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 phosphomonoesterase 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 3.8×10^7, 2.7×10^5, 2.2×10^8, 9.7×10^7 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).

130 The abundance of *ureC* was repeatedly measured in Aug from 2011 to 2014.
131 Repeated measures ANOVA indicated that year ($p<0.01$), but not N treatment
132 ($p=0.43$), significantly changed the abundance of *ureC*, with an increase from $3.2 \times$
133 10^7 per gram of dry soils in 2011 to 1.08×10^8 per gram of dry soils in 2014 (Fig. S2).

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

148 N treatment but not year significantly influenced the alpha diversity of the
149 prokaryotic community (Fig. 3). Compost treatment increased Chao1, observed OTUs,
150 and Shannon diversity in both 2011 and 2014. Bacterial community structure as
151 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

(p=0.003) (Fig. 5b). 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.

196 Top 50 *chiA* OTUs were used for detailed phylogenetic analysis (Fig. 7), which
197 accounted for 26% of the total sequences. Most of these top OTUs were assigned to
198 *Streptomyces* (*eg.* OTU 127 and OTU 265), *Lentzea* (*eg.* OTU 70 and OTU 200), and
199 *Actinoplanes* (*eg.* OTU 642) family in the *Actinobacteria.* OTU 155, the most
200 abundant OTU, was closest to *Xanthomonadaceae*. Among the top 50 OTUs, 13
201 OTUs were significantly changed by N treatments. For example, OTU 642, OTU 52
202 and OTU 42 were increased by compost treatment, while OTU 155 and OTU 433
203 were significantly increased by AS treatments.

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

217 **Microbial community composition in steer-waste compost.** The bacterial

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

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

237 **DISCUSSION**

238 In this study, the application of the organic N fertilizer (steer-waste compost)

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

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

258 We found that the overall bacterial community significantly varied between 2011
259 and 2014. Soil microbial communities often show strong seasonal and inter-annual
260 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

283 ammonium-based fertilizers.

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

300 Chitin is one of the most abundant organic N polymers in soil environments (42).
301 Previous studies have found that bacterial chitinolytic communities were significantly
302 changed by chitin amendment (22). Since compost contains multiple organic N
303 polymers, we hypothesized that compost application would also shape the chitinolytic
304 community. We did observe that compost treatment significantly increased several top

305 *chiA* OTUs (Fig. 7). However, there was no significant difference in the chitinolytic
306 community between control and compost treatment after four-years repeated compost
307 application. This is partly due to the high variability of chitinolytic community
308 composition in compost treatment. Three of the replicates in the compost treatment
309 were highly separated from the control treatment, but one of the compost-treated plots
310 was closely clustered with controls. In addition, we found that only 1.5% *chiA* OTUs
311 in steer-waste compost were recovered in compost-treated soil, indicating chitinolytic
312 microorganisms in compost are less competitive than the indigenous chitinolytic
313 community and weakly survive in soil. Interestingly, AS treatments significantly
314 changed the chitinolytic community. Several top *chiA* OTUs were strongly enriched by
315 ammonium-based fertilizers (OTU 155 and OTU 433). These results indicate that the
316 chitinolytic community in our soil may be N-limited.

317 In our study, the application of compost increased soil enzyme activities, which is
318 consistent with observations showing that organic amendments significantly increase
319 enzyme activities (15, 43, 44). This is possibly due to stimulation of microbial growth
320 and related increases in the activity of the extracellular enzyme-organo complexes (45).
321 However, we found that the abundances of functional genes involved in N
322 mineralization were not affected by the N treatments, and that the abundance of
323 functional genes involved in N mineralization did not correlate with their corresponding
324 soil enzyme activities. The lack of correlation between the abundance of functional
325 genes and their corresponding enzyme activities may be attributed to several factors.
326 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 dillution 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 $^{15}NH_4^+$ solution (containing 1.69 mM $(^{15}NH_4)_2SO_4$ at 98 atom % 15N) or $^{15}NO_3^-$ solution (containing 3.33 mM $K^{15}NO_3$ 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 NH_4^+ or NO_3^- pool. Immediately following soil mixing, one subsample was harvested and extracted with 2 M KCl to determine NH_4^+ or NO_3^- 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 NH_4^+ or NO_2^- $+NO_3^-$ 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 CO_2 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^{TM} 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

414 measured the abundance of genes encoding subtilisin (*sub*), neutral metalloprotease
415 (*npr*), chitinase (*chiA*), and urease (*ureC*) for soil sampled in Jun and Aug 2014. Primers,
416 amplification conditions, efficiencies, and calibration standards are summarized in
417 Table 2**.** Standard curves were constructed with plasmids containing cloned gene
418 products from genomic DNA of bacterial isolates (*ureC*, *sub*, and *chiA*) or from
419 environmental DNA (*npr*), and R^2 values ranged from 0.990 to 0.999 for all genes
420 targeted. Duplicate assays for each gene and calibration standard series were measured
421 in a single run.

422 **Soil metagenome processing and gene targeted assembly.** Metagenomes were also
423 obtained from soils samples in Jun 2014. DNA samples from four replicates of each N
424 treatment were pooled with equal amount of DNA. DNA were then sequenced on the
425 Illumina HiSeq 2500 platform with 2 × 150 bp paired-end format at the Joint Genome
426 Institute. Quality-filtered metagenomes were downloaded and used for gene targeted
427 assembly (55). Five genes involved in N mineralization (*sub*, *npr*, *chiA*, *ureC*, and *rocF*)
428 and *rplB* were included for the assembly. For each gene of interest, seed sequences,
429 HMMs, and nucleotide and protein reference sequences were downloaded from
430 FunGene (56). Default assembly parameters were used and sequences were clustered
431 at 95% amino acid similarity. Representative sequence from each cluster was searched
432 against the reference gene database and the non-redundant database (nr) from NCBI
433 using BLAST (57). In overall, the top hit of these representative sequences to the
434 reference gene database had a similarity higher than 49% and a e-valuate higher than
435 1.5 E-46.

436 **Illumina sequencing and data analysis for *ureC* and *chiA*.** Sequencing of the *ureC*
437 and *chiA* amplicon libraries was accomplished for steer-waster compost used in 2013
438 and soils sampled in Jun 2014. The same *ureC* and *chiA* primers described above were
439 used for high-throughput sequencing. Linkers were added to primers for *ureC* and
440 *chiA* genes, while tags were added to separate different soils samples (58). The same
441 amount of soil DNA was used for *ureC* and *chiA* amplifications and then the PCR
442 products were further purified using size selection (Agencourt® Ampure® XP PCR
443 purification). Pooled purified products were sequenced on the Illumina MiSeq
444 platform (Illumina Inc., San Diego, CA, USA) using V3 chemistry (2x300 paired
445 end).

446 For a comparison, the bioinformatic analysis of *ureC* and *chiA* amplicons was
447 performed using the same Xander post-assembly processes based on the RDP Pipeline
448 (59). Raw reads were split based on the tags (soil samples), and then forward and
449 reverse reads were merged using the USEARCH workflow (60). High quality *ureC*
450 and *chiA* sequences were extracted from merged reads in each sample using the RDP
451 SeqFilters with a read Q score cutoff of 25. Chimera sequences were removed using
452 UCHIME (61) with the *ureC* and *chiA* nucleotide reference databases downloaded
453 from the FunGene (56). The obtained sequences were further processed using the
454 FrameBot tool (62) to fix frame shifts and translate DNA to protein. The remaining
455 quality-screened protein sequences in each sample were aligned based on *ureC* and
456 *chiA* hidden Markov models using HMMER3 (63). The aligned sequences from each
457 sample were merged together using the RDP AlignmentTools. Sequences were further
458 dereplicated and singletons were removed. Operational taxonomic units (OTU) were
459 clustered at 95% amino acid similarity using the RDP Clustering. The longest

460 sequence from each OTU was chosen as a representative sequence. To obtain the
461 phylum-level classification of representative sequences, the taxonomy from the
462 closest match to the protein reference downloaded from FunGene was used. If the
463 percent identity to the reference sequences was less than 80% (percent
464 alignment >90%), we defined the phylum as unclassified. A maximum-likelihood
465 phylogenetic tree was constructed from representative sequences using FastTree with
466 default parameters (64). OTU table and taxonomy file were further organized for
467 diversity analysis using R package phyloseq (65).

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

479 **Statistical analysis.** For microbial diversity analyses, all samples were randomly
480 rarefied to lowest reads per sample (27366 reads for 16S, 25000 reads for *ureC*, and
481 1278 reads for *chiA*) to compare differences between samples. Alpha-diversity and
482 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.

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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.

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

Rate or Activity	Treatment			
	Control	AS100	AS200	Compost
GMR (mg N kg^{-1} d^{-1})	1.32	1.83	1.40	1.40
GACR (mg N kg^{-1} d^{-1})	2.51	2.88	2.14	2.71
GNR (mg N kg^{-1} d^{-1})	0.50 a	0.67 ab	1.00 b	0.99 b
(mg N kg^{-1} d^{-1})	0.60 b	0.25 a	0.23 a	0.80 b
NMR (mg N kg^{-1} d^{-1})	0.30 a	0.46 b	0.50 b	0.52 b
NNR (mg N kg^{-1} d^{-1})	0.36 a	0.49 b	0.54 b	0.53 b
RR (mg CO_2-C kg^{-1} d^{-1})	7.21 a	7.81 a	7.32 a	11.45 b
Dehydrogenase (mg TPF $kg^{-1}h^{-1}$)	2.35 a	2.50 a	3.01 ab	3.82 b
Acid phosphomonoesterase (mg p-nitrophenol kg^{-1} h^{-1})	39.90 a	42.95 a	51.76 ab	63.05 b
Alkaline phosphomonoesterase (mg p-nitrophenol kg^{-1} h^{-1})	150.95 a	158.78 a	178.12 ab	189.50 b

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.

734 Table 2. Real-time PCR amplification conditions, efficiencies, calibration standard and primers.

735

Target genes	Primer conc. µM	Size (bp)	Cycles	Denaturation 95°C	Annealing	Elongation at 72°C	Eff. (%)	Calibration standard	Primers and references
npr	0.75	233	40	20s	30s at 55°C	30s	108	Environmental DNA clone	Fp nprI, Rp nprII (70)
sub	0.75	319	40	20s	30s at 55°C	30s	88	*Bacillus subtilis* ATCC 6051	Fp subIa, RP subII (70)
chiA	0.5	417	40	60s	60s at 55°C	60s	110	*Stenotrophomonas rhizophila* ATCC BAA 473	GA1F, GA1R (71)
ureC	0.5	317	35	60s	60s at 60°C	120s	92	*Pseudomonas chloroaphis* O6	ureC1F, ureC2R (40)

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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.

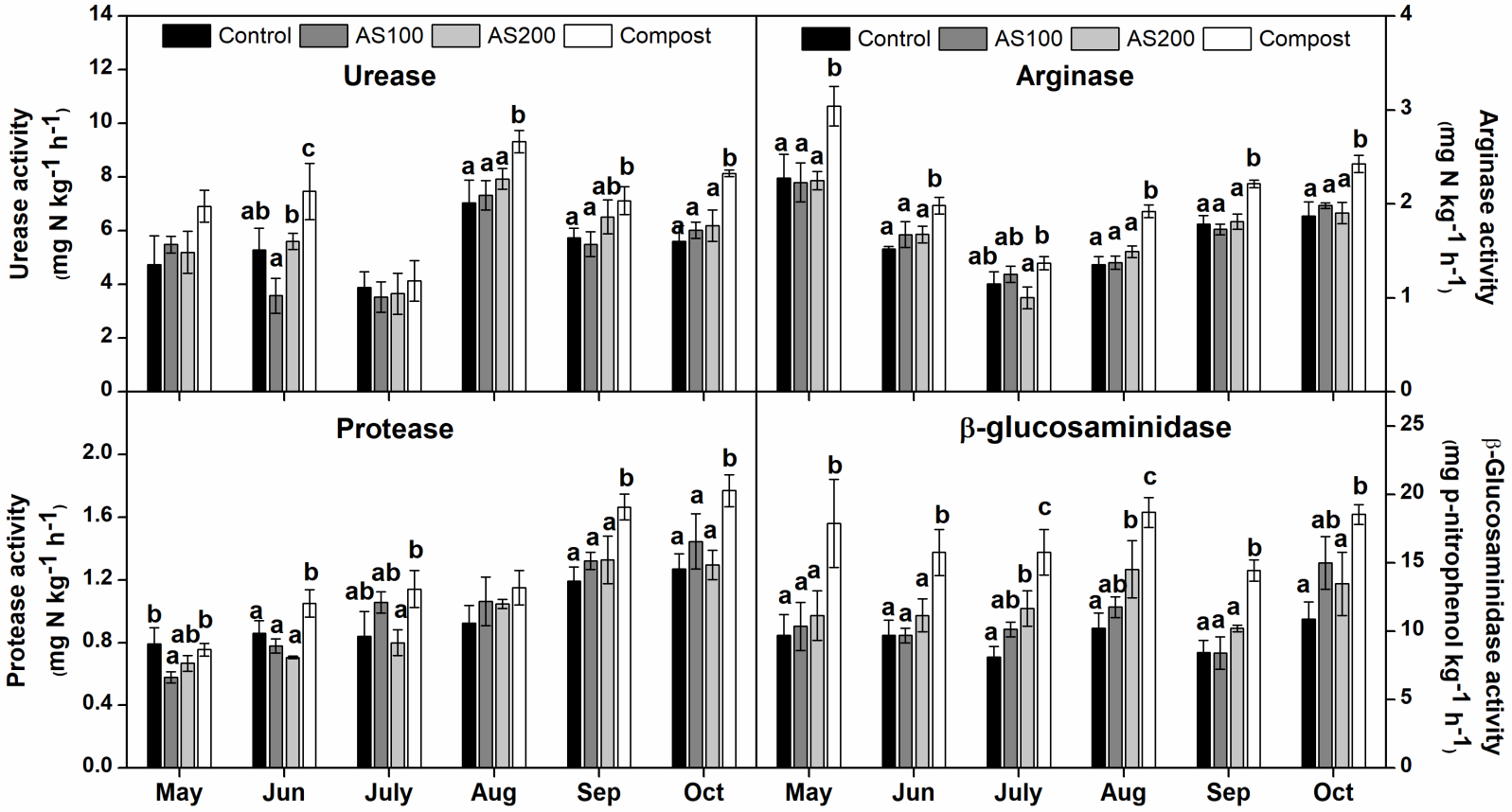
Fig. 3 Alpha diversity of soil bacterial communities across N treatments. Error bars represent standard errors (n=4). Lowercase letters indicate significant differences among treatments in a specific year ($p<0.05$).

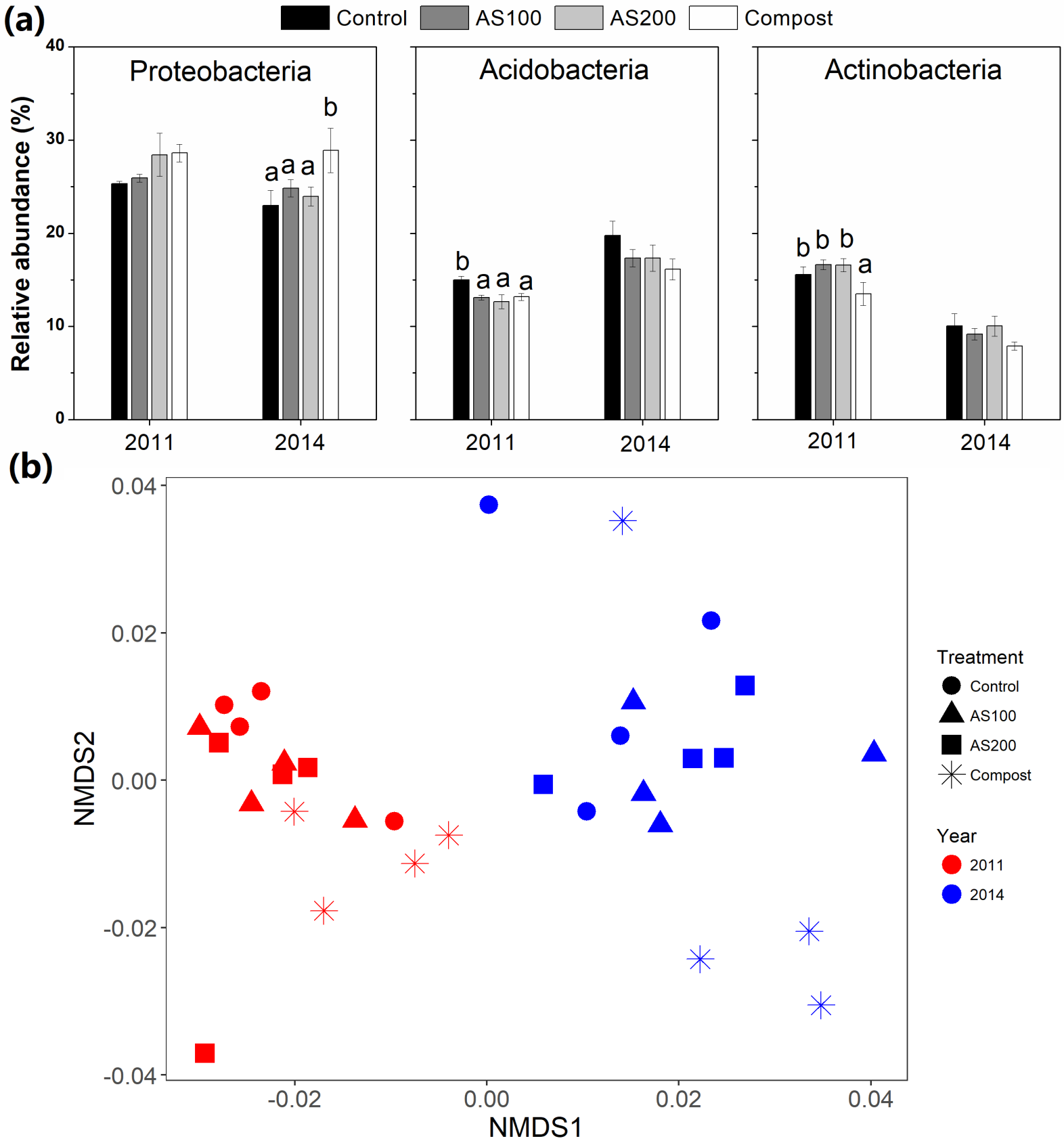
Fig.4 Log_2-fold change in relative abundance of OTUs as compared with control treatment in both 2011 and 2014. Each circle represents a single OTU with adjusted p values < 0.1. Dash and dot lines indicate increases or decreases of 2x and 10x, respectively.

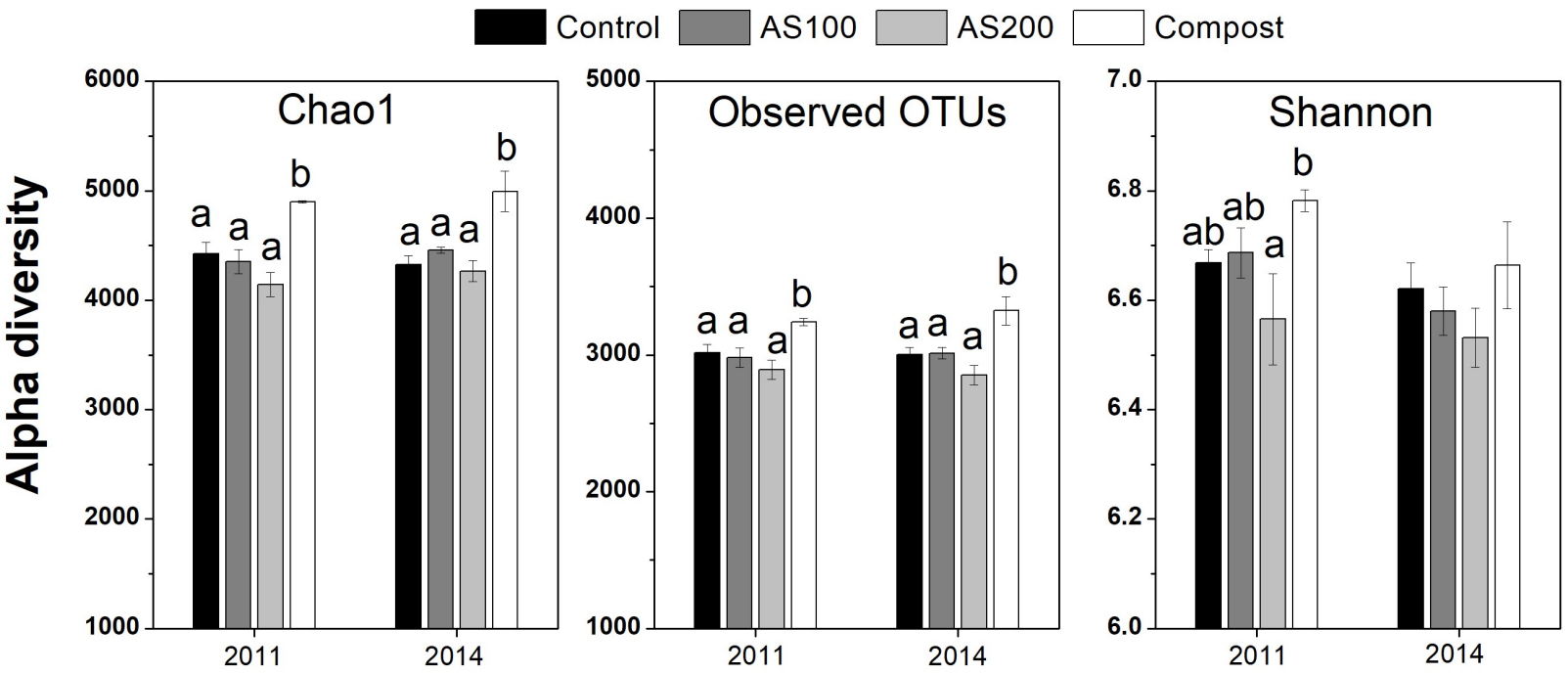
Fig.5 (**a**) Relative abundance of the dominant phyla (>1%) for bacterial *ureC*. (**b**) Nonmetric multidimensional scaling (NMDS) ordination (stress = 0.09) of weighted UniFrac distance for bacterial *ureC* under four N treatment. (**c**) Relative abundance of the dominant phyla (>1%) for bacterial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

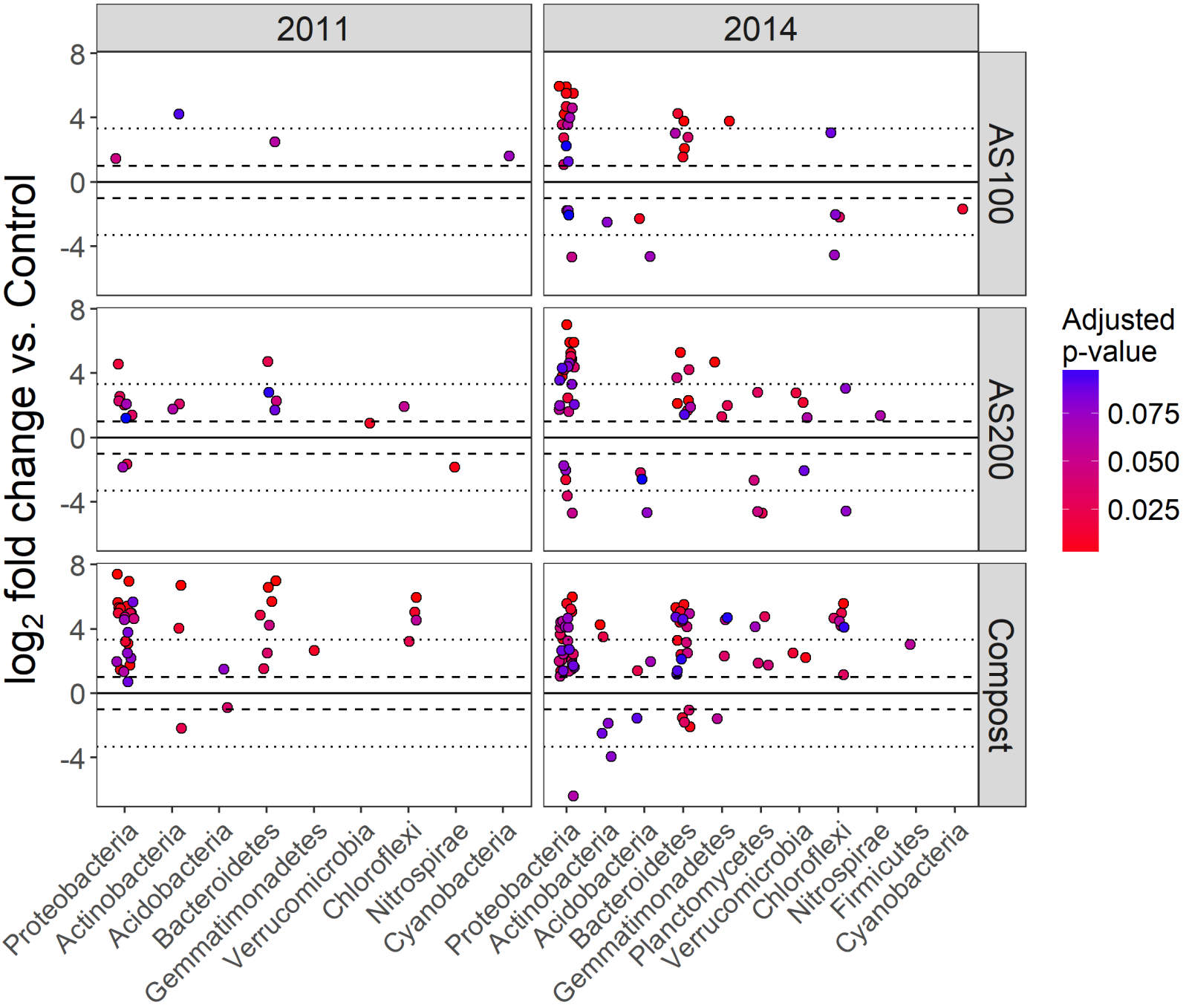
Fig. 6 Maximum likelihood tree of top 50 most abundant partial *ureC* OTUs. Different colors of branches and leaves indicate different phyla of *ureC* (check Fig. 5a for color coding). The heatmap presents the relative abundance of OTUs of *ureC* among four N treatments in Jun-2014 (mean values, n=4).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

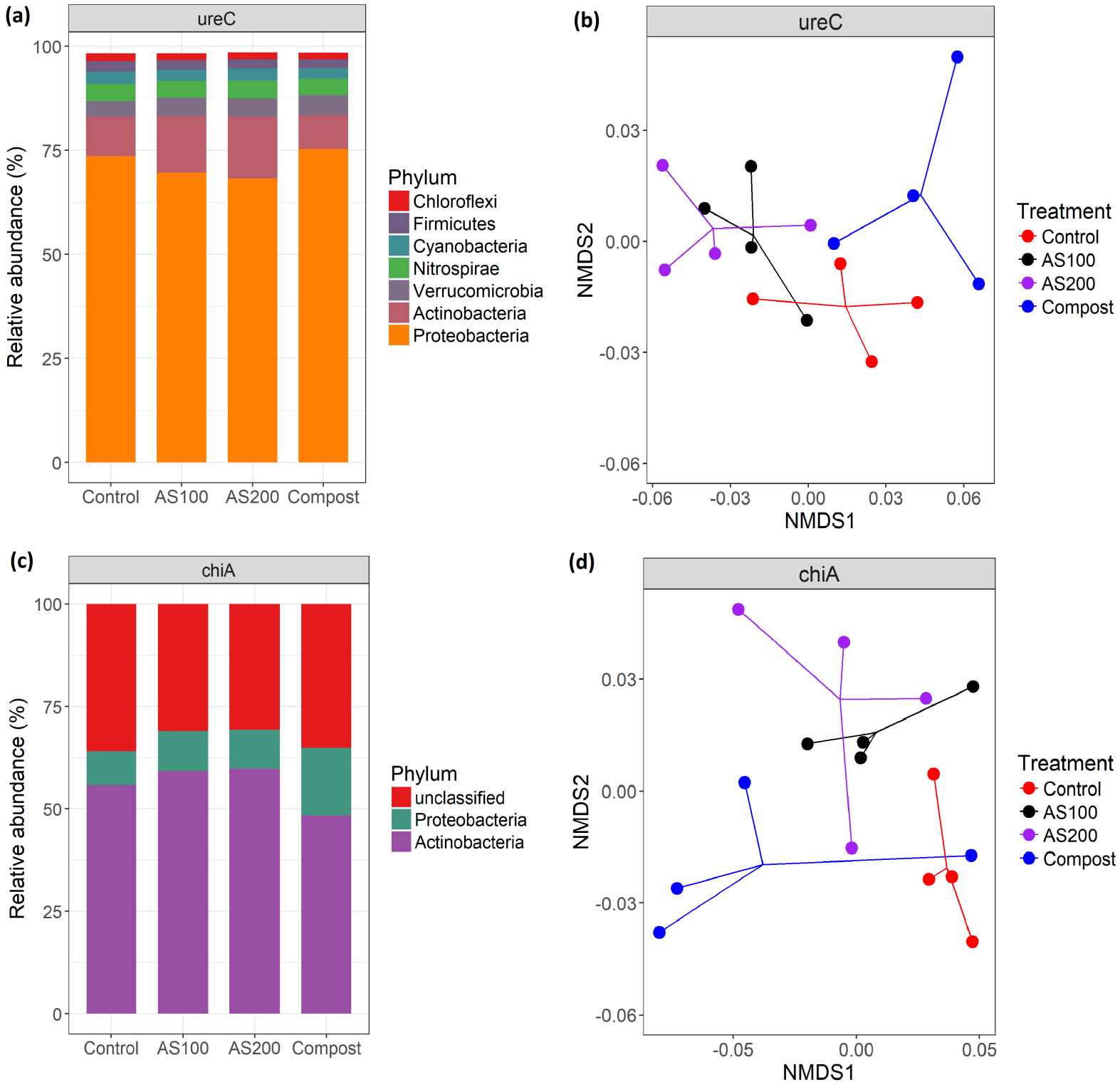
Fig. 7 Maximum likelihood tree of top 50 most abundant partial *chiA* OTUs. Different colors of branches and leaves indicate different phyla of *chiA*: *Actinobacteria* (purple), *Proteobacteria* (green), *Firmicutes* (blue), and unclassified (red). The heatmap presents the relative abundance of OTUs 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.

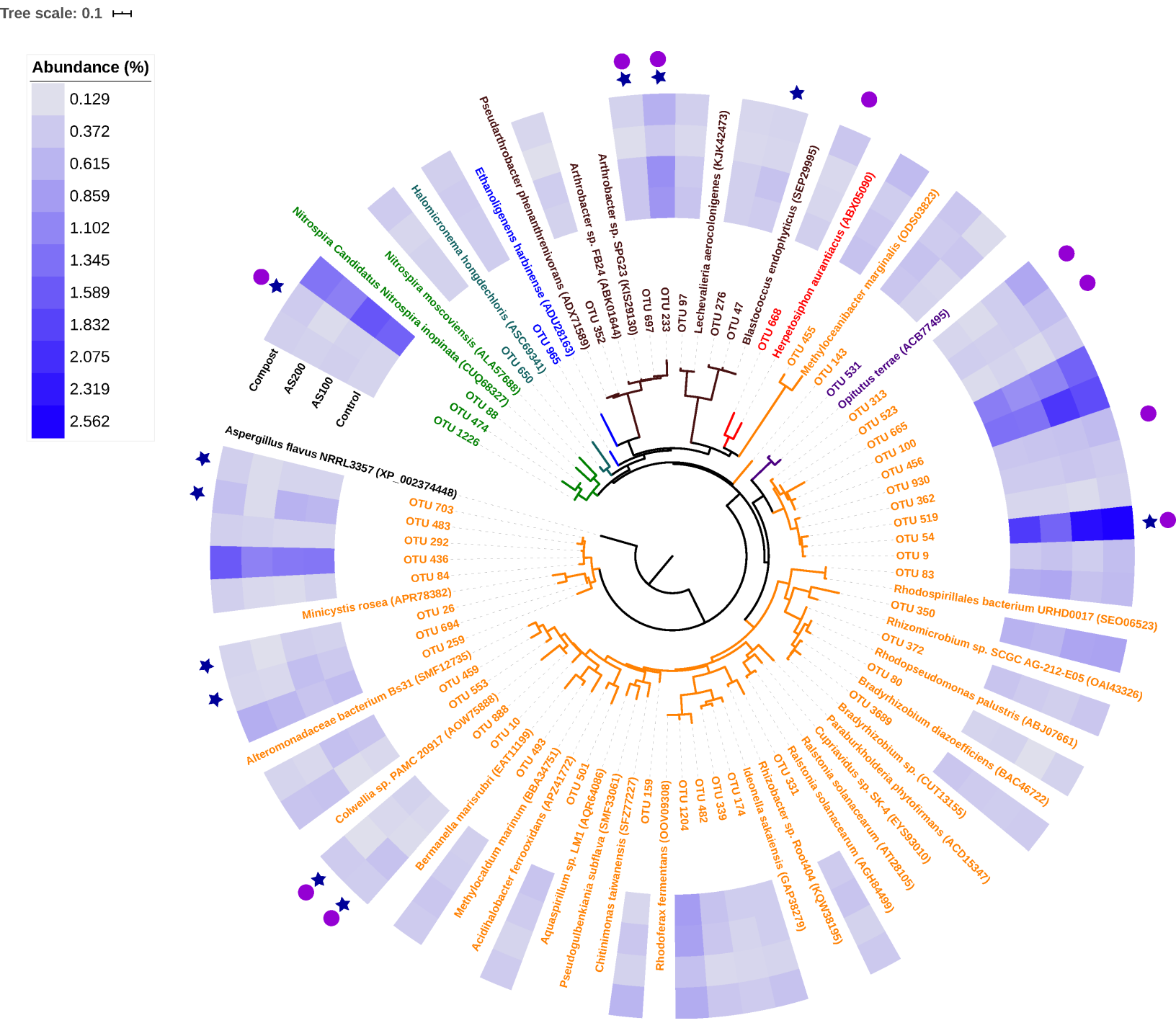

Control
AS100
AS200
Compost
Urease
Arginase
Protease
β-glucosaminidase
Urease activity (mg N kg-1 h-1)
Arginase activity (mg N kg-1 h-1)
Protease activity (mg N kg-1 h-1)
β-Glucosaminidase activity (mg p-nitrophenol kg-1 h-1)
May
Jun
July
Aug
Sep
Oct


(a)
Control
AS100
AS200
Compost
Proteobacteria
Acidobacteria
Actinobacteria
Relative abundance (%)
0
10
20
30
40
a a a b
b a a a
b b b a
2011
2014
(b)
NMDS1
NMDS2
0.04
0.02
0.00
-0.02
-0.04
Treatment
Control
AS100
AS200
Compost
Year
2011
2014


Control
AS100
AS200
Compost
Alpha diversity
Chao1
Observed OTUs
Shannon
6000
5000
4000
3000
2000
1000
7.0
6.8
6.6
6.4
6.2
6.0
a a a b
a a a b
a a a b
ab ab a b
2011
2014


2011
2014
AS100
AS200
Compost
log2 fold change vs. Control
8
4
0
-4
Adjusted p-value
0.075
0.050
0.025
Proteobacteria
Actinobacteria
Acidobacteria
Bacteroidetes
Gemmatimonadetes
Verrucomicrobia
Chloroflexi
Nitrospirae
Cyanobacteria
Planctomycetes
Firmicutes


(a)
ureC
Relative abundance (%)
100
75
50
25
0
Control
AS100
AS200
Compost
Phylum
Chloroflexi
Firmicutes
Cyanobacteria
Nitrospirae
Verrucomicrobia
Actinobacteria
Proteobacteria
(b)
ureC
NMDS2
0.03
0.00
-0.03
-0.06
-0.06
-0.03
0.00
0.03
0.06
NMDS1
Treatment
Control
AS100
AS200
Compost
(c)
chiA
Relative abundance (%)
100
75
50
25
0
Control
AS100
AS200
Compost
Phylum
unclassified
Proteobacteria
Actinobacteria
(d)
chiA
NMDS2
0.03
0.00
-0.03
-0.06
-0.05
0.00
0.05
NMDS1
Treatment
Control
AS100
AS200
Compost


Tree scale: 0.1
Abundance (%)
0.129
0.372
0.615
0.859
1.102
1.345
1.589
1.832
2.075
2.319
2.562
Compost
AS200
AS100
Control
Nitrospira Candidatus Nitrospira inopinata (CUQ68327)
Nitrospira moscoviensis (ALA57688)
Halomicronema hongdechloris (ASC69341)
Ethanoligenens harbinense (ADU28163)
Pseudarthrobacter phenanthrenivorans (ADX71589)
Arthrobacter sp. FB24 (ABK01644)
Arthrobacter sp. SPG23 (KIS29130)
Lechevalieria aerocolonigenes (KJK42473)
Blastococcus endophyticus (SEP29995)
Herpetosiphon auranticus (ABX05090)
Methyloceanibacter marginalis (ODS03823)
Opitutus terrae (ACB77495)
Rhodospirillales bacterium URHD0017 (SEO06523)
Rhizomicrobium sp. SCGC AG-212-E05 (OAI43326)
Rhodopseudomonas palustris (ABJ07661)
Bradyrhizobium diazoefficiens (BAC46722)
Bradyrhizobium sp. (CUT13155)
Paraburkholderia phytofirmans (ACD15347)
Cupriavidus sp. SK-4 (EYS93010)
Ralstonia solanacearum (AIT28105)
Ralstonia solanacearum (AGH84499)
Rhizobacter sp. Root404 (KQW98195)
Ideonella sakaiensis (GAP38279)
Rhodoferax fermentans (OOV09308)
Chitinimonas taiwanensis (SFZ77227)
Pseudogulbenkiania subflava (SMF33061)
Aquaspirillum sp. LM1 (AQR64086)
Acidihalobacter ferrooxidans (APZ41772)
Methylocaldum marinum (BBA34751)
Bermanella marisrubri (EAT11199)
Colwellia sp. PAMC 20917 (AOW75888)
Alteromonadaceae bacterium Bs31 (SMF12735)
Minicystis rosea (APR78382)
Aspergillus flavus NRRL3357 (XP_002374448)
OTU 1226
OTU 474
OTU 88
OTU 650
OTU 965
OTU 352
OTU 697
OTU 233
OTU 97
OTU 276
OTU 47
OTU 668
OTU 455
OTU 143
OTU 531
OTU 313
OTU 523
OTU 665
OTU 100
OTU 456
OTU 930
OTU 362
OTU 519
OTU 54
OTU 9
OTU 83
OTU 350
OTU 372
OTU 80
OTU 3689
OTU 331
OTU 174
OTU 339
OTU 482
OTU 1204
OTU 159
OTU 501
OTU 493
OTU 10
OTU 888
OTU 553
OTU 459
OTU 259
OTU 694
OTU 26
OTU 84
OTU 436
OTU 292
OTU 483
OTU 703

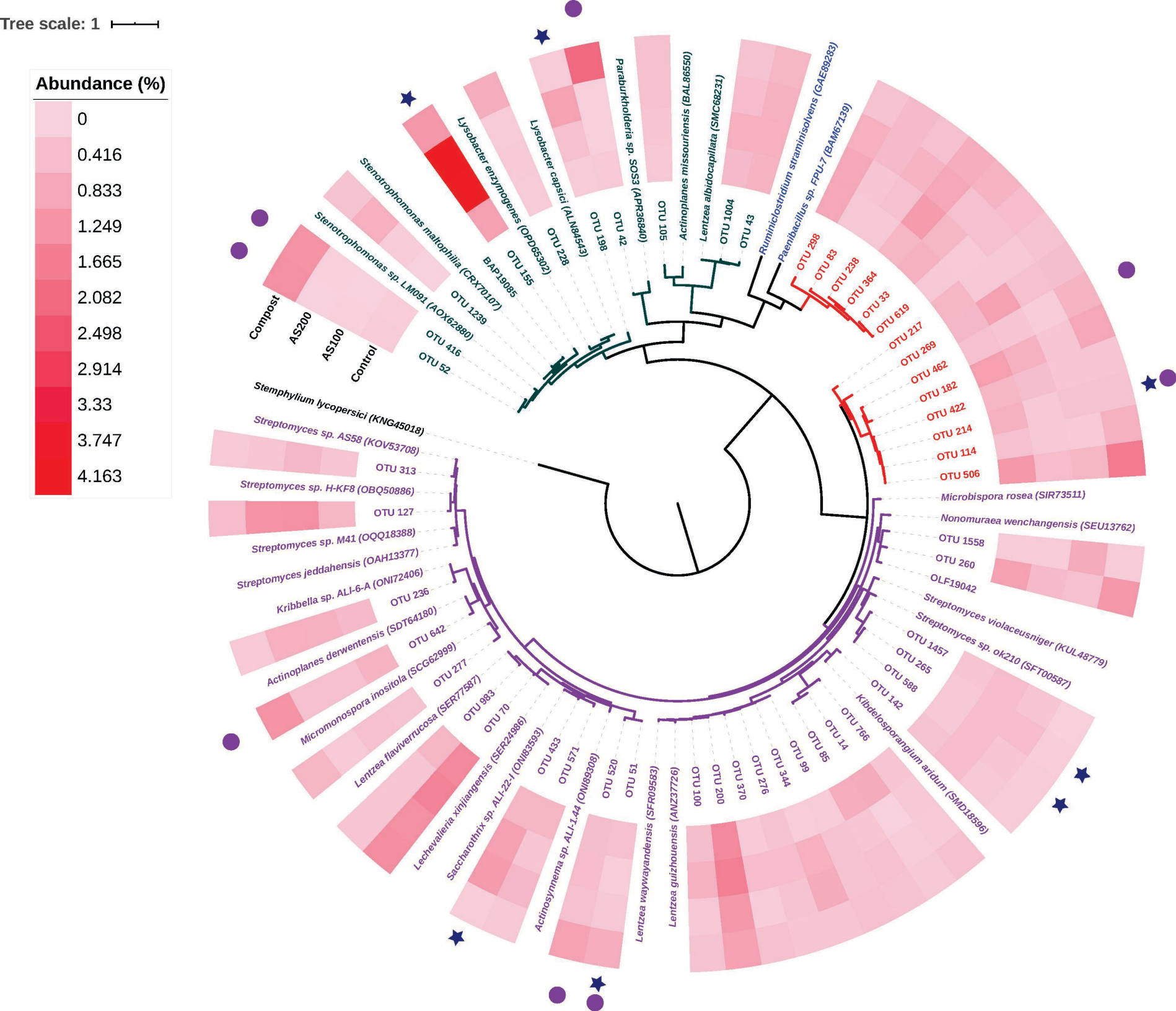

Tree scale: 1
Abundance (%)
0
0.416
0.833
1.249
1.665
2.082
2.498
2.914
3.33
3.747
4.163
Compost
AS200
AS100
Control
Stenotrophomonas maltophilia (CRX70107)
Stenotrophomonas sp. LM091 (AOX62880)
Lysobacter enzymogenes (OPD65302)
Lysobacter capsici (ALN84543)
Paraburkholderia sp. SOS3 (APR36940)
Actinoplanes missouriensis (BAL86550)
Lentzea albidocapillata (SMC68231)
Ruminiclostridium straminisolvens (GAE89283)
Paenibacillus sp. FPU-7 (BAM67139)
OTU 1239
OTU 416
OTU 52
BAP19085
OTU 155
OTU 228
OTU 198
OTU 42
OTU 105
OTU 1004
OTU 43
OTU 298
OTU 83
OTU 238
OTU 364
OTU 33
OTU 619
OTU 217
OTU 269
OTU 462
OTU 182
OTU 422
OTU 214
OTU 114
OTU 506
Stemphylium lycopersici (KNG45018)
Streptomyces sp. AS58 (KOV53708)
OTU 313
Streptomyces sp. H-KF8 (OBQ50886)
OTU 127
Streptomyces sp. M41 (OQQ18388)
Streptomyces jeddahensis (OAH13377)
Kribbella sp. ALI-6-A (ONI72406)
OTU 236
Actinoplanes derwentensis (SDT64180)
OTU 642
Micromonospora inositola (SCG62999)
OTU 277
Lentzea flaviverrucosa (SER77587)
OTU 983
OTU 70
Lechevalieria xinjiangensis (SER24986)
Saccharothrix sp. ALI-22-I (ONI83593)
OTU 433
OTU 571
Actinosynnema sp. ALI-1.44 (ONI89308)
OTU 520
OTU 51
Lentzea waywayandensis (SFR09583)
Lentzea guizhouensis (ANZ37726)
OTU 100
OTU 200
OTU 370
OTU 276
OTU 344
OTU 99
OTU 85
OTU 14
OTU 766
Kibdelosporangium aridum (SMD18596)
OTU 142
OTU 588
OTU 265
OTU 1457
Streptomyces sp. ok210 (SFT00587)
Streptomyces violaceusniger (KUL48779)
OLF19042
OTU 260
OTU 1558
Nonomuraea wenchangensis (SEU13762)
Microbispora rosea (SIR73511)

Table S1 Results of the repeated measures ANOVA of the effect of N sources and sampling time on soil enzyme activities.

Factors	Protease		β-glucosaminidase		Arginase		Urease	
	F	P	F	P	F	P	F	P
Treatment	4.25	**	21.13	***	26.57	***	9.80	***
Sampling time	138.37	***	15.75	***	47.15	***	32.10	***
Treatment* time	6.90	***	1.39	ns	2.76	***	1.52	ns

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.

Factors	*sub*		*npr*		*chiA*		*ureC*	
	F	P	F	P	F	P	F	P
Treatment	2.41	0.09	0.96	0.43	0.94	0.44	1.31	0.29
Sampling time	0.29	0.59	0.05	0.82	2.40	0.14	0.05	0.83
Treatment* time	0.47	0.70	0.20	0.90	0.90	0.46	0.66	0.59

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

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

Genes	Enzymes	r	p	n
sub	Protease	0.22	0.22	32
npr	Protease	0.17	0.35	32
chiA	β-glucosaminidase	-0.13	0.48	32
ureC	Urease	0.18	0.33	32

Table S4 Results of Two-way ANOVA of the effect of N treatment and year on the relative abundance of selected prokaryotic phyla (>1%).

Factors	Proteobacteria		Acidobacteria		Actinobacteria		Bacteroidetes		Gemmatimonadetes		Planctomycetes		Verrucomicrobia	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Treatment	3.61	**	3.31	**	3.38	**	2.29	ns	1.58	ns	1.09	ns	0.31	ns
Year	3.42	ns	37.35	***	96.93	***	23.31	***	15.14	***	4.26	ns	97.63	***
Treatment* Year	0.97	ns	0.38	ns	0.52	ns	1.17	ns	1.19	ns	0.72	ns	0.73	ns

Factors	Chloroflexi		Crenarchaeota		Nitrospirae		Firmicutes		Cyanobacteria	
	F	P	F	P	F	P	F	P	F	P
Treatment	0.29	ns	2.03	ns	2.91	ns	0.75	ns	0.36	ns
Year	1.39	ns	4.5	**	29.28	***	32.22	***	1.59	ns
Treatment* Year	0.36	ns	1.93	ns	1.81	ns	0.75	ns	0.9	ns

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

Table S5 Xander assembly of N mineralization genes in soil metagenomes.

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

[a] 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.

OTUs	Control (%)	AS100 (%)	AS200 (%)	Compost (%)	Accession number	Identity (%)	Phylum
npr1	0.00	20.00	37.50	40.00	APH03530	55.80	Firmicutes
npr2	20.00	20.00	0.00	0.00	EDL62708	54.22	Firmicutes
npr3	40.00	0.00	0.00	0.00	APH03530	52.63	Firmicutes
npr4	0.00	0.00	25.00	0.00	ESU33670	52.36	Firmicutes
chiA1	17.39	6.25	13.04	4.30	BAK53887	47.66	Proteobacteria
chiA2	8.70	6.25	0.00	4.30	KQV05794	46.15	Actinobacteria
chiA3	0.00	0.00	0.00	3.23	SEN14635	54.11	Firmicutes
chiA4	0.00	0.00	2.17	2.15	KMN82353	64.89	Proteobacteria
chiA5	0.00	0.00	0.00	2.15	SDP35911	99.07	Actinobacteria
chiA6	0.00	0.00	0.00	2.15	KLI99810	74.03	Proteobacteria
chiA7	0.00	0.00	0.00	2.15	CBG90311	72.63	Proteobacteria
chiA8	0.00	0.00	0.00	2.15	EGL13042	50.69	Firmicutes
chiA9	0.00	0.00	0.00	2.15	APR36840	79.29	Proteobacteria
chiA10	0.00	0.00	0.00	2.15	SFK38221	57.31	Proteobacteria
rocF1	1.50	1.49	1.17	1.12	CDM65343	74.50	Acidobacteria
rocF2	1.68	1.34	1.04	0.91	CDM65343	73.18	Acidobacteria
rocF3	1.12	0.00	0.52	0.91	OFW02708	75.42	Acidobacteria
rocF4	0.75	0.45	0.78	0.49	OIN96242	78.72	Chloroflexi
rocF5	0.93	0.45	0.52	0.42	KPF94140	74.33	Proteobacteria
rocF6	0.75	0.74	0.13	0.35	CDM65343	73.42	Acidobacteria
rocF7	0.75	0.30	0.26	0.42	OGF13763	77.59	Candidatus Eisenbacteria
rocF8	0.56	0.30	0.13	0.49	OFW02708	83.72	Acidobacteria
rocF9	0.56	0.45	0.39	0.28	OHB25893	64.68	Proteobacteria
rocF10	0.56	0.59	0.39	0.21	OLD24873	67.68	Acidobacteria
ureC1	3.76	2.98	5.16	1.40	OLC92811	83.83	Thaumarchaeota

ureC2	0.00	2.98	1.29	0.84	AFU57556	84.94	Thaumarchaeota
ureC3	0.00	1.19	1.29	1.40	CUQ68327	84.32	Nitrospirae
ureC4	0.00	0.00	0.00	1.96	OLC28686	90.49	Acidobacteria
ureC5	0.00	1.19	1.29	0.84	AFU57556	91.77	Thaumarchaeota
ureC6	1.50	0.60	1.29	0.56	ODT89782	80.66	Proteobacteria
ureC7	0.00	0.60	0.00	1.40	ALA57688	95.79	Nitrospirae
ureC8	1.50	0.00	0.65	0.84	KZM51172	80.18	Proteobacteria
ureC9	0.00	1.19	1.29	0.56	AFU57556	92.22	Thaumarchaeota
ureC10	3.01	0.60	0.00	0.28	ALA66374	84.75	Nitrospirae

Fig. S1 Abundance of genes encoding subtilisin (*sub*), neutral metalloprotease (*npr*), chitinase (*chiA*), and urease (*ureC*) across four N treatments in 2014.

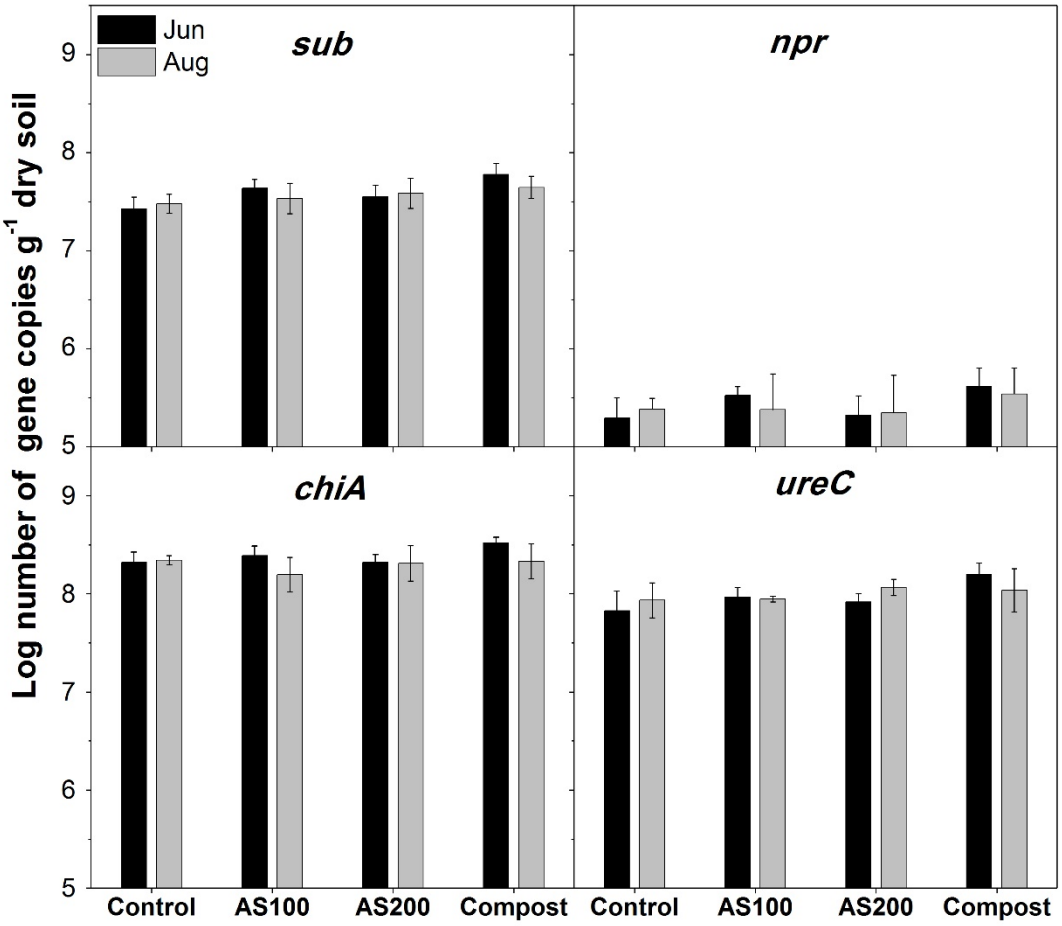


Fig. S2 Abundance of *ureC*.gene copy numbers (log_{10} transformed) across four N treatment from soils sampled in August of 2011 to 2014.

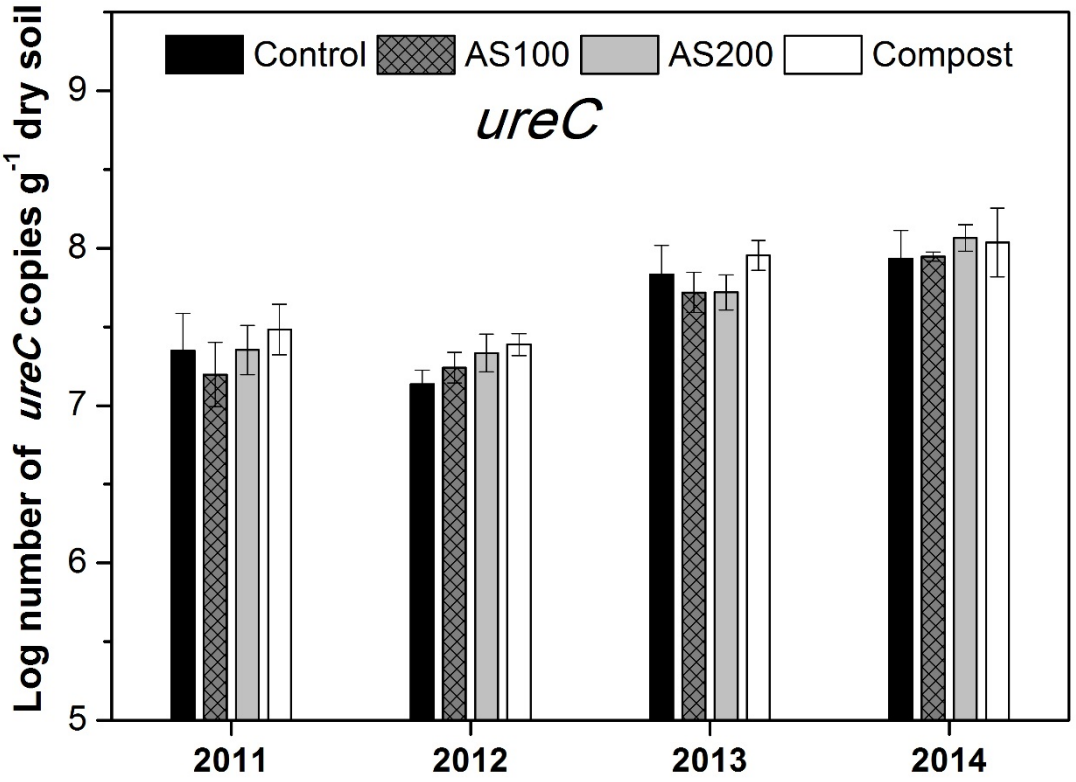


Fig. S3 Relative abundance of the dominant phyla (>1%) for bacterial communities.

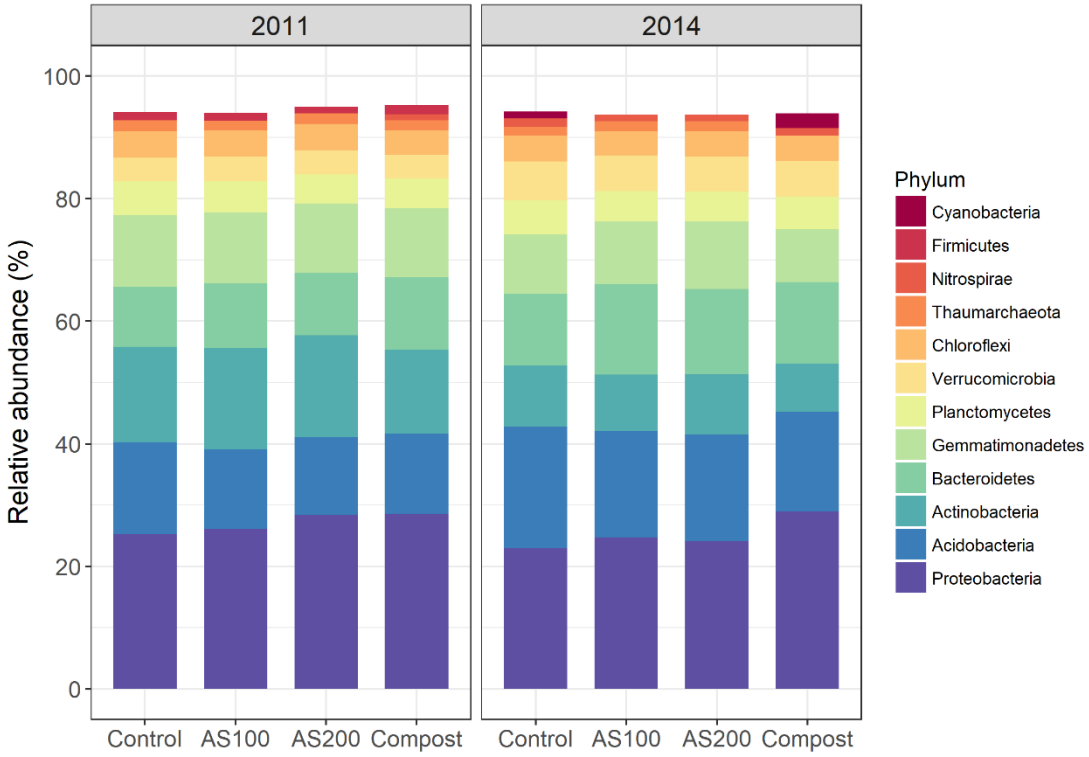
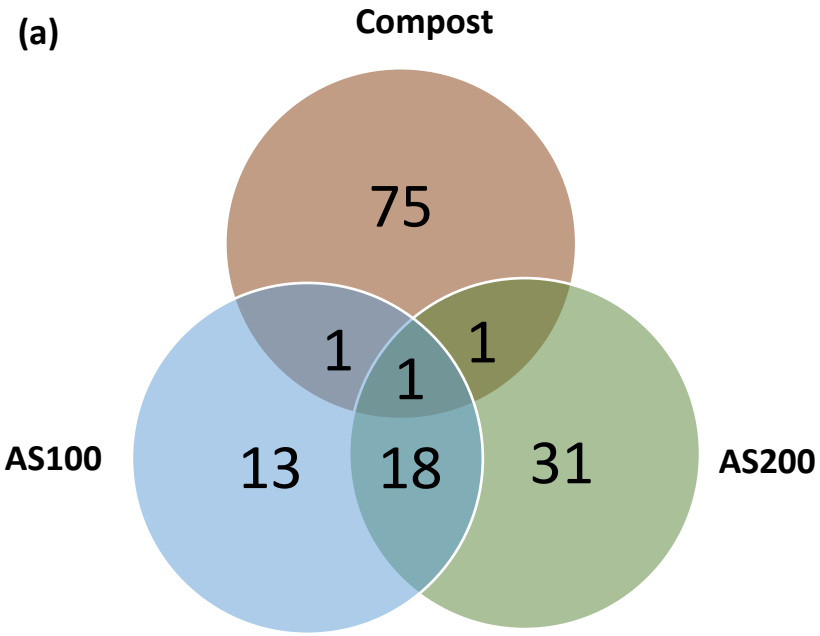


Fig S4 Venn diagram of responsive OTUs among N fertilization treatments in 2014 (a). Venn diagram of responsive OTUs between 2011 and 2014 in compost treatment (b).



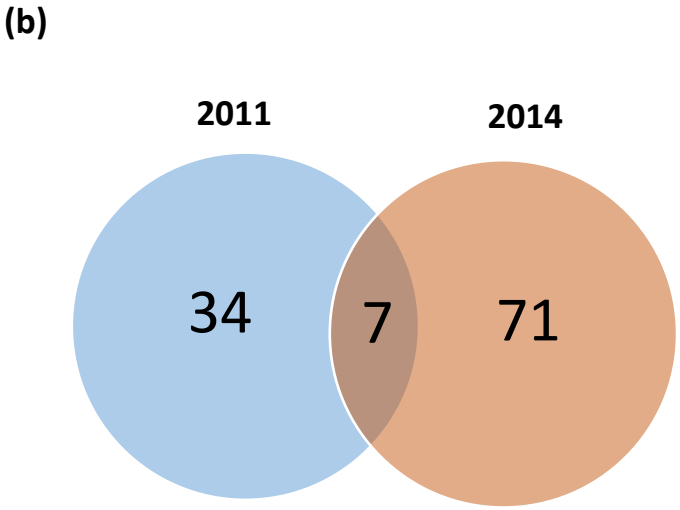
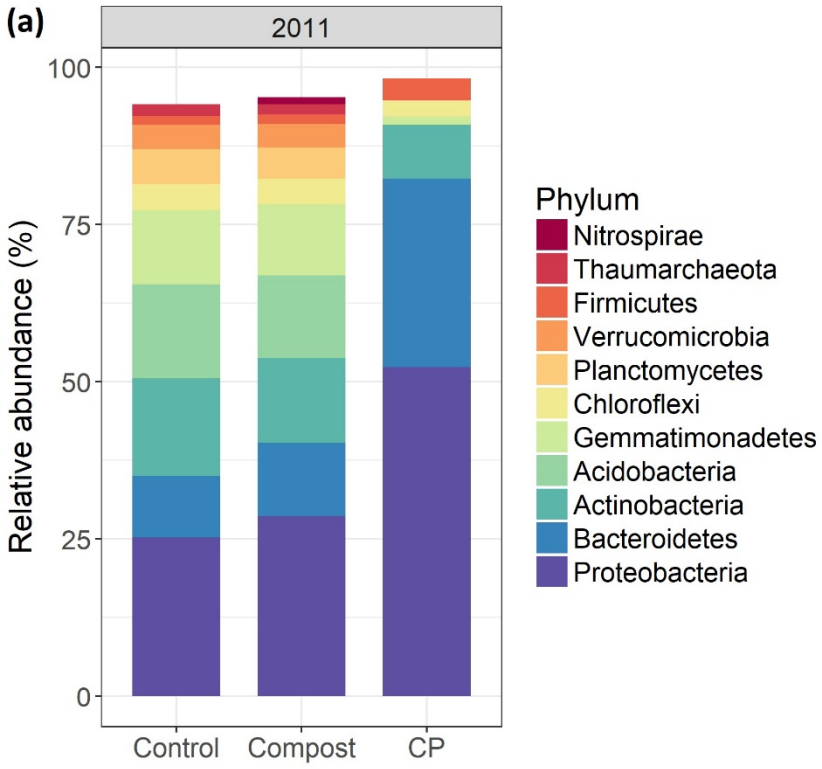


Fig S5 Relative abundance of the dominant phyla (>1%) for bacterial communities from steer-waster compost (CP) and compost treated soils in 2011(**a**). Venn diagram of OTUs in steer-waster compost (CP) and compost treated soils (**b**).



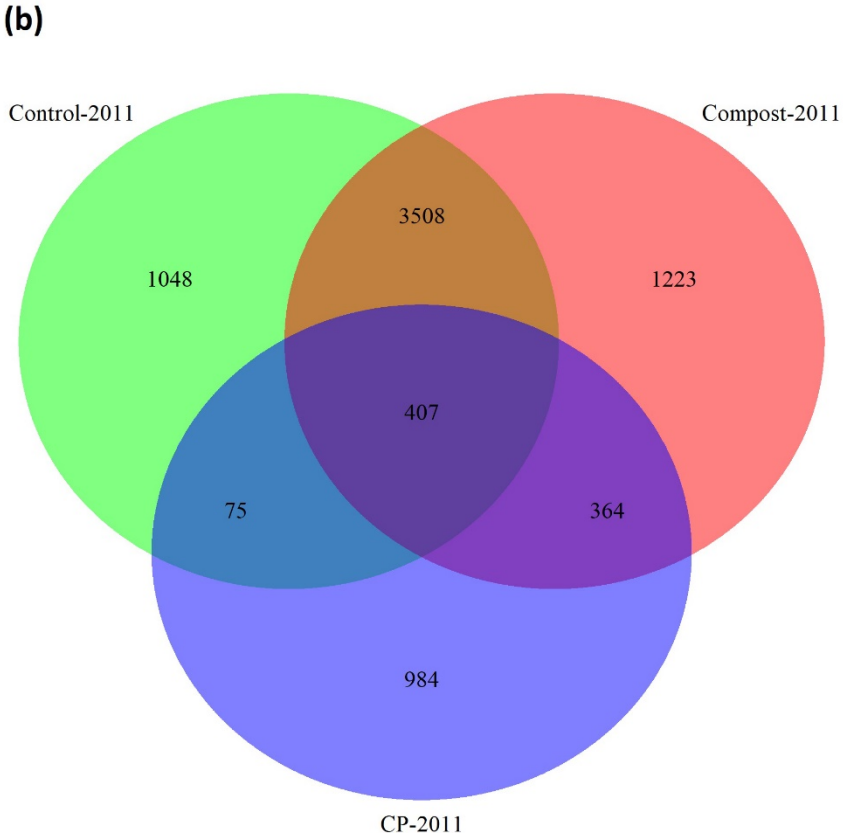
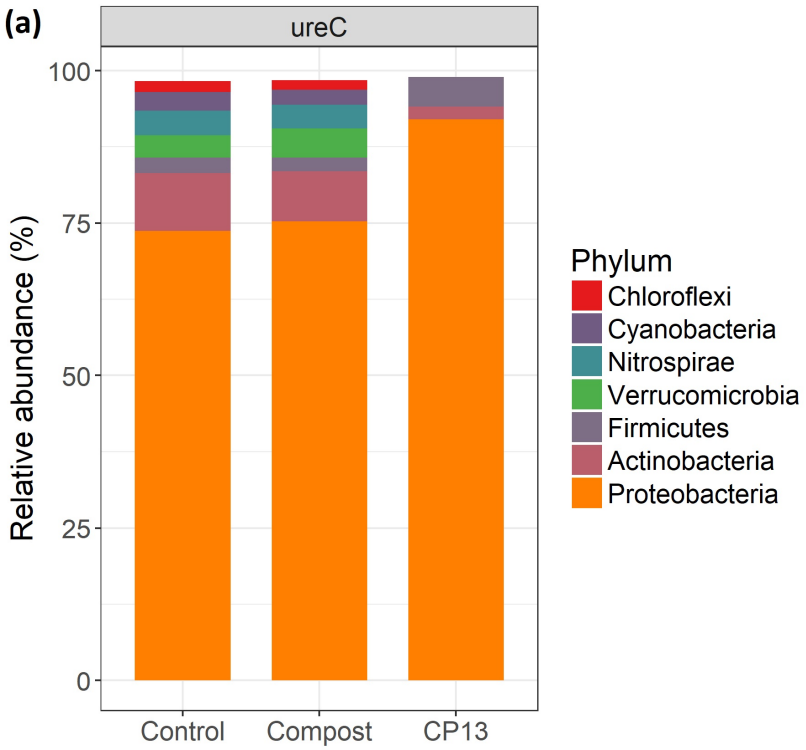


Fig S6 Relative abundance of the dominant phyla (>1%) for bacterial ureolytic communities from steer-waster compost (CP-2013) and compost treated soils (**a**). Venn diagram of *ureC* OTUs in steer-waster compost (CP) and compost treated soils (**b**).



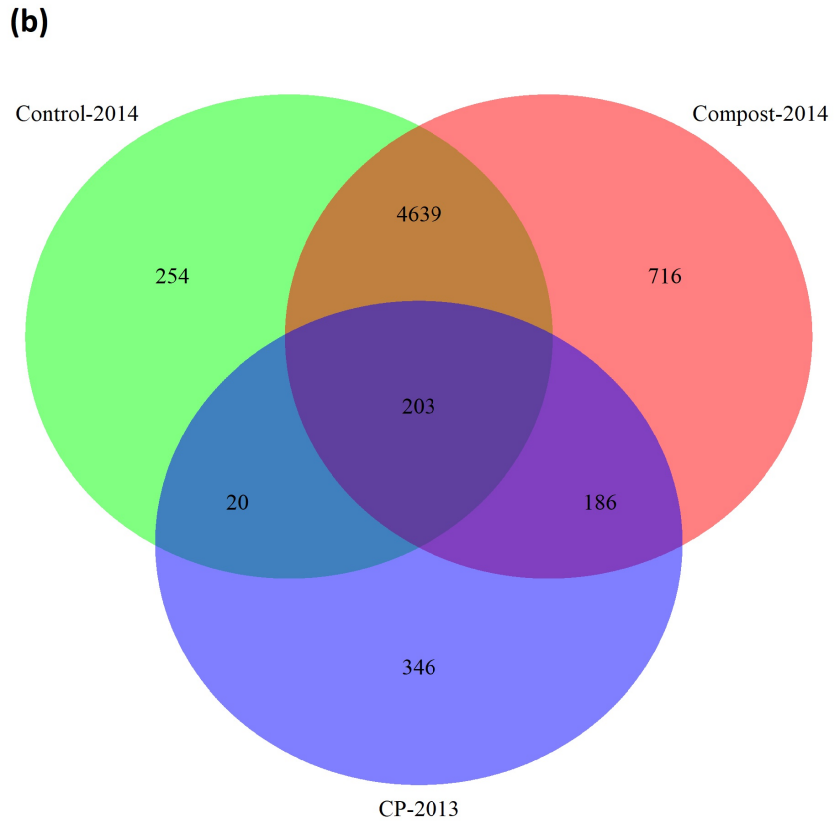


Fig S7 Relative abundance of the dominant phyla (>1%) for bacterial chitinolytic communities from steer-waster compost (CP-2013) and compost treated soils (**a**). Venn diagram of *chiA* OTUs in steer-waster compost (CP) and compost treated soils (**b**).