilizers (OTU 155 and OTU 433). These results indicate that the chitinolytic community in our soil may be N-limited.

In our study, the application of compost increased soil enzyme activities, which is consistent with observations showing that organic amendments significantly increase enzyme activities (15, 43, 44). This is possibly due to stimulation of microbial growth and related increases in the activity of the extracellular enzyme-organo complexes (45). However, we found that the abundances of functional genes involved in N mineralization were not affected by the N treatments, and that the abundance of functional genes involved in N mineralization did not correlate with their corresponding soil enzyme activities. The lack of correlation between the abundance of functional genes and their corresponding enzyme activities may be attributed to several factors. First, primers used to target the functional genes did not cover all of the microbial
community responsible for the specific enzyme function. For example, there are many
different groups of protease (46, 47), but only limited proteolytic gene primers have
been developed and identified for soil microbiome, including serine peptidase (sub) and
neutral metallopeptidase (npr) (48). In addition, the primer pairs used in our study did
not cover the fungal community. Metagenomic analysis for the both prokaryotic and
fungal communities may provide a better coverage for the functional groups producing
the specific enzymes, although the depth of sequencing remains an issue. Second,
DNA-based analyses do not differentiate inactive from active members of the soil
community. Proteomic or RNA-based techniques may be more appropriate to link the
abundance of active functional groups with their corresponding enzyme function (45).
Third, production of extracellular enzymes is regulated by genes encoding the
corresponding enzyme, but once they are secreted out of the cells, their stabilization
and degradation are controlled by physical and chemical conditions of the environment
(16). Fuka et al. (25) reported that a significant correlation between sub and npr genes
and potential protease was only found for sandy soils but not clay soil suggesting that
these relations may be soil specific.

In summary, the application of organic N fertilizer, but not inorganic N fertilizer,
increased the diversity of the bacterial community and the activities of soil enzymes. N
fertilization significantly changed ureolytic and chitinolytic bacterial communities. The
abundance of selected functional genes involved in N mineralization was not affected
by the N treatments, regardless of the inorganic and organic fertilizer form used. The
abundance of targeted functional genes was not correlated with the corresponding
enzyme activities. Metagenomics or metatranscriptomics associated with high-throughput sequencing targeting functional genes including those from fungi are needed to provide better coverage for the novel responsible members of the microbial community. With this additional information our ability to link microbial functional genes to their associated enzyme activity should be strengthened.

MATERIALS AND METHODS

Soil characterization. The details of the agricultural site (North Logan, Utah, USA), experimental design, treatments, soil sampling, and soil characteristics have been previously described (38, 39). Briefly, the experimental design is a randomized complete block with four blocks and four nitrogen treatments: control (no N fertilization), ammonium sulfate (AS 100 and 200 kg N ha$^{-1}$), and steer-waste compost (200 kg total N ha$^{-1}$). Treatments were surface applied in May of each year and incorporated by tilling immediately after application. The soil is an irrigated, very strongly calcareous Millville silt loam (Coarse-silty, carbonatic, mesic Typic Haploxeroll). Soils were sampled in August from 2011 to 2014, and soils were also sampled monthly during the growing season of 2014. Six soil cores (0-15 cm depth, three cores in the intervals between rows and three cores in the row between plants) were taken from each plot, composited and thoroughly mixed, and a sample of soil was stored at -80 °C immediately after soils were brought to the laboratory.

Gross and net N transformation rates. Gross N transformation rates were determined in laboratory incubations using N$^{15}$ pool dilution for soil sampled in August 2014.
Three well-mixed 40 g dry-weight equivalent subsamples were weighed into plastic specimen cups. Then, 1.6 ml of $^{15}$NH₄⁺ solution (containing 1.69 mM ($^{15}$NH₄)₂SO₄ at 98 atom % $^{15}$N) or $^{15}$NO₃⁻ solution (containing 3.33 mM K$^{15}$NO₃ at 99 atom % $^{15}$N) were added to the soils and carefully mixed, creating a final soil water content of 0.18 kg kg⁻¹. The quantity of $^{15}$N added approximately doubled the soil NH₄⁺ or NO₃⁻ pool. Immediately following soil mixing, one subsample was harvested and extracted with 2 M KCl to determine NH₄⁺ or NO₃⁻ concentration and $^{15}$N enrichment at time-0. The other subsample was placed in 1-L Mason jars with lids containing butyl rubber septa and with 1 ml water at the bottom of the jar to minimize loss of moisture from the soil. Jars were incubated for 48 h at 25°C before extraction in 2M KCl. Soil NH₄⁺ or NO₃⁻ +NO₃⁻ were measured with a flow injection analyzer. The extracts were prepared for $^{15}$N analyses using a diffusion procedure described in Stark and Hart (49), and the $^{15}$N enrichment was measured by continuous-flow direct dry combustion and mass spectrometry with an ANCA 2020 system (Europa Scientific, Cincinnati, OH). Gross N transformation rates were calculated using the equation of Norton and Stark (50).

Net mineralization and nitrification were determined by a 21-day incubation. Fifteen grams of moist soil (0.18 kg kg⁻¹ water content) in a plastic specimen container was placed in a 1-L Mason jar with a lid containing butyl rubber septum and 1 ml water at the bottom. Soil was extracted with 2 M KCl before and after incubation. Headspace CO₂ was measured at 3 days, 7 days, 14 days, and 21 days by a gas chromatograph with a thermal conductivity detector to determine the soil respiration rate.
Soil enzyme activities. We measured activity of protease (EC 3.4.21), arginase (EC 3.5.3.1), urease (EC 3.5.1.5), and β-glucosaminidase (EC 3.21.30), dehydrogenase (EC 1.1.1), acid phosphomonoesterase (EC 3.1.3.2) and alkaline phosphomonoesterase (EC 3.1.3.1). The details of the protocol for measurement of these enzyme activities have been previously described (26). Briefly, for protease assay, soil samples were incubated at 37 °C with 0.6% casein. Protease activity was calculated from the difference between amino acid concentrations over 2 hours. The arginase activity was measured as reported by Bonde et al (51). Soil slurries were incubated with final concentration of 1.0 mM L-arginine at 37°C for 1 hour. Urease activity was determined as shown by Gianfreda et al. (52). Fresh soil was incubated at 37 °C with 0.2 M urea solution for 2 hours. β-glucosaminidase activity was determined by the method of Parham and Deng (53). Fresh soil was mixed with sodium acetate buffer (pH 5.5) and p-nitrophenyl-N-acetyl-β-D-glucosaminide solution in 50 ml centrifuge tubes and kept at 37 °C for 1 hour. Activities of dehydrogenase, acid phosphomonoesterase and alkaline phosphomonoesterase were measured at 37 °C as previously described (54).

Soil DNA extraction and real-time quantitative PCR. Soil DNA was extracted using a MoBio PowerSoil DNA isolation kit (MoBio Laboratories Inc, Carlsbad, USA). DNA extracts were quantified by using the Quant-iT™ PicoGreen dsDNA BR Assay Kit (Molecular Probes, Inc. Eugene OR, USA) according to the manufacturer’s protocol. Quantitative PCR of genes encoding enzymes involved in soil N mineralization was performed using the SsoAdvanced SYBR Green Supermix and a CFX CONNECT Real-Time PCR Detection System (Bio-Rad laboratories, Hercules, CA, USA). We
measured the abundance of genes encoding subtilisin (\textit{sub}), neutral metalloprotease (\textit{npr}), chitinase (\textit{chiA}), and urease (\textit{ureC}) for soil sampled in Jun and Aug 2014. Primers, amplification conditions, efficiencies, and calibration standards are summarized in Table 2. Standard curves were constructed with plasmids containing cloned gene products from genomic DNA of bacterial isolates (\textit{ureC}, \textit{sub}, and \textit{chiA}) or from environmental DNA (\textit{npr}), and R$^2$ values ranged from 0.990 to 0.999 for all genes targeted. Duplicate assays for each gene and calibration standard series were measured in a single run.

\textbf{Soil metagenome processing and gene targeted assembly.} Metagenomes were also obtained from soils samples in Jun 2014. DNA samples from four replicates of each N treatment were pooled with equal amount of DNA. DNA were then sequenced on the Illumina HiSeq 2500 platform with 2 × 150 bp paired-end format at the Joint Genome Institute. Quality-filtered metagenomes were downloaded and used for gene targeted assembly (55). Five genes involved in N mineralization (\textit{sub}, \textit{npr}, \textit{chiA}, \textit{ureC}, and \textit{rocF}) and \textit{rplB} were included for the assembly. For each gene of interest, seed sequences, HMMs, and nucleotide and protein reference sequences were downloaded from FunGene (56). Default assembly parameters were used and sequences were clustered at 95\% amino acid similarity. Representative sequence from each cluster was searched against the reference gene database and the non-redundant database (\textit{nr}) from NCBI using BLAST (57). In overall, the top hit of these representative sequences to the reference gene database had a similarity higher than 49\% and a e-value higher than 1.5 E-46.
Illumina sequencing and data analysis for ureC and chiA. Sequencing of the ureC and chiA amplicon libraries was accomplished for steer-waster compost used in 2013 and soils sampled in Jun 2014. The same ureC and chiA primers described above were used for high-throughput sequencing. Linkers were added to primers for ureC and chiA genes, while tags were added to separate different soils samples (58). The same amount of soil DNA was used for ureC and chiA amplifications and then the PCR products were further purified using size selection (Agencourt® Ampure® XP PCR purification). Pooled purified products were sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using V3 chemistry (2x300 paired end).

For a comparison, the bioinformatic analysis of ureC and chiA amplicons was performed using the same Xander post-assembly processes based on the RDP Pipeline (59). Raw reads were split based on the tags (soil samples), and then forward and reverse reads were merged using the USEARCH workflow (60). High quality ureC and chiA sequences were extracted from merged reads in each sample using the RDP SeqFilters with a read Q score cutoff of 25. Chimera sequences were removed using UCHIME (61) with the ureC and chiA nucleotide reference databases downloaded from the FunGene (56). The obtained sequences were further processed using the FrameBot tool (62) to fix frame shifts and translate DNA to protein. The remaining quality-screened protein sequences in each sample were aligned based on ureC and chiA hidden Markov models using HMMER3 (63). The aligned sequences from each sample were merged together using the RDP AlignmentTools. Sequences were further dereplicated and singletons were removed. Operational taxonomic units (OTU) were clustered at 95% amino acid similarity using the RDP Clustering. The longest
sequence from each OTU was chosen as a representative sequence. To obtain the phylum-level classification of representative sequences, the taxonomy from the closest match to the protein reference downloaded from FunGene was used. If the percent identity to the reference sequences was less than 80% (percent alignment >90%), we defined the phylum as unclassified. A maximum-likelihood phylogenetic tree was constructed from representative sequences using FastTree with default parameters (64). OTU table and taxonomy file were further organized for diversity analysis using R package phyloseq (65).

Illumina sequencing of 16S rRNA. The variable V4 region of the 16S was amplified with 515F and 816R universal primers for the bacterial community (66). The 16S amplicon sequencing was performed on an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA). The Illumina raw reads were processed using a custom pipeline developed at the Joint Genome Institute (https://bitbucket.org/berkeleylab/jgi_itagger). Briefly, raw reads were first quality-filtered, and then the high quality sequences were clustered into operational taxonomic units (OTUs) based on 97% identity for prokaryotic dataset using the USEARCH pipeline (60). Taxonomies were assigned to each OTU using the RDP Classifier with a confidence threshold of 0.60 (67). All data files were then organized using R package phyloseq (65).

Statistical analysis. For microbial diversity analyses, all samples were randomly rarefied to lowest reads per sample (27366 reads for 16S, 25000 reads for ureC, and 1278 reads for chiA) to compare differences between samples. Alpha-diversity and beta-diversity were then calculated. Nonmetric Multidimensional Scaling (NMDS) and
PerMANOVA were conducted to visualize and assess the distances matrices in vegan package of R software. Fold change in relative abundance of OTU under N fertilization was performed using R package DESeq2 (68). We removed OTUs that were sparsely represented across samples (baseMean < 1.7) and adjusted the P values with the Benjamini and Hochberg correlation method (68, 69).

Statistical analysis for seasonal dynamics of soil enzyme activities was analyzed using repeated measures analysis of variance (ANOVA) with Proc Mixed model. Treatment and year were used as fixed effects and block as a random effect. Data were log transformed as necessary to meet normality assumptions. Two-way ANOVA was used to analyze effect of treatment and time on functional gene abundances and alpha-diversity of prokaryotic communities. One-way ANOVA followed by Tukey’s HSD was performed to compare soil biological properties measured in Aug 2014. Pearson correlation coefficients were determined for the relationships between functional gene abundances and enzyme activities. ANOVA and Pearson correlation were carried out with the SAS 9.2 software (SAS Institute, Inc., Cary, NC, USA).

Data availability. Illumina sequence data can be accessed from NCBI BioProject PRJNA510146.

ACKNOWLEDGMENTS

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functional genes for nitrification and nitrogen mineralization. Utah State University, Logan, Utah USA. Some of the work including 16S Illumina sequencing and metagenomics was conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, and was supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

REFERENCES


Table 1. Soil N transformation rates and enzyme activities in Aug 2014 (Mean values, N=4, lowercase letters indicate significant differences among treatments, p<0.05).

<table>
<thead>
<tr>
<th>Rate or Activity</th>
<th>Control</th>
<th>AS100</th>
<th>AS200</th>
<th>Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMR (mg N kg(^{-1}) d(^{-1}))</td>
<td>1.32</td>
<td>1.83</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>GACR (mg N kg(^{-1}) d(^{-1}))</td>
<td>2.51</td>
<td>2.88</td>
<td>2.14</td>
<td>2.71</td>
</tr>
<tr>
<td>GNR (mg N kg(^{-1}) d(^{-1}))</td>
<td>0.50 b</td>
<td>0.67 ab</td>
<td>1.00 b</td>
<td>0.99 b</td>
</tr>
<tr>
<td>(\cdot) (mg N kg(^{-1}) d(^{-1}))</td>
<td>0.60 b</td>
<td>0.25 a</td>
<td>0.23 a</td>
<td>0.80 b</td>
</tr>
<tr>
<td>NMR (mg N kg(^{-1}) d(^{-1}))</td>
<td>0.30 a</td>
<td>0.46 b</td>
<td>0.50 b</td>
<td>0.52 b</td>
</tr>
<tr>
<td>NNR (mg N kg(^{-1}) d(^{-1}))</td>
<td>0.36 a</td>
<td>0.49 b</td>
<td>0.54 b</td>
<td>0.53 b</td>
</tr>
<tr>
<td>RR (mg CO(_2)-C kg(^{-1}) d(^{-1}))</td>
<td>7.21 a</td>
<td>7.81 a</td>
<td>7.32 a</td>
<td>11.45 b</td>
</tr>
<tr>
<td>Dehydrogenase (mg TPF kg(^{-1})h(^{-1}))</td>
<td>2.35 a</td>
<td>2.50 a</td>
<td>3.01 ab</td>
<td>3.82 b</td>
</tr>
<tr>
<td>Acid phosphomonoesterase (mg p-nitrophenol kg(^{-1})h(^{-1}))</td>
<td>39.90 a</td>
<td>42.95 a</td>
<td>51.76 ab</td>
<td>63.05 b</td>
</tr>
<tr>
<td>Alkaline phosphomonoesterase (mg p-nitrophenol kg(^{-1})h(^{-1}))</td>
<td>150.95 a</td>
<td>158.78 a</td>
<td>178.12 ab</td>
<td>189.50 b</td>
</tr>
</tbody>
</table>

Abbreviation: GMR-gross mineralization rate, GACR-gross ammonium consumption rate, GNR-gross nitrification rate, GNCR-gross nitrate consumption rate, NMR-net mineralization rate, NNR-net nitrification rate, RR-respiration rate.
Table 2. Real-time PCR amplification conditions, efficiencies, calibration standard and primers.

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Primer conc. μM</th>
<th>Size (bp)</th>
<th>Cycles</th>
<th>Denaturation 95°C</th>
<th>Annealing</th>
<th>Elongation at 72°C</th>
<th>Eff. (%)</th>
<th>Calibration standard</th>
<th>Primers and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>npr</td>
<td>0.75</td>
<td>233</td>
<td>40</td>
<td>20s</td>
<td>30s at 55°C</td>
<td>30s</td>
<td>108</td>
<td>Environmental DNA clone</td>
<td>Fp nprI, Rp nprII (70)</td>
</tr>
<tr>
<td>sub</td>
<td>0.75</td>
<td>319</td>
<td>40</td>
<td>20s</td>
<td>30s at 55°C</td>
<td>30s</td>
<td>88</td>
<td>Bacillus subtilis ATCC 6051</td>
<td>Fp subIa, RP subII (70)</td>
</tr>
<tr>
<td>chiA</td>
<td>0.5</td>
<td>417</td>
<td>40</td>
<td>60s</td>
<td>60s at 55°C</td>
<td>60s</td>
<td>110</td>
<td>Stenotrophomonas rhizophila ATCC BAA 473</td>
<td>GA1F, GA1R (71)</td>
</tr>
<tr>
<td>ureC</td>
<td>0.5</td>
<td>317</td>
<td>35</td>
<td>60s</td>
<td>60s at 60°C</td>
<td>120s</td>
<td>92</td>
<td>Pseudomonas chloroaphis O6</td>
<td>ureC1F, ureC2R (40)</td>
</tr>
</tbody>
</table>
Fig. 1 Soil enzyme activities across four N treatments in 2014. Error bars represent standard errors (n=4). Different letters above the bars indicate a significant difference among N treatments in a specific month (p<0.05), based on repeated measures ANOVA.

Fig. 2 (a) Relative abundance of *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, which are significantly changed by N treatment. Error bars represent standard errors (n=4). Lowercase letters indicate significant differences among N treatments in a specific year (p<0.05). (b) Nonmetric multidimensional scaling (NMDS) ordination (stress = 0.1) of weighted UniFrac distance for bacterial communities under four N treatment in both 2011 and 2014.

Fig. 3 Alpha diversity of soil bacterial communities across N treatments. Error bars represent standard errors (n=4). Lowercase letters indicate significant differences among treatments in a specific year (p<0.05).

Fig. 4 Log2-fold change in relative abundance of OTUs as compared with control treatment in both 2011 and 2014. Each circle represents a single OTU with adjusted p values < 0.1. Dash and dot lines indicate increases or decreases of 2x and 10x, respectively.

Fig. 5 (a) Relative abundance of the dominant phyla (>1%) for bacterial *ureC*. (b) Nonmetric multidimensional scaling (NMDS) ordination (stress = 0.09) of weighted UniFrac distance for bacterial *ureC* under four N treatment. (c) Relative abundance of the dominant phyla (>1%) for bacterial *chiA*. (d) Nonmetric multidimensional scaling (NMDS) ordination (stress = 0.05) of weighted UniFrac distance for bacterial *chiA* under four N treatment.

Fig. 6 Maximum likelihood tree of top 50 most abundant partial *ureC* OTUs. Different colors of branches and leaves indicate different phyla of *ureC* (check Fig. 5a for color coding). The heatmap presents the relative abundance of OTUs of *ureC* among four N treatments in Jun-2014 (mean values, n=4). Stars indicate a significant difference between control and AS treatments, while circles indicate a significant difference between control and compost treatment.

Fig. 7 Maximum likelihood tree of top 50 most abundant partial *chiA* OTUs. Different colors of branches and leaves indicate different phyla of *chiA*: *Actinobacteria* (purple), *Proteobacteria* (green), *Firmicutes* (blue), and unclassified (red). The heatmap presents the relative abundance of OTUs of *chiA* among four N treatments in Jun-2014 (mean values, n=4). Stars indicate a significant difference between control and AS treatments, while circles indicate a significant difference between control and compost treatment.
Table S1 Results of the repeated measures ANOVA of the effect of N sources and sampling time on soil enzyme activities.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Protease</th>
<th>β-glucosaminidase</th>
<th>Arginase</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Treatment</td>
<td>4.25 **</td>
<td>21.13 ***</td>
<td>26.57 ***</td>
<td>9.80 ***</td>
</tr>
<tr>
<td>Sampling time</td>
<td>138.37 ***</td>
<td>15.75 **</td>
<td>47.15 ***</td>
<td>32.10 ***</td>
</tr>
<tr>
<td>Treatment* time</td>
<td>6.90 ***</td>
<td>1.39 ns</td>
<td>2.76 ***</td>
<td>1.52 ns</td>
</tr>
</tbody>
</table>

Asterisks highlight significant P values (*** P < 0.01, ** P< 0.05)

Table S2 Results of Two-way ANOVA of the effect of N sources and sampling time on functional gene abundances.

<table>
<thead>
<tr>
<th>Factors</th>
<th>sub</th>
<th>npr</th>
<th>chiA</th>
<th>ureC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Treatment</td>
<td>2.41 0.09</td>
<td>0.96 0.43</td>
<td>0.94 0.44</td>
<td>1.31 0.29</td>
</tr>
<tr>
<td>Sampling time</td>
<td>0.29 0.59</td>
<td>0.05 0.82</td>
<td>2.40 0.14</td>
<td>0.05 0.83</td>
</tr>
<tr>
<td>Treatment* time</td>
<td>0.47 0.70</td>
<td>0.20 0.90</td>
<td>0.90 0.46</td>
<td>0.66 0.59</td>
</tr>
</tbody>
</table>

Asterisks highlight significant P values (*** P < 0.01, ** P< 0.05)

Table S3 Pearson Correlation Coefficients between enzyme activities and corresponding gene abundance.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Enzymes</th>
<th>r</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>sub</td>
<td>Protease</td>
<td>0.22</td>
<td>0.22</td>
<td>32</td>
</tr>
<tr>
<td>npr</td>
<td>Protease</td>
<td>0.17</td>
<td>0.35</td>
<td>32</td>
</tr>
<tr>
<td>chiA</td>
<td>β-glucosaminidase</td>
<td>-0.13</td>
<td>0.48</td>
<td>32</td>
</tr>
<tr>
<td>ureC</td>
<td>Urease</td>
<td>0.18</td>
<td>0.33</td>
<td>32</td>
</tr>
</tbody>
</table>
Table S4 Results of Two-way ANOVA of the effect of N treatment and year on the relative abundance of selected prokaryotic phyla (>1%).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Proteobacteria</th>
<th>Acidobacteria</th>
<th>Actinobacteria</th>
<th>Bacteroidetes</th>
<th>Gemmatimonadetes</th>
<th>Planctomycetes</th>
<th>Verrucomicrobia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.61</td>
<td>**</td>
<td>3.31</td>
<td>**</td>
<td>3.38</td>
<td>**</td>
<td>2.29</td>
</tr>
<tr>
<td>Year</td>
<td>3.42</td>
<td>ns</td>
<td>37.35</td>
<td>***</td>
<td>96.93</td>
<td>***</td>
<td>23.31</td>
</tr>
<tr>
<td>Treatment* Year</td>
<td>0.97</td>
<td>ns</td>
<td>0.38</td>
<td>ns</td>
<td>0.52</td>
<td>ns</td>
<td>1.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>Chloroflexi</th>
<th>Crenarchaeota</th>
<th>Nitrospirae</th>
<th>Firmicutes</th>
<th>Cyanobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.29</td>
<td>ns</td>
<td>2.03</td>
<td>ns</td>
<td>2.91</td>
</tr>
<tr>
<td>Year</td>
<td>1.39</td>
<td>ns</td>
<td>4.5</td>
<td>**</td>
<td>29.28</td>
</tr>
<tr>
<td>Treatment* Year</td>
<td>0.36</td>
<td>ns</td>
<td>1.93</td>
<td>ns</td>
<td>1.81</td>
</tr>
</tbody>
</table>

Asterisks highlight significant P values (** P < 0.05, *** P < 0.01)
Table S5: Xander assembly of N mineralization genes in soil metagenomes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>AS100</th>
<th>AS200</th>
<th>Compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>File size (GB)</td>
<td>33</td>
<td>39</td>
<td>43</td>
<td>76</td>
</tr>
<tr>
<td>npr OTUs number</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>sub OTUs number</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>chiA OTUs number</td>
<td>19</td>
<td>28</td>
<td>34</td>
<td>71</td>
</tr>
<tr>
<td>rocF OTUs number</td>
<td>78</td>
<td>93</td>
<td>103</td>
<td>236</td>
</tr>
<tr>
<td>ureC OTUs number</td>
<td>113</td>
<td>139</td>
<td>126</td>
<td>280</td>
</tr>
<tr>
<td>npr abundance</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>sub abundance</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>chiA abundance</td>
<td>0.06</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>rocF abundance</td>
<td>0.35</td>
<td>0.29</td>
<td>0.29</td>
<td>0.44</td>
</tr>
<tr>
<td>ureC abundance</td>
<td>0.34</td>
<td>0.32</td>
<td>0.26</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* The abundance for N mineralization genes was normalized to total abundance of the rplB gene.
Table S6 The relative abundance of top OTUs of N mineralization genes among four N treatments in Jun-2014 soil samples. Best match of top OTUs to the reference databases in Fungene.

<table>
<thead>
<tr>
<th>OTUs</th>
<th>Control (%)</th>
<th>AS100 (%)</th>
<th>AS200 (%)</th>
<th>Compost (%)</th>
<th>Accession number</th>
<th>Identity (%)</th>
<th>Phylum</th>
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</thead>
<tbody>
<tr>
<td>npr1</td>
<td>0.00</td>
<td>20.00</td>
<td>37.50</td>
<td>40.00</td>
<td>APH03530</td>
<td>55.80</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>npr2</td>
<td>20.00</td>
<td>20.00</td>
<td>0.00</td>
<td>0.00</td>
<td>EDL62708</td>
<td>54.22</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>npr3</td>
<td>40.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>APH03530</td>
<td>52.63</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>npr4</td>
<td>0.00</td>
<td>0.00</td>
<td>25.00</td>
<td>0.00</td>
<td>ESU33670</td>
<td>52.36</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>chiA1</td>
<td>17.39</td>
<td>6.25</td>
<td>13.04</td>
<td>4.30</td>
<td>BAK53887</td>
<td>47.66</td>
<td>Proteobacteria</td>
</tr>
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<td>6.25</td>
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<td>0.00</td>
<td>KQV05794</td>
<td>46.15</td>
<td>Actinobacteria</td>
</tr>
<tr>
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<td>0.00</td>
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<td>SEN14635</td>
<td>54.11</td>
<td>Firmicutes</td>
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<td>0.00</td>
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<td>2.15</td>
<td>KMN82353</td>
<td>64.89</td>
<td>Proteobacteria</td>
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<td>Actinobacteria</td>
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<td>0.00</td>
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<td>SFK38221</td>
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</tr>
<tr>
<td>rocF1</td>
<td>1.50</td>
<td>1.49</td>
<td>1.17</td>
<td>1.12</td>
<td>CDM65343</td>
<td>74.50</td>
<td>Acidobacteria</td>
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<tr>
<td>rocF2</td>
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<td>1.34</td>
<td>1.04</td>
<td>0.91</td>
<td>CDM65343</td>
<td>73.18</td>
<td>Acidobacteria</td>
</tr>
<tr>
<td>rocF3</td>
<td>1.12</td>
<td>0.00</td>
<td>0.52</td>
<td>0.91</td>
<td>OFW02708</td>
<td>75.42</td>
<td>Acidobacteria</td>
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<tr>
<td>rocF4</td>
<td>0.75</td>
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<td>0.78</td>
<td>0.49</td>
<td>OIN96242</td>
<td>78.72</td>
<td>Chloroflexi</td>
</tr>
<tr>
<td>rocF5</td>
<td>0.93</td>
<td>0.45</td>
<td>0.52</td>
<td>0.42</td>
<td>KPF94140</td>
<td>74.33</td>
<td>Proteobacteria</td>
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<tr>
<td>rocF6</td>
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<td>0.74</td>
<td>0.13</td>
<td>0.35</td>
<td>CDM65343</td>
<td>73.42</td>
<td>Acidobacteria</td>
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<tr>
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<td>0.30</td>
<td>0.26</td>
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<td>77.59</td>
<td>Candidatus Eisenbacteria</td>
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Fig. S1 Abundance of genes encoding subtilisin (sub), neutral metalloprotease (npr), chitinase (chiA), and urease (ureC) across four N treatments in 2014.
Fig. S2 Abundance of *ureC* gene copy numbers (log$_{10}$ transformed) across four N treatment from soils sampled in August of 2011 to 2014.
Fig. S3 Relative abundance of the dominant phyla (>1%) for bacterial communities.
Fig S4 Venn diagram of responsive OTUs among N fertilization treatments in 2014 (a). Venn diagram of responsive OTUs between 2011 and 2014 in compost treatment (b).
Fig S5 Relative abundance of the dominant phyla (>1%) for bacterial communities from steer-waster compost (CP) and compost treated soils in 2011 (a). Venn diagram of OTUs in steer-waster compost (CP) and compost treated soils (b).
Fig S6 Relative abundance of the dominant phyla (>1%) for bacterial ureolytic communities from steer-waster compost (CP-2013) and compost treated soils (a). Venn diagram of ureC OTUs in steer-waster compost (CP) and compost treated soils (b).
Fig S7 Relative abundance of the dominant phyla (>1%) for bacterial chitinolytic communities from steer-waster compost (CP-2013) and compost treated soils (a). Venn diagram of chiA OTUs in steer-waster compost (CP) and compost treated soils (b).