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Title: Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil.

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Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil.

Abstract

In the majority of agricultural soils, ammonium (NH_4^+) is rapidly converted to nitrate (NO_3^-) in the biological ammonia and nitrite oxidation processes known as nitrification. The often rate-limiting step of ammonia oxidation to nitrite is mediated by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). The response of AOA and AOB communities to organic and conventional nitrogen (N) fertilizers, and their relative contributions to the nitrification process were examined for an agricultural silage corn system using a randomized block design with 4 N treatments: control (no additional N), ammonium sulfate (AS) fertilizer at 100 and 200 kg N ha^{-1}, and steer-waste compost (200 kg total N ha^{-1}) over four seasons. DNA was extracted from the soil, and real-time PCR and 454-pyrosequencing were used to evaluate the quantity and diversity of the *amoA* gene which encodes subunit A of ammonia monooxygenase. Soil pH, nitrate pools, and nitrification potentials were influenced by ammonium and organic fertilizers after the first fertilization, while changes in AOB abundance and community structure were not apparent until after the second fertilization or later. The abundance of AOA was always greater than AOB but was unaffected by N treatments. In contrast, AOB abundance and community structure were changed significantly by ammonium fertilizers. Specific inhibitors of nitrification were used to evaluate the relative contribution of AOA and AOB to nitrification. We found that AOB dominantly contributed to potential nitrification activity determined at 1 mM ammonium in soil slurries and nitrification potential activity was higher in soils treated with ammonium fertilizers relative to control soils. However, AOA dominated gross nitrification activity in moist soils. Our result

suggests that AOB activity and community are more responsive to ammonium fertilizers than AOA but that *in situ* nitrification rate is controlled by ammonium availability in this agricultural soil. Understanding this response of AOA and AOB to N fertilizers may contribute to improving strategies for the management of nitrate production in agricultural soils.

Keywords: Nitrification, ammonia oxidizing archaea, ammonia oxidizing bacteria, nitrogen fertilizers, *Nitrosospira*, octyne.

1. Introduction

Nitrification, the biological oxidation of ammonia to nitrite and subsequently to nitrate, influences the fate of N in terrestrial systems and often promotes the loss of nitrate from soils. In soils, the first step of autotrophic nitrification is mediated by ammonia oxidizing bacteria (AOB) of the *Betaproteobacteria* and ammonia oxidizing archaea (AOA) of the *Thaumarcheota* (Norton, 2011; Schleper and Nicol, 2010). Since both AOA and AOB contain the ammonia monooxygenase enzyme, the *amoA* gene is frequently used as a molecular marker to explore the abundance and diversity of ammonia oxidizers. Leininger et al (2006) first revealed that AOA were quantitatively dominant in a variety of soils from diverse ecosystems. The abundance and communities of AOA and AOB and their relative contributions to soil nitrification are influenced by complex factors in agricultural soils (Giguere et al., 2015; Taylor et al., 2012).

Comparisons of N dynamics under organic N sources versus mineral fertilizers have consistently shown changes in N transformation rates and associated microbial communities (Burger and Jackson, 2003; Chu et al., 2007; Geisseler and Scow, 2014; Habteselassie et al., 2006b; Habteselassie et al., 2013; Reeve et al., 2010; Shi and Norton, 2000). AOB and AOA have been found to co-exist in most agricultural soils

78 and may have functional differences in their response to nitrogen management (Di et
79 al., 2009; Habteselassie et al., 2013; Offre et al., 2009; Xia et al., 2011). The
80 application of mineral fertilizers or urea changed the abundance and composition of
81 AOB, but it did not significantly influence the AOA community in several agricultural
82 soils (Ai et al., 2013; Shen et al., 2008; Wang et al., 2009; Xia et al., 2011). In
83 contrast, AOA growth was detected when ammonia was supplied by mineralization of
84 organic N derived from composted manure or soil organic matter (Jiang et al., 2014b;
85 Levicnik-Hoefferle et al., 2012; Offre et al., 2009; Schleper, 2010). However, these
86 studies were conducted in microcosms or after long term (>5 years) field fertilization
87 experiments. We have limited information about how AOA and AOB population and
88 community respond to mineral and organic fertilization temporally in the field.
89 While previous studies have shown that the archaeal *amoA* is often more
90 abundant than bacterial *amoA* in soils (Leininger et al., 2006; Schleper and Nicol,
91 2010; Shen et al., 2012), the rate of ammonia oxidation may or may not be linked
92 directly to current AOA and AOB populations (Myrold et al., 2014; Nicol et al.,
93 2008). Xia et al. (2011) found AOB dominantly contributed to nitrification activity in
94 an agricultural soil by using a stable isotope probing technique. However, AOA were
95 found to be the more dominant players in nitrification in other agricultural soils (Offre
96 et al., 2009; Zhang et al., 2010). Taylor et al. (2010) developed a short-term assay
97 based on the recovery of the nitrification potential (RNP) after inhibition with
98 acetylene in the presence and absence of bacterial protein synthesis inhibitors. They
99 discovered that in recently N fertilized and cropped soils the majority of RNP activity
100 was due to AOB, and that in pasture and grassland soils, RNP was due primarily to
101 AOA or to a mixture of AOA and AOB. Recent findings by Taylor and colleagues
102 (2015; 2013) showed that AOA pure cultures were more resistant to the C8 alkyne

inhibitor, 1-octyne, in comparison to AOB, and that therefore octyne can be used to distinguish AOA and AOB contributions to soil nitrification in short-term assays. These differential inhibitors allow us to assess the relative contribution of AOA and AOB to nitrification.

In the present study, our goal was to investigate effects of conventional and organic N sources on AOA and AOB populations and community composition in a replicated field experiment over 4 years. Concurrently, we assessed nitrification rates and the relative contribution of AOA and AOB under these contrasting N treatments. We hypothesized that the AOA and AOB communities and their relative contributions to nitrification would respond differentially to these contrasting N sources. Our expectation was that AOB abundance and activity would increase in response to ammonium fertilizer while AOA would increase in response to an organic N source. Understanding the relative contribution of AOA and AOB to nitrification may contribute to improving strategies for the management of nitrate production in agricultural soils.

2. Materials and methods

2.1 Experimental field plots and soil sampling

Field plots were established in 2011 at the Utah Experiment Station Greenville farm located in North Logan, Utah (41°45' N, 111°48' W). The soil is an irrigated, very strongly calcareous Millville silt loam (Coarse-silty, carbonatic, mesic Typic Haploxeroll). Previously, the field was used for conventional small grain production with the annual application of 70 kg N ha^{-1} as urea. 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}). Each plot is 3.8 m wide and 9.1 m long with a 4.6 m alley between each plot and a 1.2 m alley between each block. Composted steer manure (obtained from Miller Co. Hyrum, UT) was analyzed for moisture and N content immediately before application to determine the mass needed to supply the 200 kg total N ha^{-1} rate. Treatments were surface applied in May of each year (2011 - 2014), incorporated by tilling immediately after application, and silage corn was planted within one week after treatment application as previously described (Habteselassie et al., 2006a).

Soil samples were collected in May (0-30 cm depth) and August (0-15 cm depth) from 2011 to 2014. We collected 0-30 cm soil depth in May before tillage to coincide with standard practice for fertility determinations and to assess winter leaching of nitrate. In August, we sampled the 0-15 cm soil depth approximately 90 days after fertilization. Fertilizers are tilled into this depth and nitrification activity is generally higher in this surface layer (Habteselassie et al., 2006a; 2006b). Six soil cores were randomly taken from each plot, composited and mixed, placed on ice, and brought to the laboratory for immediate processing.

2.2 Soil chemical properties

Soil ammonium and nitrate were extracted immediately after sampling with 2M KCl (1:5 of soil:solution by mass). Soil ammonium and nitrate were measured with a flow injection analyzer (QuikChem 8500, methods 12-107-06-1-A, 12-107-04-1-J Lachat Instrument, Loveland CO). The soil moisture was determined by drying soils at 105°C for 24 h. Soil samples were then sieved (2.0 mm) and stored at 4°C or air-

149 dried for other measurements. Soil pH was determined on a 1:2 soil-water suspension.
150 Soil organic C and total N were determined by dry combustion (Primacs^{SLC} for
151 organic carbon, Primacs^{SN} for total N, Skalar, Inc, GA, USA).
152 *2.3 Nitrification potential and recovered nitrification potential assays*
153 Nitrification potential was determined by the shaken soil slurry method as
154 described previously (Hart et al., 1994; Norton and Stark, 2011). Briefly, 15 g sieved
155 fresh soil was placed into a 250 ml flask, and 100 ml 1mM phosphate buffer (pH=7.2)
156 containing 1 mM NH_4^+ was added to the flask. Flasks were shaken for 24 h at 200
157 rpm and sampled four times (2, 4, 22, 24 h) after the beginning of shaking. The
158 concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) were measured with a flow
159 injection analyzer (QuikChem 8500 method 10-107-04-1-C Lachat Instruments,
160 Loveland CO).
161 We measured the recovery of nitrification potentials (RNP) as described by
162 Taylor et al (2010; 2012). Briefly, 9 g of moist soil were added to 60 ml of 30 mM
163 TES buffer (pH 7.2) with 1 mM NH_4^+ in 150 ml bottles with caps fitted with gray
164 butyl stoppers. Soil slurry was then exposed to acetylene (0.025 KPa) for 6 h.
165 Acetylene was removed by placing the soil slurries under a vacuum and degassing for
166 6 min. After degassing, all bottles were shaken and incubated with caps loosened at
167 30°C. Once acetylene is removed, AMO may be synthesized again allowing
168 nitrification to resume and nitrite and nitrate to accumulate after a delay of
169 approximately 24 h. Thus the RNP was calculated by assessing the rate of nitrite and
170 nitrate accumulation 24 to 48 h after acetylene removal. In some samples, two

bacterial protein synthesis inhibitors, kanamycin (at a final concentration of 800 μg/ml) and spectinomycin (at a final concentration of 200 μg/ml) were used together to prevent synthesis of ammonia monooxygenase (AMO) by AOB, and thus the recovery in these samples is due to AOA (RNP_{AOA}). RNP of AOB was calculated as the difference between total RNP and RNP_{AOA}.

Nitrification potential with and without 4 μM aqueous concentration (C_{aq}) of 1-octyne was measured to distinguish the contribution of AOA and AOB to NP, using the modified method of Taylor and colleagues (2013). Briefly, 4.5 g sieved soil samples were placed in bottles with caps fitted with butyl stoppers, and then 30 ml of 30 mM TES buffer with ammonium sulfate (1.0 mM final NH_4^+-N) was added. Bottles were shaken for 2 h to equilibrate the soil and solution at 30°C. One group of these soil slurries was then exposed to 4 μM C_{aq} of octyne, and the other group served as a control. Five ml aliquots were sampled two times (2 and 24 h) after the beginning of shaking. The aliquots were centrifuged at 8000 g for 8 min, and the supernatants were frozen until analysis. The concentration of $NO_2^- + NO_3^-$ was measured with a flow injection analyzer as above. The nitrification potentials were determined by linear regression of $NO_2^- + NO_3^-$ accumulation over time. Nitrification activity in the presence of octyne was considered to be contributed by AOA (which are octyne-resistant), and the difference between nitrification activity with and without octyne was considered to be contributed by AOB (octyne-sensitive).

2.4 Gross and net nitrification rates

Gross nitrification rates were determined in laboratory incubations for soil

193 sampled in August 2011 and 2014. Three well-mixed 40 g dry-weight equivalent
194 subsamples were weighed into plastic specimen cups. Then, 1.6 ml of a $^{15}NO_3^-$
195 solution (containing 3.33 mM $K^{15}NO_3$ at 99 atom % ^{15}N) was added to the soils and
196 carefully mixed, creating a final soil water content of 0.18 kg kg^{-1}. The quantity of ^{15}N
197 added approximately doubled the soil NO_3^- pool. Immediately following soil mixing,
198 one subsample was harvested and extracted with 2 M KCl to determine NO_3^-
199 concentration and ^{15}N enrichment at time-0. The other two subsamples were placed in
200 1-L Mason jars with lids containing butyl rubber septa and with 1 ml water at the
201 bottom of the jar to minimize loss of moisture from the soil. Octyne was immediately
202 added to the subsample in one of the jars after the lid was sealed (final concentration
203 was 4 μM C_{aq}). Jars were incubated for 48 h at 25°C before extraction in 2M KCl.
204 Soil NO_2^-+NO_3^- were measured as described above. The extracts were prepared for
205 ^{15}N analyses using a diffusion procedure described in Stark and Hart (1996), and the
206 ^{15}N enrichment was measured by continuous-flow direct dry combustion and mass
207 spectrometry with an ANCA 2020 system (Europa Scientific, Cincinnati, OH). Gross
208 nitrification rates were calculated using the equation of Norton and Stark (2011). Net
209 nitrification was determined by a 21-day incubation. Fifteen grams of moist soil (0.18
210 kg kg^{-1} water content) in a plastic specimen container was placed in a 1-L Mason jar
211 with 1 ml water at the bottom of it to minimize loss of moisture from the soil. Soil
212 was extracted before and after incubation and NO_2^-+NO_3^- accumulation determined
213 over the incubation time.
214 *2.4 Soil DNA extraction and real-time quantitative PCR*

Soil samples were brought from the field to the laboratory, and immediately a 10 g sample of soil was frozen at -80 °C until DNA extraction. Soil DNA was extracted following the MoBio Power Soil DNA isolation protocol (MoBio Laboratories Inc, Carlsbad, USA) using 0.25 g moist soil. DNA extracts were quantified by using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR of AOA and AOB *amoA* genes was performed using the SsoAdvanced SYBR Green Supermix and a CFX CONNECT Real-Time PCR Detection System (Bio-Rad laboratories, Hercules, CA, USA). Primers amoA19F and amoA643R were used to quantify AOA *amoA* gene abundance, while primers amoA189F and amoA2R were used to quantify AOB *amoA* gene abundance (Habteselassie et al., 2013; Leininger et al., 2006; Okano et al., 2004). Standard curves were constructed with plasmids containing cloned *amoA* products from genomic DNA of *Nitrosospira multiformis* ATCC 25196 or from environmental DNA. The 25 µl reaction mixture contained 12.5 µl SsoAdvanced SYBR Green Supermix (Bio-Rad laboratories, Hercules, CA, USA), 0.5 µM of both reverse and forward primers, and 10 µl of 10-fold diluted soil DNA extract. Amplifications were carried out as follows: an initial denaturation step of 95°C for 10 min, 40 cycles of 95°C for 45 s, 60°C for 1 min for AOB or 58°C for AOA, and 72°C for 45 s, and a final extension step of 72°C for 10 min. Fluorescence intensity was read during the 72°C step of each cycle. Reaction efficiencies ranged from 99%-105%, and R^2 values ranged from 0.990-0.999 for AOB. For AOA, efficiencies ranged from 98-106% and R^2 values ranged from 0.992-0.999.

2.5 Pyrosequencing and bioinformatic analysis

Sequencing of *amoA* amplicon libraries was accomplished using the 454 sequencing system (Roche Life Sciences, Branford, CT). Amplicons of AOA and

AOB *amoA* genes were obtained with the same primers as mentioned above, but 10 bp barcodes and linkers were added to each forward primer. The 25 μl PCR reaction mixture contained 1×PCR buffer ($MgCl_2$ plus), 0.2 mM dNTPs, 1 μM of each forward and reverse primers, 0.05 U of enzyme (FastStart High Fidelity Enzymes Blend (Roche Life Sciences, Branford, CT), and 2μl of template DNA. Amplification was carried out as follows: an initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min, and a final extension step of 72°C for 10 min. The PCR products were checked by agarose gel electrophoresis, and cleaned by Agencourt AMPure XP (Beckman Coulter, Inc.). The DNA concentration of the purified PCR product was measured using the Quant-iT^{TM} PicoGreen dsDNA BR Assay Kit (Invitrogen) according to the manufacturer's protocol. Finally, the purified PCR products were pooled in an equal mole concentration, and then sequenced unidirectionally from the forward primer on a GS FLX+/XLR70 Instrument.

The bioinformatic analysis was performed using QIIME (Caporaso et al., 2010). Raw pyrosequencing reads were split based on the barcodes, and the sequences of low quality (quality score < 25, length < 350 bp) were removed. The obtained sequences were further denoised (Reeder and Knight, 2010), and then submitted to RDP pipeline using the FrameBot tool to fix frame shifts (Wang et al., 2013). The remaining quality-screened sequences were clustered into operational taxonomic units (OTU) based on 90% sequence identity. The 90% similarity level was chosen because AOB pure cultures with about 90% *amoA* identity have ~97% identity of 16S rRNA genes (Norton et al., 2002). The longest sequence from each OTU was chosen as a representative sequence. AOA *amoA* representatives were trimmed and aligned to an existing high–quality *amoA* database (Pester et al., 2012) using the ARB programs

(Ludwig et al., 2004). AOB *amoA* representatives were also trimmed and aligned with reference sequences, which were retrieved from GenBank. Phylogenetic trees were constructed by neighbor-joining using a Kimur 2-parameter distance with 1000 bootstrap replicates with MEGA 6 (Tamura et al., 2013).

2.6 Statistical analysis

Statistical analysis of the effect of N source on soil properties and *amoA* gene copy numbers were completed using a repeated measures analysis of variance (ANOVA) with the Proc Mixed model in SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). 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 effects of treatment and year on AOA and AOB *amoA* relative abundance. Two-way ANOVA was used to analyze effects of treatment and 1-octyne on gross and potential nitrification rates in soils sampled in Aug 2014. One-way ANOVA was used to evaluate the relative contribution of AOB to RNP and NP among treatments. P values <0.05 were considered to be statistically significant. To carry out the beta-diversity analysis, the AOA and AOB libraries were first normalized to 359 and 219 reads respectively (fewest sequences per sample) and then weighted UniFrac distance matrices were produced using QIIME. PCA and two-way PerMANOVA were conducted to visualize and assess the weighted Unifrac distances matrices in *vegan* package of R software (http://www.r-project.org).

3. Results

3.1 Soil chemical properties

Soil N treatments showed no significant effects on soil organic C over four years (Fig.1A and table S1) although soil organic C was higher in soils receiving compost in

2014 when the year was considered separately. Total N was affected by both treatment and year (Fig.1B). Soil pH was lowest in the AS200 treatment in all four years ranging from 8.11 to 8.15 (Fig.1C), while control and compost treatments had a relative higher soil pH (8.15-8.35). Soil extractable NH_4^+ was not influenced by treatment in either May and Aug soil samples over four years (Fig.1D and Table S1). Soil extractable nitrate showed significant treatment and year effect in soils sampled both in May and Aug (Fig. 1E, 1F and Table S1). AS200 often had a higher extractable nitrate than other treatments over four years due to excessive nitrate not used by plants.

3.2 Nitrification potential and recovered nitrification potential

Nitrification potentials reflect the short-term rate of nitrate formation in a well-mixed system supplied with 1 mM NH_4^+. The actual solution concentration of ammonium during the assay is closer to 0.5 mM due to soil adsorption. Repeated ANOVA analysis showed that both treatment and year significantly affected NP, and there was also a treatment and year interaction effect (Table S1). As early as August 2011, NPs were significantly elevated by AS or compost treatments compared with control (Fig.2). Over all four years, AS200 had the highest NP. Compost treatment had a significantly higher NP than control treatment in 2011 and 2013.

Like the NP assay, recovered nitrification potential measured nitrate production contributed by AOA or AOB under 1 mM NH_4^+ in a well-mixed system. The RNP assay indicated that both AOB and AOA were involved in ammonia oxidation in August 2013 (Fig.3A). RNP_{AOA} showed no significant differences among the

311 treatments, and averaged 5.63 mg N kg^{-1} d^{-1}. However, the RNP_{AOB} were higher in AS
312 treatments, ranging from 5.59 to 20.08 mg N kg^{-1} d^{-1}. RNP_{AOA} and RNP_{AOB}
313 contributed almost equally to total RNP in control soils, while RNP_{AOB} contributed
314 more (above 70%) to total RNP in soil from the fertilized treatments.
315 The soil nitrification potential assays with the differential inhibitor 1-octyne
316 showed similar results to the RNP assay for August 2013 soil samples (Fig.3B).
317 Generally, RNP_{AOA} was significantly correlated with octyne-resistant NP (r=0.56,
318 p=0.02), but octyne-resistant NP was lower than RNP_{AOA} averaging 3.0 mg N kg^{-1} d^{-1}.
319 Therefore, the NP assay with octyne suggested that octyne-sensitive NP (i.e. AOB)
320 contributed dominantly (74-88%) to NP under all treatments. ANOVA analysis also
321 indicated that octyne-sensitive NP showed a higher fraction of total NP in N added
322 treatments (Fig.3B). The NP measured in May 2014 from 0-30 cm depth soil (Fig 3C)
323 was about 50% of NP in Aug 2013 from 0-15 cm depth soil (Fig 3B), and the octyne-
324 resistant NP ranged from 1.3 to 2.3 mg N kg^{-1} d^{-1} in May 2014. Again, octyne-
325 sensitive NP dominated under all treatments. NP assay in August 2014 soil samples
326 showed the similar result as August 2013 soil samples (Fig. 4A).
327 Net and gross nitrification rates, measured in whole soils without NH_4^+ addition,
328 were elevated after four years repeated fertilization and cropping (Table 1). There was
329 no significant treatment effect in Aug 2011. In Aug 2014, both net and gross
330 nitrification rates were higher in AS and compost treatments, but net and gross rates
331 showed no difference between AS and compost treated soils. Under ambient NH_4^+
332 condition, almost all of the gross nitrification rate was due to octyne-resistant activity

333 (Fig.4B). Octyne-resistant gross nitrification rates were higher in N-addition
334 treatments.
335 *3.3 Abundance of AOA and AOB*
336 In May 2011 before treatment application, the copy numbers of *amoA* from AOA
337 and AOB were 4.1×10^7 g^{-1} soil and 3.9×10^6 g^{-1} soil, respectively (Fig.5A and 5B).
338 AOA *amoA* numbers were significantly affected by year in May and August soil
339 samples, respectively (Fig. 5A and 5C; Table S2). While treatment showed no
340 significant effects on AOA *amoA* numbers, AOA *amoA* numbers were higher than
341 AOB for all time points. In contrast, AOB *amoA* numbers were significantly affected
342 by both treatment and year (Table S2). After the first fertilization, AOB *amoA*
343 numbers showed no significant difference among treatments (August 2011 and May
344 2012; Fig 5B and 5D). However, after the second fertilization, AOB numbers were
345 higher in the AS200 treatment. This effect lasted through the third and fourth years,
346 over all sampling times and depths. The ratios of AOA to AOB *amoA* copy numbers,
347 varying from 2.9 to 158.8, were strongly affected by treatment and year, and were
348 higher under control and compost treatments than under AS treatments after the
349 second fertilization (Table 2, Table S2). AOB *amoA* copy numbers (but not AOA)
350 were significantly correlated with NP rates ($p<0.001$) (Fig S1).
351 *3.4 Community structure of AOA and AOB*
352 The structure of AOA and AOB communities was revealed by 454-
353 pyrosequencing analysis of *amoA* genes for Aug 2011 and 2013 samples. Totals of
354 22,925 and 25,903 high-quality raw sequence reads were obtained for AOB and AOA,

355 respectively, with 27 and 19 unique OTUs respectively (90% identity cut off).
356 Consensus representative sequences for the most abundant OTUs are available in
357 GenBank KU291532-54.

358 Phylogenetic analysis showed that the dominant AOB OTUs were affiliated with
359 the *Nitrosospira* Cluster 3 lineage (Fig.6A). The relative abundance of AOB OTUs
360 was not significantly affected by treatment in 2011, but the five most abundant OTUs
361 (BamoA14, BamoA25, BamoA26, BamoA27, BamoA4) were all significantly
362 affected by treatment in 2013 (Fig.6B). For example, BamoA14, BamoA27 and
363 BamoA4 were higher under AS treatments, while BamoA25 and BamoA26 were
364 higher under control and compost treatments in 2013 soils. Most of AOB OTU
365 relative abundances were significantly affected by year. For example, BamoA10,
366 BamoA15, BamoA23, and BamoA26 were significantly decreased from 2011 to 2013
367 under all treatments. *Nitrosomonas* (BamoA11, 0.17% of total sequences) was present
368 in 2011 under all treatments, but it was below detection in 2013. AOB community
369 structure was different between 2011 and 2013 and treatment changed AOB
370 community structure in 2013 as revealed by weighted UniFrac distance matrices
371 (Fig.8). Two-way PerMANOVA further confirmed that AOB community structure
372 was significantly affected by year ($p=0.001$) and treatment ($p=0.05$).

373 All of AOA *amoA* OTUs were affiliated with the *Nitrososphaera* cluster
374 (Fig.7A). Most of AOA *amoA* gene sequences were grouped into AamoA OTU5
375 (44.1% of total sequences) and AamoA OTU12 (44.0% of total sequences) (Fig.7B).
376 AamoA12 was classified into *Nitrososphaera* subcluster 1.1, while AamoA5 could not

be classified into a stable *Nitrososphaera* subcluster although it was consistently associated with a group including the soil fosmid 54d9. AamoA7, which was the third most abundant OTU, belongs to *Nitrososphaera* subcluster 4.1. The relative abundance of AamoA5 decreased, but AamoA12 increased from 2011 to 2013. However, none of AOA OTU relative abundances were significantly affected by treatment. AOA community structure as revealed by weighted UniFrac distance matrics (Fig.8) and two-way PerMANOVA also showed AOA community composition was not affected by treatments ($p=0.33$) or year ($p=0.18$).

4. Discussion

In this four-year field study, we started with soil from plots with a consistent agricultural management history. We observed significant effects of the new N management on soil total N concentrations, pH, nitrate pools, nitrification activities, ammonia oxidizers populations and communities. Soil pH, nitrate pools, and nitrification potentials were quickly changed by mineral and organic fertilizers after the first fertilization, while changes in ammonia oxidizers populations and communities occurred after the second fertilization or later. Our observations suggest that NP is an useful indicator of community wide nitrification activity, and reflects changes in active nitrifiers and their abundance over time at a non-limiting substrate concentration, while substrate availability controls rates of nitrification under field conditions as indicated by gross nitrification rate measurements.

AOA and AOB abundance and community composition response to contrasting N sources

399 Overall, we observed higher abundance of AOA compared to AOB, similarly to
400 observations of Leininger et al. (2006) in various agricultural soils. We found ratios of
401 AOA to AOB were higher in the control and compost treated soils after repeated
402 fertilization, both treatments have the majority of their ammonium supplied through
403 mineralization of organic nitrogen (Habteselassie et al., 2013). In this study, AOA
404 abundance fluctuated over four years, but did not respond to ammonium or organic N
405 fertilizers. This is in contrast to previous studies in agricultural soils (Ai et al., 2013;
406 Jiang et al., 2014b), which reported that AOA abundance increased when ammonia
407 was supplied as mineralized organic N derived from composted manure or soil
408 organic matter over more than 10 years. In our study, we did not observe a significant
409 increase in AOA abundance but these changes may develop with additional years or
410 with higher amounts of manure applications.
411 In this study, AOA *amoA* OTUs were all affiliated with *Nitrososphaera*, which is
412 commonly detected in agricultural soils (Bates et al., 2011; Pester et al., 2012). Most
413 of AOA *amoA* gene sequences were equally distributed into two *Nitrososphaera*
414 clades (together 88.1% of total sequences). This pattern of low richness of AOA is a
415 common observation when soils are sampled at a single location (Jiang et al., 2014a).
416 One of our abundant OTUs (AamoA 5) was associated with the soil fosmid 54d9,
417 which is widely distributed in global soils (Alves et al., 2013; Bates et al., 2011). To
418 date, this group of AOA does not have any pure culture representatives. Another
419 abundant AOA OTU (AamoA12) was classified into *Nitrososphaera* subcluster 1.1,
420 which are often abundant in soils, and includes cultured representatives
421 *Nitrososphaera gargensis* and *Nitrososphaera viennensis* (Hatzenpichler et al., 2008;
422 Tourna et al., 2011) .

423 Ammonium fertilizer treatments strongly stimulated AOB growth rather than AOA

424 with repeated fertilizations. This is consistent with previous studies in agricultural
425 soils (Ai et al., 2013; Habteselassie et al., 2013; Shen et al., 2008; Wu et al., 2011).
426 AOB are favored by inputs of mineral ammonium or urea sources and high
427 concentrations of N. Field and soil microcosm experiments have previously shown
428 significant increases in AOB abundance under high ammonium concentration (Jia and
429 Conrad, 2009; Okano et al., 2004; Taylor et al., 2012; Verhamme et al., 2011), while
430 AOA abundance was not affected (Di et al., 2009, 2010). However, we observed that
431 AOB abundance was not increased after a single fertilization when measured near the
432 end of the season in August and in the following May, even though NP was
433 stimulated. Our observations indicate that pre-planting fertilization with soluble
434 ammonium fertilizers (e.g application of ammonium sulfate) changes the growth and
435 community of AOB but not significantly in one season. Continuous corn management
436 would stimulates nitrification in the field, possibly increase the N loss in the soil, and
437 thus reduce the N use efficiency for plants.

438 The AOB communities were mainly affiliated with the *Nitrosospira* cluster 3 in
439 this study, a finding consistent with other studies that have shown the dominance of
440 this group of AOB in a number of agricultural soils (Chu et al., 2007; Habteselassie et
441 al., 2013; Phillips et al., 2000). Tourna et al. (2010) suggested that *Nitrosospira* cluster
442 3 often outcompetes other *Nitrosospira* under high ammonium conditions. This is true
443 for most AOB OTUs (including BamoA14 and BamoA27), which were favored by
444 ammonium fertilization in the current study. However, from 2011 to 2013, BamoA26,
445 our most abundant OTU, significantly decreased under AS treatments. This OTU is

446 related to the 3A cluster containing *Nitrosospira sp.* Wyke8 (Webster et al., 2005) see
447 Fig. 6A, which was also observed to be inhibited by high ammonium concentrations.
448 Interestingly, we detected *Nitrosomonas* (0.17% of total sequences) in 2011 under all
449 treatments, but it was below detection in 2013. *Nitrosomonas* have often been
450 detected in manure treated soils (Fan et al., 2011) and in liquid waste treated soils
451 (Habteselassie et al., 2013). Our results suggest that soil *Nitrosomonas* may not be
452 favored by fertilization and conventional corn management.

453 Amplicon pyrosequencing revealed that the community composition of AOB was
454 significantly altered by three years but not one year of contrasting N treatment, while
455 AOA community was less responsive to N treatment. Previous microcosm studies for
456 agricultural soils did not show any AOB community shifts under ammonium
457 treatment with 4-6 weeks of incubation (Avrahami et al., 2002; Mendum et al., 1999),
458 while AOB community composition was altered after relatively longer incubations
459 (Avrahami et al., 2003). We observed shifts in the AOB community under AS
460 fertilization after the third year of treatment. Other studies that investigated the effect
461 of long-term field fertilization on ammonia-oxidizer communities showed contrasting
462 results. Some studies indicated repeated fertilization significantly altered AOB
463 community composition (Ai et al., 2013; Chu et al., 2007; Wu et al., 2011), while
464 others have shown changes in AOA community composition as well (Gubry-Rangin et
465 al., 2010; He et al., 2007). The temporal aspect of AOB community shifts in the field
466 that we observed suggest that responses to are likely after 2-3 years of ammonium
467 fertilizer applications.

468 *Contribution of AOA and AOB to nitrification activity*

469 In our study, nitrification potentials range from 5-31 mg N kg^{-1} d^{-1}, which falls
470 within the range of 2–45 mg N kg^{-1} d^{-1} found in many agricultural soils (Ai et al.,
471 2013; Fortuna et al., 2003; Habteselassie et al., 2006a; Taylor et al., 2010). Since NP
472 is measured under excess substrate and without diffusional limitations, it is often
473 thought to reflect the legacy of substrate availability (Fortuna et al., 2003). We found
474 that AS200 treatment had a much higher NP than compost treatment in all years, even
475 though the compost treatment received the same total N. The slow release of
476 ammonium from the compost does not stimulate NP to the same extent as applications
477 of ammonium fertilizers. Gross nitrification measures the actual rate of conversion of
478 NH_4^+/NH_3 to NO_3^-, and is often limited by substrate availability (Booth et al., 2005).
479 We found that the highest gross nitrification rates are in the compost treated soils
480 where mineralization increases substrate availability and mineralized ammonium
481 stimulates rates of nitrification under low ammonium ambient conditions. We found
482 that gross nitrification rate was only 1-11% of the NP, but also reflected the effects of
483 fertilization (Table 1). Net nitrification rate was about 60% of the gross nitrification
484 rate (Table 1), indicating an important role for other nitrate consumptive processes.

485 Interestingly, nitrification potentials were elevated by mineral and organic
486 fertilizers after the first fertilization, while AOA and AOB *amoA* gene abundance
487 showed no significant difference among treatments at this time point. Our *amoA* gene
488 abundance measurement was based on soil DNA, while the rate of ammonia oxidation
489 may be more related to the relationship among transcription, translation and enzyme

490 function (Myrold et al., 2014; Nicol et al., 2008). Our observation suggests a
491 proteomic approach might be appropriate for explaining short-term changes in
492 nitrification activity especially for the AOB.

493 In this study, NP rates significantly correlated with AOB *amoA* copy numbers, but
494 not with AOA abundance over four years of repeated N treatments (Fig. S1). The RNP
495 after acetylene inactivation in the presence and absence of bacterial protein synthesis
496 inhibitors suggested that AOB were responsible for approximately 69-76% of
497 nitrifying activity in fertilized soils. The soil slurry approach with or without 1-octyne
498 also indicated that AOB contributed approximately 82-91% to NP in N treated soils.
499 This is consistent with several previous studies (Taylor et al., 2010; Taylor et al.,
500 2012; Xia et al., 2011). Xia et al. (2011) found AOB dominantly contributed to
501 nitrification activity (about 76%) in an agricultural soil with the addition of 100 mg
502 NH_4^+-N kg^{-1} soil every week. Taylor et al. (2010) discovered that in recently N
503 fertilized cropped soils, the majority of RNP activity was due to AOB. A subsequent
504 study has shown the relative contributions of AOA and AOB to RNP were affected by
505 cropping treatments, the time since N fertilization, and soil conditions in the field
506 (Taylor et al., 2012). Assuming each AOB cell has 2.5 *amoA* gene copies, and using
507 the octyne-sensitive NP, we calculated ammonia oxidation rate of AOB was about 41
508 fmol NH_3 oxidized $cell^{-1}$ h^{-1}. This rate is comparable to ammonia oxidation rates (4-23
509 fmol NH_3 oxidized $cell^{-1}$ h^{-1}) of AOB pure cultures (Belser and Schmidt, 1980; Jiang
510 and Bakken, 1999). We observed that AOA also play a role in nitrification potential,
511 especially in control and compost treated soils. Assuming each AOA cell has one

512 *amoA* gene copy, and using our estimate of octyne-resistant nitrification potential at
513 30 ^{o}C, we calculated ammonia oxidation rate of AOA was about 0.2 fmol NH_3
514 oxidized cell^{-1} h^{-1}. This is similar to specific rates found for AOA pure cultures
515 (Hatzenpichler et al., 2008; Konneke et al., 2005; Tourna et al., 2011) and estimated
516 rates from soils (Jia and Conrad, 2009).
517 Three months after fertilization, readily available ammonium was consumed, and
518 the majority of ammonium was supplied though mineralization of organic N. Since
519 AOA may have a higher affinity for ammonia than AOB (Martens-Habbena et al.,
520 2009), we expected AOA might dominate *in situ* nitrification, and this hypothesis was
521 confirmed by the findings that the addition of 1-octyne did not inhibit gross
522 nitrification rate in whole soils sampled in August. Taylor et al. (2013) discovered that
523 in cropped soil AOB dominantly contributed to the nitrification activity under
524 saturated ammonium conditions, while AOA activity dominated without ammonium
525 addition based on whole soil octyne inhibition assays. This is consistent with other
526 studies that show that AOA dominate nitrification activity when ammonia is produced
527 by mineralization of soil organic matter (Gubry-Rangin et al., 2010; Offre et al., 2009;
528 Zhang et al., 2010).
529 The contrasting results of octyne inhibition of nitrification activity under ambient
530 and nitrification potential conditions suggests that ammonium concentration and
531 diffusional supply is a key factor determining the relative contribution of AOA and
532 AOB to measured nitrification. Immediately following ammonium fertilizations AOB
533 activity increases and growth may occur. AOA are still functioning and at low

concentrations of ammonium such as found in August they are responsible for a higher relative amount of the gross nitrification activity. We also observe that at about 1 mM concentrations AOA are still responsible for about 20% of the nitrification potential (Fig 3B,C). but at these concentrations their capacity to respond to additional ammonium is likely saturated Therefore, AOA's relative contributions to nitrification activity decreases rapidly with increasing ammonium availability such as occurs in recently fertilized soils (Giguere et al., 2015).). While inhibition of nitrification activity may occur at high ammonium concentrations (i.e. > 5 mM (Tourna et al., 2010; Tourna et al., 2011 Koper et al., 2010; Prosser and Nicol, 2012)) we did not reach these levels of ammonium in the current study. Inhibition at high ammonium levels may further affect the relative contributions to nitrification of AOA and AOB and this effect requires further investigation especially under field conditions. Our observations suggest controlling the activity of AOB in the field immediately after the application of mineral N fertilizers would be an effective strategy to reduce and delay nitrification and therefore improve the synchronization of N mobility with crop N uptake. However, our study also showed that AOA contributed most to nitrification at low ammonium availability which is typical over a longer portion of the season. Practices to control AOA activity would need to be an extended effort, and may be more important under organic N management. Further studies examining seasonal changes in nitrification kinetics and the role of AO communities in this experimental system are in progress.

In conclusion, our investigation of AOA and AOB population and community composition under mineral and organic N sources for 4 years in the field, found that

one fertilization did not significantly alter the abundance and community composition of AOB and AOA, but significantly elevated nitrification potential. After repeated fertilization, N treatment significantly affected abundance and community composition of AOB, but had little effect on AOA. By evaluating the relative contribution of AOA and AOB to NP after 3 or 4 years of repeated N treatments, we found that AOB dominantly contributed to potential nitrification. However, this dominant role of AOB in NP does not indicate the same role for *in situ* nitrification, and AOA dominated gross nitrification activity at low levels of ambient ammonium. Our results suggests that AOB activity and community are more responsive to ammonium fertilizers than AOA in agricultural soil, and practices to control nitrification should focus attention on AOB, especially in the short term after application of ammonium fertilizers.

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Table 1. Net and gross nitrification rates in August 2011 and 2014.

Treatments	NNR*		GNR*		NNR/GNR		GNR /NP	
	2011	2014	2011	2014	2011	2014	2011	2014
Control	0.14	0.36 a	0.19	0.50 a	0.77	0.71	0.02	0.08 b
AS100	0.20	0.49 b	0.19	0.67 ab	1.09	0.73	0.01	0.05 a
AS200	0.17	0.54 b	0.38	1.00 b	0.45	0.54	0.02	0.05 a
Compost	0.15	0.53 b	0.63	0.99 b	0.24	0.53	0.05	0.11 b

Abbreviation: NNR-Net nitrification rate, GNR-Gross nitrification rate, NP-nitrification potential. Different letters within a column indicate significantly different treatment means within the year.

*Unit: mg N $kg^{-1}d^{-1}$.

Table 2. Ratios of AOA to AOB *amoA* copy numbers from May 2011 to Aug 2013 (mean values, ±SD, N=4, lowercase letters indicate significant difference among treatments, P<0.05).

Sample Time	Ratios of AOA to AOB *amoA* copy number			
	Control	AS100	AS200	Compost
May-2011	10.1 a	13.3 a	16.4 a	20.3 a
May-2012	7.0 a	3.2 a	6.9 a	8.0 a
May-2013	65.6 a	27.1 a	23.5 a	126.4 a
May-2014	122.3 b	18.0 a	15.6 a	94.5 b
Aug-2011	2.8 a	2.9 a	4.8 a	4.0 a
Aug-2012	41.6 b	7.7 a	4.5 a	30.7 b
Aug-2013	36.0 b	9.6 a	6.5 a	34.7 b
Aug-2014	158.8 b	37.1 a	14.4 a	109.9 b

Figure 1

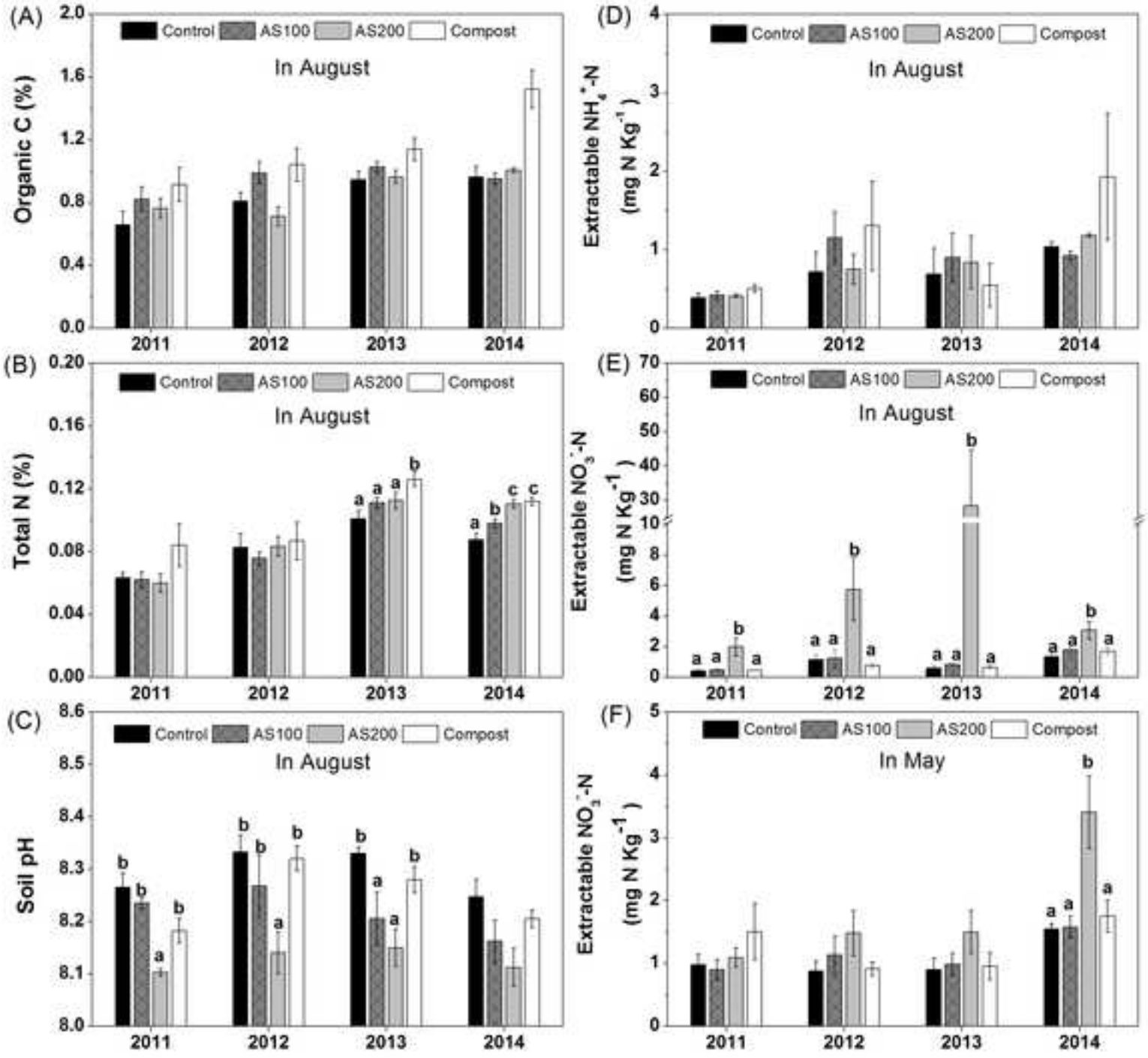
(A)
Organic C (%)
Control
AS100
AS200
Compost
In August
2011
2012
2013
2014
(B)
Total N (%)
(C)
Soil pH
(D)
Extractable NH4+-N (mg N Kg-1)
(E)
Extractable NO3--N (mg N Kg-1)
(F)
In May

Figure 2

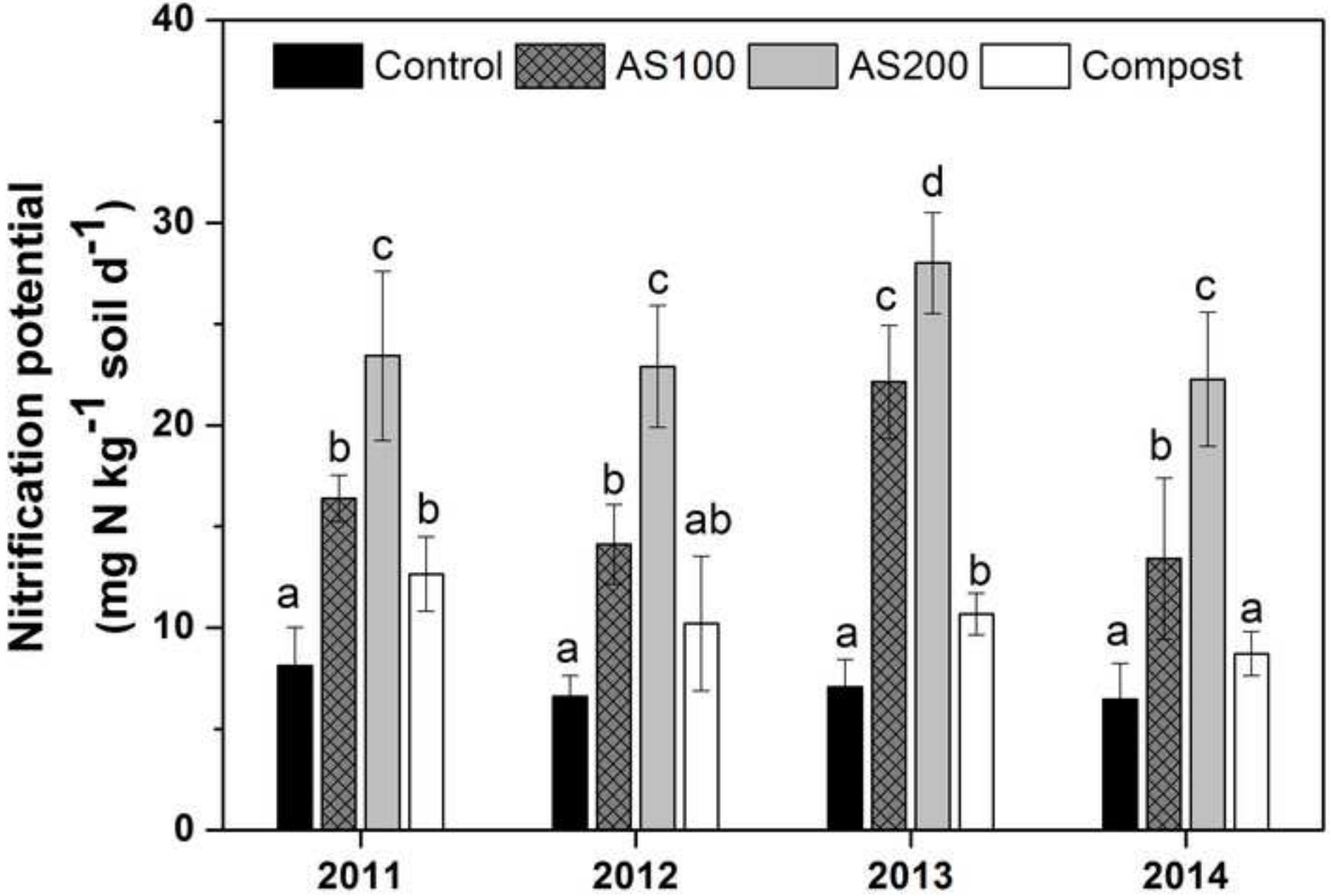
Control
AS100
AS200
Compost
Nitrification potential
(mg N kg-1 soil d-1)
40
30
20
10
0
a
b
c
b
a
b
c
ab
a
c
d
b
a
b
c
a
2011
2012
2013
2014

Figure 3

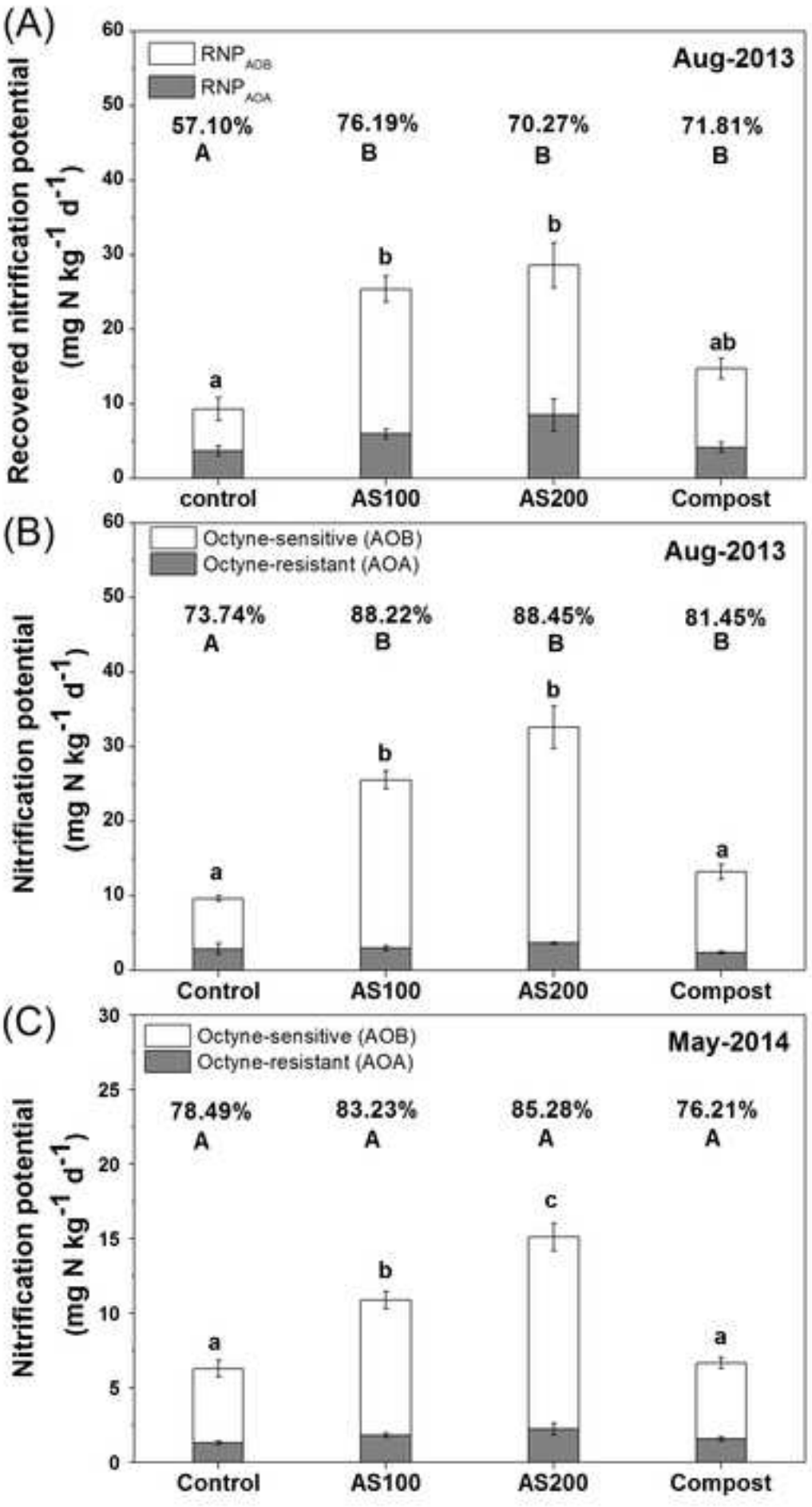
(A)
Recovered nitrification potential (mg N kg-1 d-1)
RNPAOB
RNPAOA
Aug-2013
57.10%
A
76.19%
B
70.27%
B
71.81%
B
a
b
b
ab
control
AS100
AS200
Compost
(B)
Nitrification potential (mg N kg-1 d-1)
Octyne-sensitive (AOB)
Octyne-resistant (AOA)
Aug-2013
73.74%
A
88.22%
B
88.45%
B
81.45%
B
a
b
b
a
Control
AS100
AS200
Compost
(C)
Nitrification potential (mg N kg-1 d-1)
Octyne-sensitive (AOB)
Octyne-resistant (AOA)
May-2014
78.49%
A
83.23%
A
85.28%
A
76.21%
A
a
b
c
a
Control
AS100
AS200
Compost

Figure 4

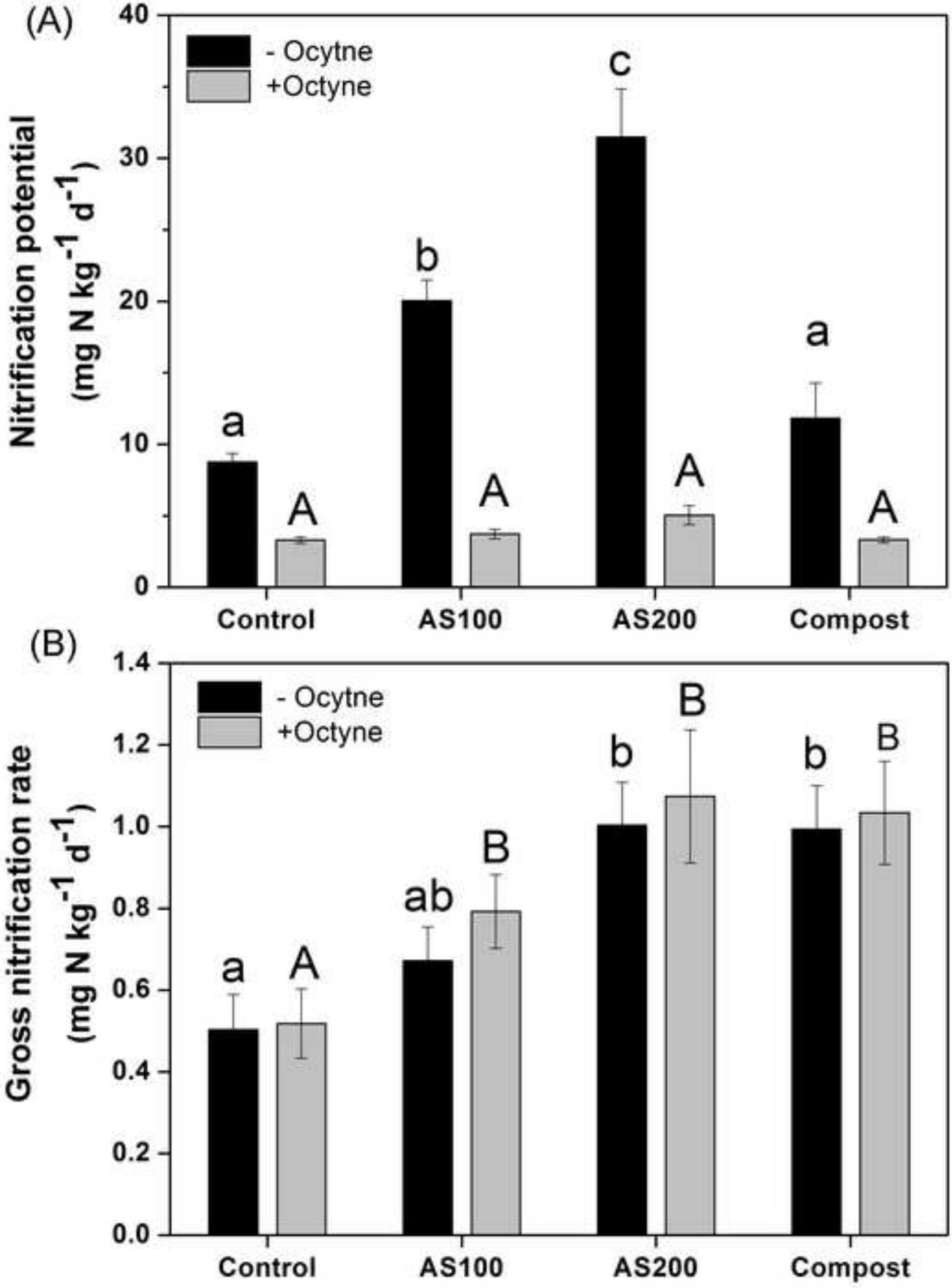
(A)
- Ocytne
+Octyne
Nitrification potential
(mg N kg-1 d-1)
40
30
20
10
0
a
A
b
A
c
A
a
A
Control
AS100
AS200
Compost
(B)
- Ocytne
+Octyne
Gross nitrification rate
(mg N kg-1 d-1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
A
ab
B
b
B
b
B
Control
AS100
AS200
Compost

Figure 5

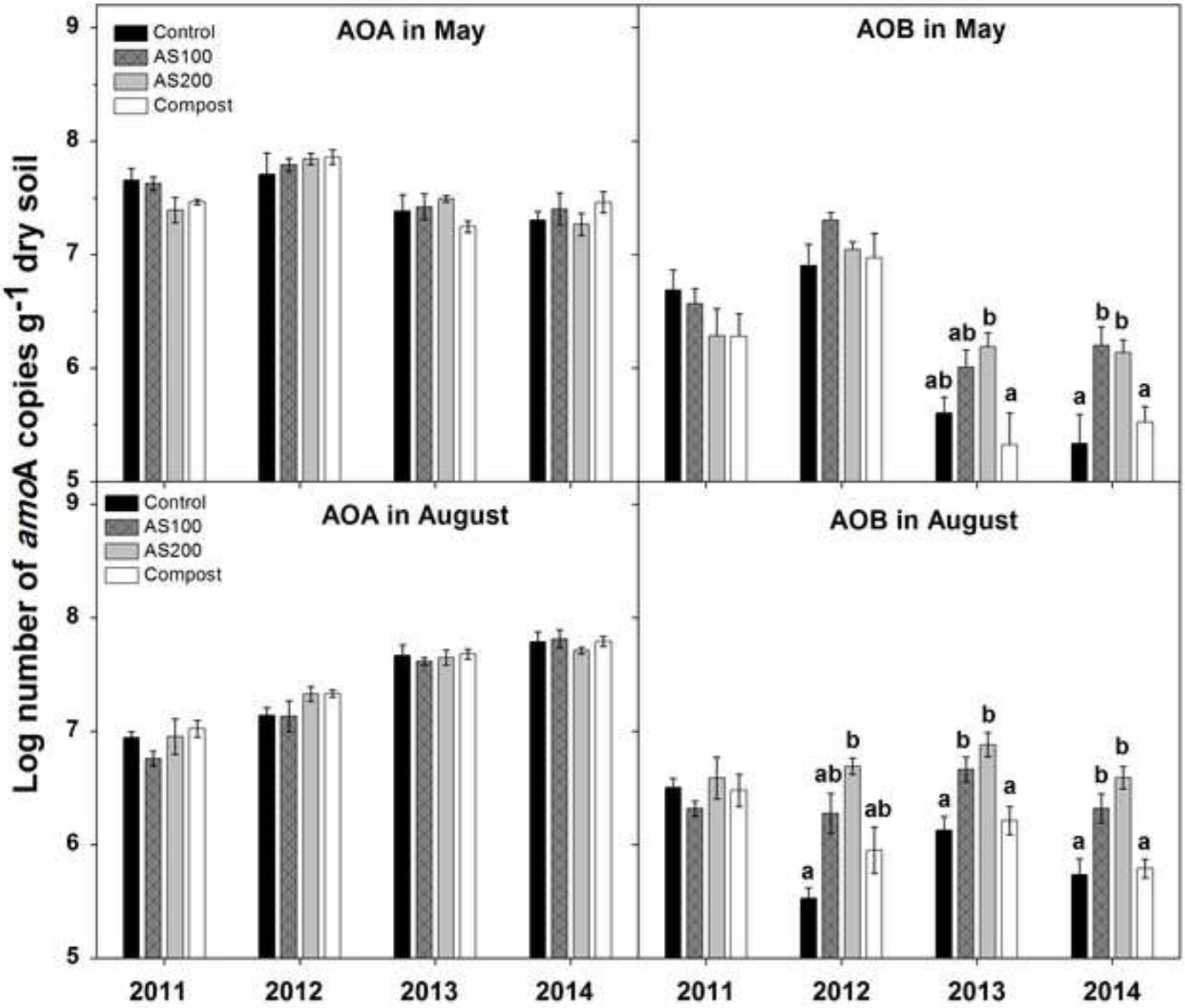
AOA in May
AOB in May
AOA in August
AOB in August
Control
AS100
AS200
Compost
Log number of amoA copies g-1 dry soil
9
8
7
6
5
2011
2012
2013
2014
a
ab
b

Figure 6A

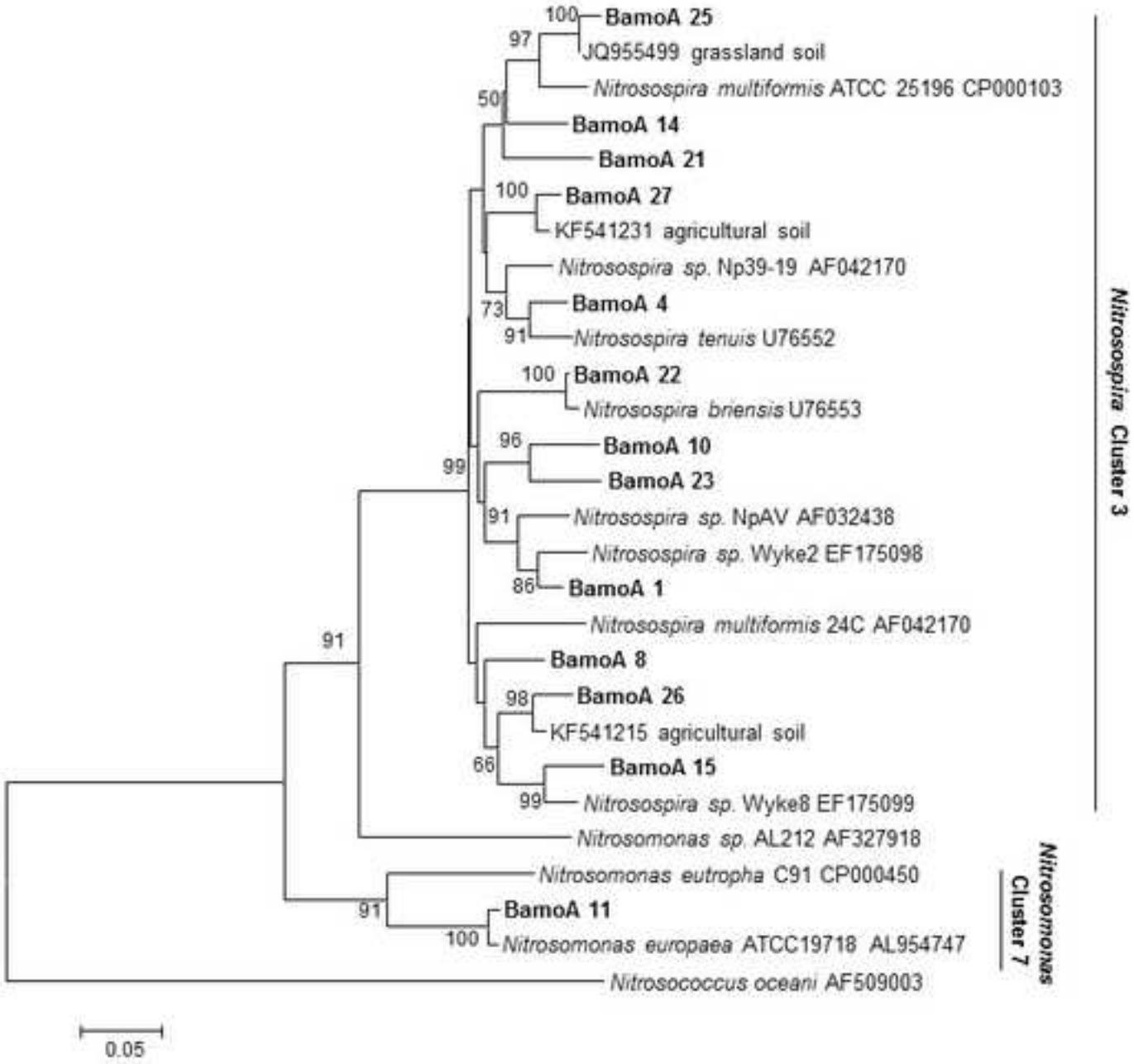
BamoA 25
JQ955499 grassland soil
Nitrosospira multiformis ATCC 25196 CP000103
BamoA 14
BamoA 21
BamoA 27
KF541231 agricultural soil
Nitrosospira sp. Np39-19 AF042170
BamoA 4
Nitrosospira tenuis U76552
BamoA 22
Nitrosospira briensis U76553
BamoA 10
BamoA 23
Nitrosospira sp. NpAV AF032438
Nitrosospira sp. Wyke2 EF175098
BamoA 1
Nitrosospira multiformis 24C AF042170
BamoA 8
BamoA 26
KF541215 agricultural soil
BamoA 15
Nitrosospira sp. Wyke8 EF175099
Nitrosomonas sp. AL212 AF327918
Nitrosomonas eutropha C91 CP000450
BamoA 11
Nitrosomonas europaea ATCC19718 AL954747
Nitrosococcus oceani AF509003
0.05
Nitrosospira Cluster 3
Nitrosomonas Cluster 7

Figure 6B

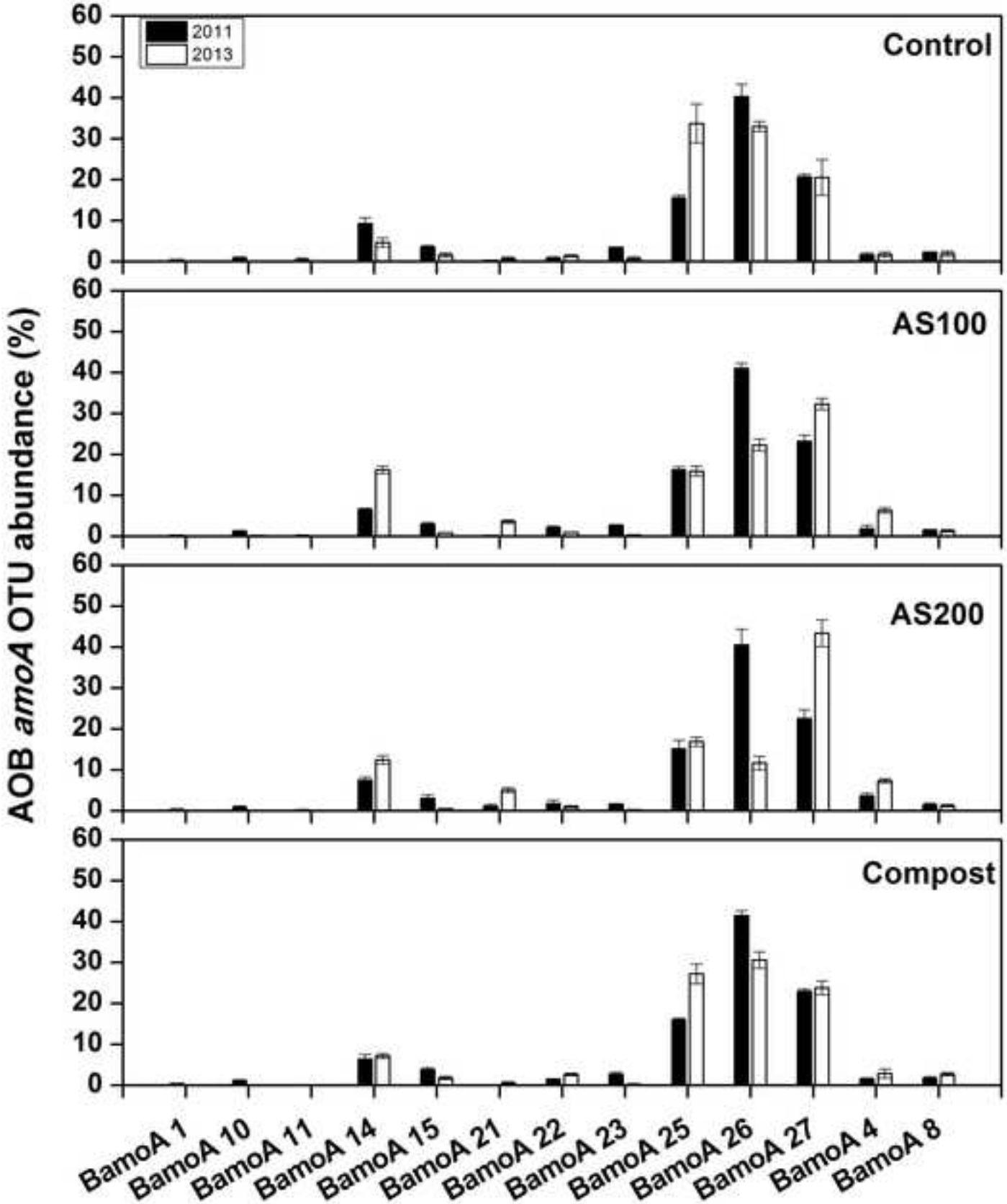
2011
2013
Control
AS100
AS200
Compost
AOB amoA OTU abundance (%)
0
10
20
30
40
50
60
BamoA 1
BamoA 10
BamoA 11
BamoA 14
BamoA 15
BamoA 21
BamoA 22
BamoA 23
BamoA 25
BamoA 26
BamoA 27
BamoA 4
BamoA 8

Figure 7A

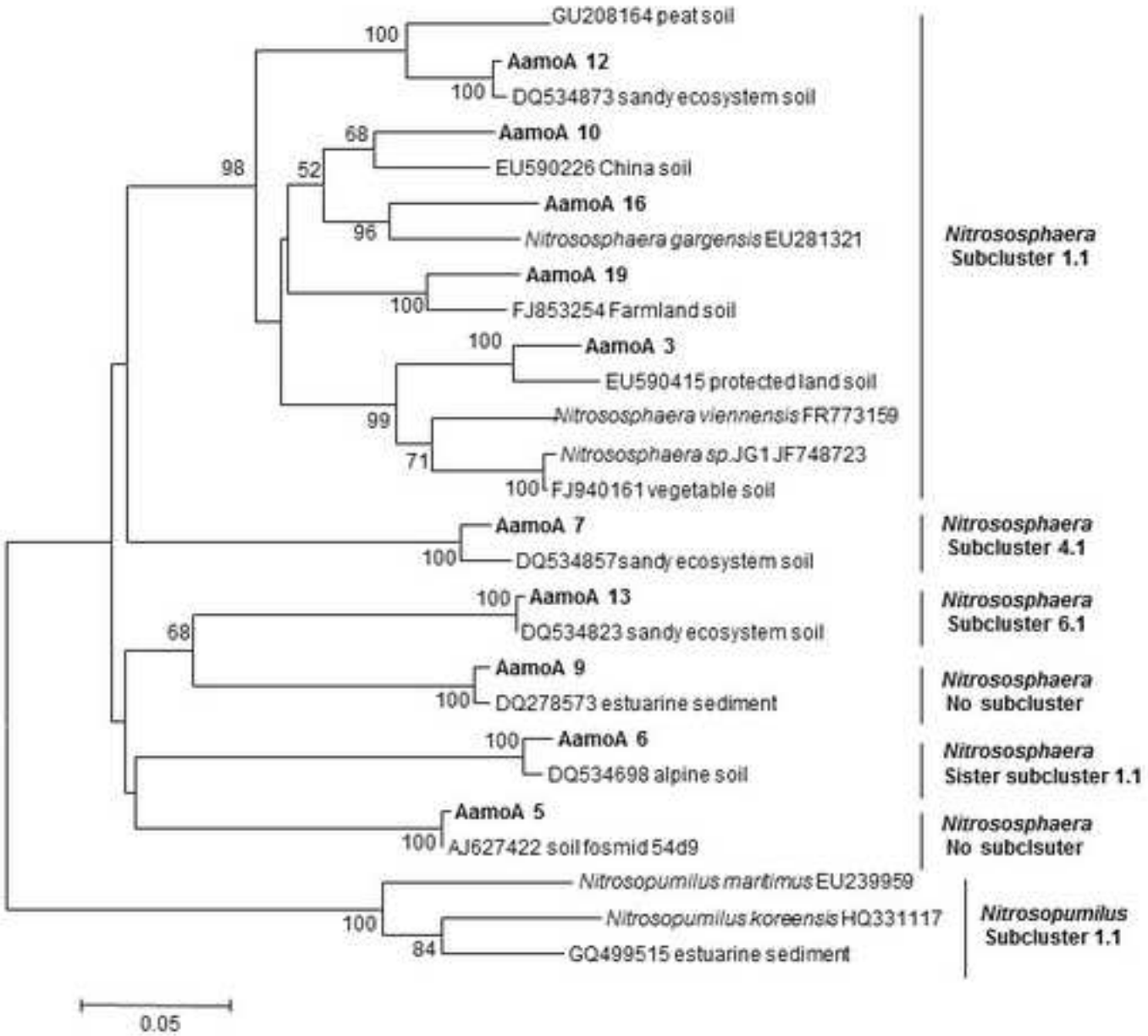
GU208164 peat soil
AamoA 12
DQ534873 sandy ecosystem soil
AamoA 10
EU590226 China soil
AamoA 16
Nitrososphaera gargensis EU281321
AamoA 19
FJ853254 Farmland soil
AamoA 3
EU590415 protected land soil
Nitrososphaera viennensis FR773159
Nitrososphaera sp. JG1 JF748723
FJ940161 vegetable soil
AamoA 7
DQ534857 sandy ecosystem soil
AamoA 13
DQ534823 sandy ecosystem soil
AamoA 9
DQ278573 estuarine sediment
AamoA 6
DQ534698 alpine soil
AamoA 5
AJ627422 soil fosmid 54d9
Nitrosopumilus maritimus EU239959
Nitrosopumilus koreensis HQ331117
GQ499515 estuarine sediment
0.05
Nitrososphaera Subcluster 1.1
Nitrososphaera Subcluster 4.1
Nitrososphaera Subcluster 6.1
Nitrososphaera No subcluster
Nitrososphaera Sister subcluster 1.1
Nitrososphaera No subclsuter
Nitrosopumilus Subcluster 1.1

Figure 7B

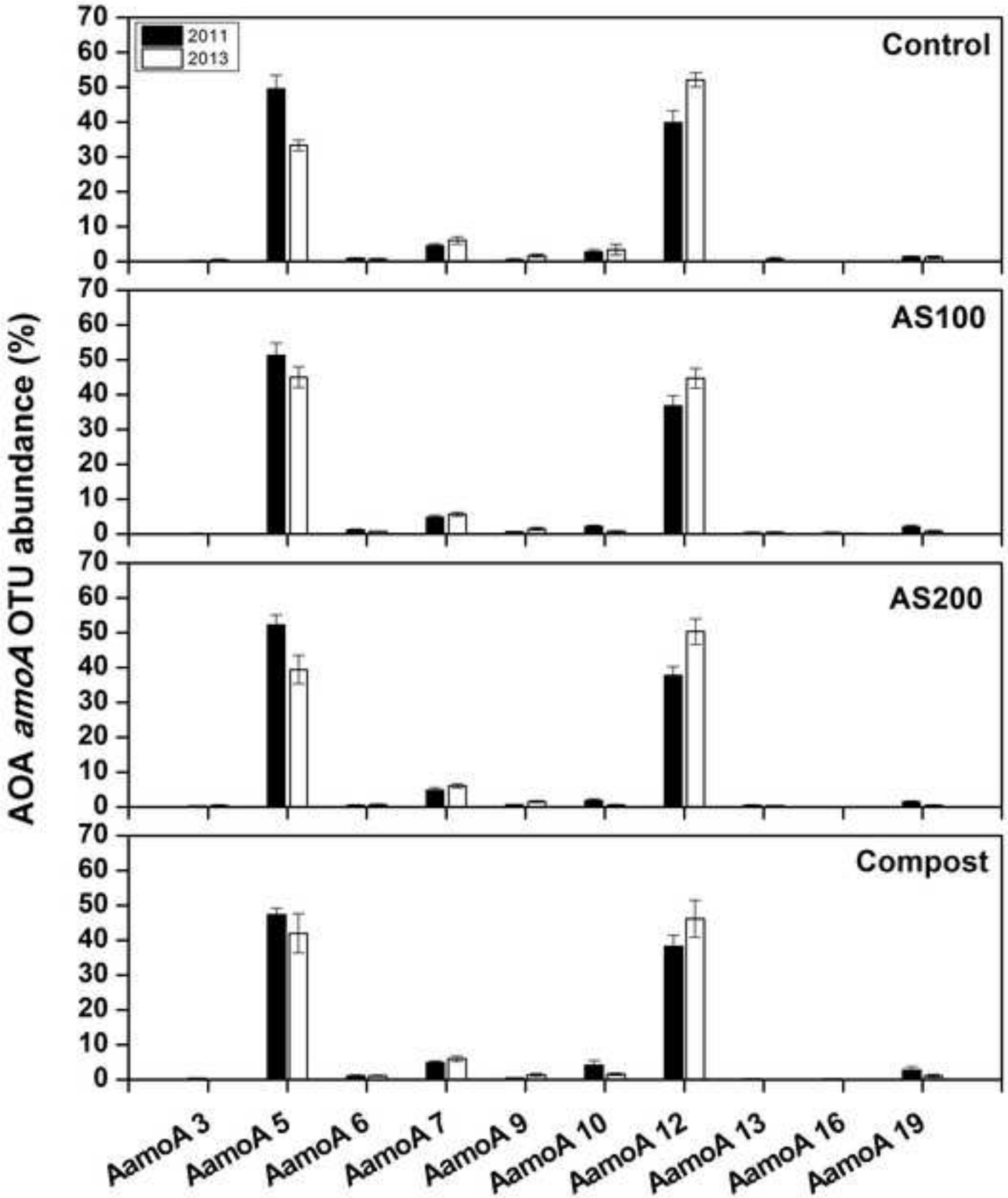
AOA amoA OTU abundance (%)
2011
2013
Control
AS100
AS200
Compost
0
10
20
30
40
50
60
70
AamoA 3
AamoA 5
AamoA 6
AamoA 7
AamoA 9
AamoA 10
AamoA 12
AamoA 13
AamoA 16
AamoA 19

Figure 8

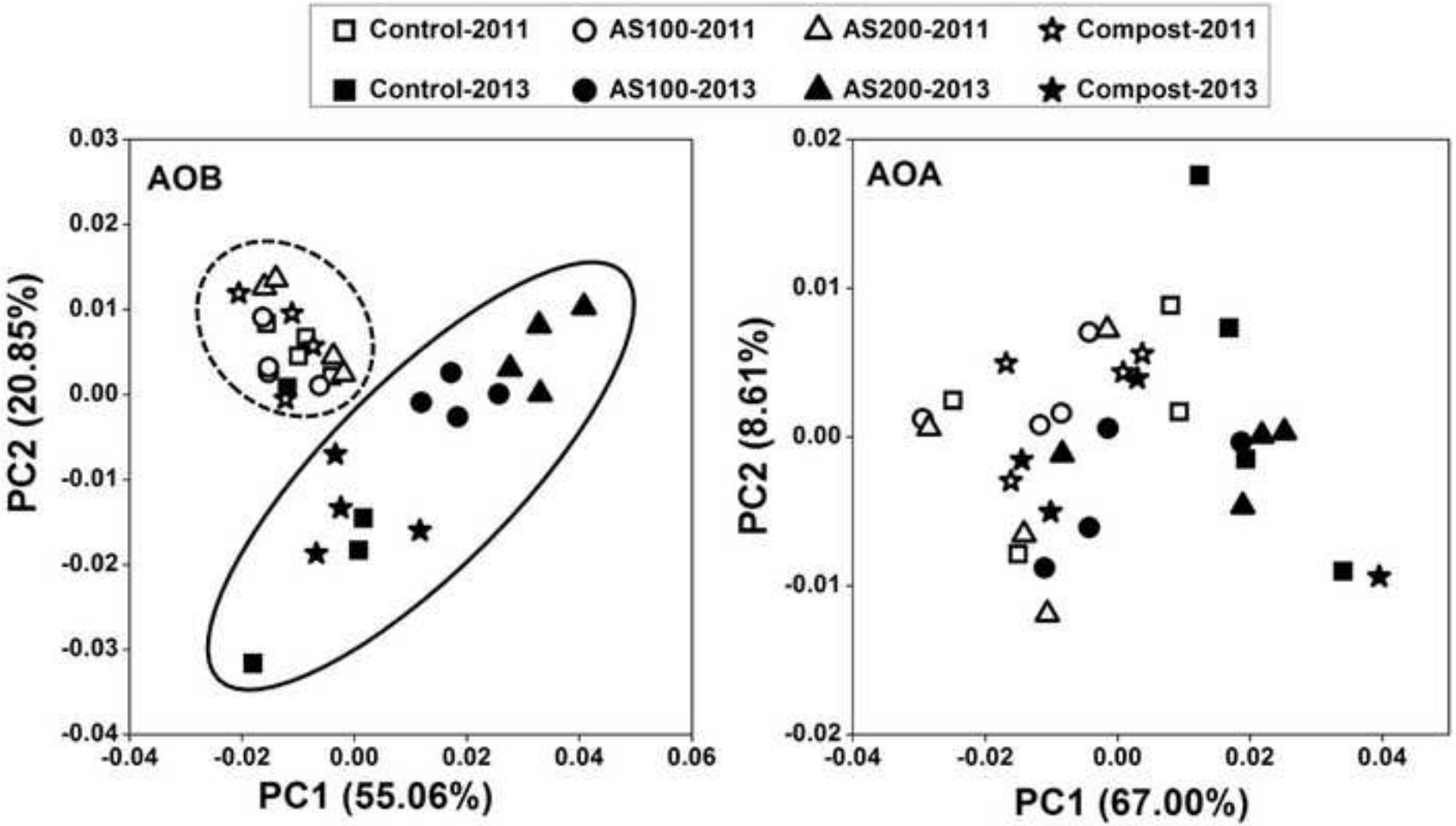
Control-2011
AS100-2011
AS200-2011
Compost-2011
Control-2013
AS100-2013
AS200-2013
Compost-2013
AOB
PC2 (20.85%)
PC1 (55.06%)
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
-0.04
-0.02
0.00
0.02
0.04
0.06
AOA
PC2 (8.61%)
PC1 (67.00%)
0.02
0.01
0.00
-0.01
-0.02
-0.04
-0.02
0.00
0.02
0.04

Figure legends

Fig.1. Soil organic C (**A**), total N (**B**), soil pH (**C**), extractable ammonium (**D**), and extractable nitrate (**E**) in August, and extractable nitrate (**F**) in May for four N treatments (control (no N fertilization), ammonium sulfate (AS 100 & 200 kg N ha^{-1}), and compost (200 kg N ha^{-1})). Error bars represent standard errors (n=4). Different lowercases above the bars indicate a significant difference among treatments in a specific year ($p<0.05$), based on repeated measures ANOVA.

Fig. 2. Nitrification potentials in August over four years. Nitrification potential rate was measured by soil slurry assay supplemented with 1 mM NH_4^+. Error bars represent standard errors (n=4). Different letters above the bars indicate a significant difference among treatments in a specific year ($p<0.05$), based on repeated measures ANOVA.

Fig.3. Recovery of nitrification potential (RNP) in Aug-2013 soil samples (**A**), nitrification potential (NP) with and without 1-octyne in Aug-2013 (**B**), and in May-2014 (**C**). Percentages above the bars indicates the relative contribution of AOB to total RNP or NP. Different uppercases indicate a significant difference in relative contribution of AOB among treatments ($p<0.05$), based on one-way ANOVA.

Error bars represent standard errors (n=4). Different letters above the bars indicate a significant difference in total NP or RNP among treatments ($p<0.05$), based on one-way ANOVA.

Fig. 4. Effect of N treatment and 1-octyne on nitrification potential (**A**), and gross nitrification rate (**B**) in soils sampled in August 2014. Nitrification potential rate was measured by soil slurry assay supplemented with 1 mM NH_4^+. Gross nitrification rate was determined by using N^{15} pool dilution technique with soils incubated at 25 °C for two days. Error bars represent standard errors (n=4). Different lowercases indicate a significant difference among treatments without exposure to octyne ($p<0.05$), based one-way ANOVA. Different uppercases letters indicate a significant difference among treatments with soil samples exposed to octyne ($p<0.05$), based on one-way ANOVA.

Fig. 5. Abundance of AOA and AOB *amoA* gene copy numbers (log_{10} transformed) across four N treatments. Error bars represent standard errors (n=4). Different letters above the bars indicate a significant difference among treatments in a specific year ($p<0.05$), based on repeated measures ANOVA.

Fig. 6. A) Neighbor joining tree for representative AOB partial *amoA* OTUs (representatives with relative abundance > 0.1%). OTUs from this study are shown in bold. The scale bar represents 5% nucleic acid sequence divergence, and bootstrap values (>50%) are showed at branch points. (**B**) The relative abundance of partial AOB *amoA* OTUs (relative abundance > 0.1%) among four N treatments in Aug-2011 and Aug-2013 soil samples. Error bars represent standard errors (n=4).

Fig.7. A) Neighbor joining tree for AOA partial *amoA* OTU (representatives with relative abundance > 0.1%). AOA *amoA* OTU representative sequences from this study are shown in bold. The scale bar represents 5% nucleic acid sequence divergence, and bootstrap values (>50%) are showed at branch points. **B**). The relative abundance of partial AOA *amoA* OTUs (relative abundance > 0.1%) among four N treatments in Aug-2011 and Aug-2013 soil samples Error bars represent standard errors (n=4).

Fig. 8. Principal coordinate analysis based on the abundance of all AOA and AOB *amoA* gene OTUs (weighted UniFrac). For this analysis, The AOA and AOB *amoA* OTUs were normalized to 359 and 219 reads per sample, respectively. Shapes denote treatment (square=Control, circle=AS100, up triangle=AS200, star=Compost). Fill denote sampling time (open=2011, solid=2013).