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Response of microbial biomass and bacterial community composition to fertilization in a salt marsh in China

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Abstract

The effects of nitrogen (N) addition on microbial biomass, bacterial abundance, and community composition in sediment colonized by *Suaeda heteroptera* were examined by chloroform fumigation extraction method, real-time quantitative polymerase chain reaction, and denaturing gradient gel electrophoresis (DGGE) in a salt marsh located in Shuangtai Estuary, China. The sediment samples were collected from plots treated with different amounts of a single N fertilizer (urea supplied at 0.1, 0.2, 0.4 and 0.8 g/kg (nitrogen content in sediment) and different forms of N fertilizers (urea, $(NH_4)_2SO_4$, and NH_4NO_3, each supplied at 0.2 g/kg (calculated by nitrogen). The fertilizers were applied 1–4 times during the plant-growing season in May, July, August, and September of 2013. Untreated plots were included as a control. The results showed that both the amount and form of N positively influenced microbial biomass carbon, microbial biomass nitrogen, and bacterial abundance. The DGGE profiles revealed that the bacterial community composition was also affected by the amount and form of N. Thus, our findings indicate that short-term N amendment increases microbial biomass and bacterial abundance, and alters the structure of bacterial community.

Key words: fertilization, microbial biomass, 16S rRNA gene abundance, bacterial community, salt marsh

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1 Introduction

Salt marshes are among the most abundant, fertile, and accessible coastal habitats on earth, and provide a high number of valuable ecosystem benefits to coastal populations, including raw materials and food, coastal protection, erosion control, water purification, maintenance of fisheries, carbon sequestration, tourism, recreation, education, and research (Gedan et al., 2009; Barbier et al., 2011). However, despite their economic importance, salt marshes are under considerable threat from anthropogenic activities (Gedan et al., 2009; Deegan et al., 2012). Located at the interface between terrestrial uplands and marine waters, salt marshes are vulnerable to perturbations from both the environments (Bowen et al., 2013). The movement of land-derived nutrients to estuaries and other coastal waters has increased owing to the expansion of human activities in the coastal zone (Valiela et al., 1992; Howarth et al., 2002; Cole et al., 2006). Nitrogen (N) loading to estuaries is a major concern among coastal planners. With urban development on coastal watershed, estuaries and bays are becoming more eutrophic, and the cascading effects are being felt at every trophic level (Bowen and Valiela, 2004). In salt marshes, N inputs have been shown to accelerate the turnover of the litter of *Spartina alterniflora* (Valiela et al., 1985) and alter the abundances of *Triglochin maritimum* and *S. alterniflora* (Fitch et al., 2009). Recently, different responses of the bacterial and denitrifying communities to increased N supply in different marsh habitats have been reported (Bowen et al., 2009, 2011, 2013). Irvine et al. (2012) found that N enrichment to salt marsh sediments increased methane flux. Furthermore, Lage et al. (2010) and Peng et al. (2012) reported differential responses of ammonia-oxidizing bacteria/ammonia-oxidizing archaea communities and abundance to fertilization. Ammonia oxidation, carried out by ammonia-oxidizing bacteria and ammonia-oxidizing archaea, is a central process in the sediment N cycle. However, much less is known about how salt marsh sediment microbial biomass and bacterial community respond to fertilizer inputs. In the present study, we hypothesize that N additions at different amounts and forms affect microbial biomass, bacterial abundance, and community composition in the sediment colonized by *Suaeda heteroptera* in a salt marsh located in Shuangtai Estuary, China.

2 Materials and methods

2.1 *Experimental design*

The experiment was conducted in a salt marsh located in Shuangtai Estuary, China, and the study area (~4 800 m^2) was

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dominated by *S. heteroptera*. We selected six fertilization treatments, along with an unfertilized control. Each treatment was applied to three replicate plots. At each plot, a polyvinyl chloride plastic pipe (15 cm×20 cm, inner diameter×height) was driven into the sediments , leaving a height of 2–3 cm of pipe above the ground. The plots were treated with (1) different amounts of a single N fertilizer (urea: 0.1 (A1), 0.2 (A2), 0.4 (A3) and 0.8 (A4) g/kg, and (2) different forms of N fertilizers (urea, $(NH_4)_2SO_4$ (B2), and NH_4NO_3 (B3), each applied at 0.2 g/kg, calculated by nitrogen). Treatments were applied 1–4 times during the plant-growing season (May 15, July 4, August 14, and September 26 of 2013). Untreated plots (A0) were included as controls. Each N amendment was blended in 50 mL of water and injected into the appropriate pipes using a spinal needle. An equivalent volume of deionized water was injected into the control pipes.

2.2 *Sediment sampling*

Before the nitrogen applicatien, the sediment samples (10–15 cm) were collected from each polyvinyl chloride pipe on July 4, August 14, September 26, and November 22 of 2013, placed in sealed plastic bags, and labeled. All the samples were kept on ice in the dark during transport to the laboratory and stored at –20°C until further analysis.

2.3 *Estimation of microbial biomass by chloroform fumigation extraction method*

The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were estimated by the chloroform fumigation extraction method (Wu et al., 2006). A total of six portions of 50 g wet sediment samples were taken from each treatment. Three portions were fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the sediment was extracted with 100 mL of 0.5 mol/L K_2SO_4 by horizontal shaking for 30 min at 300 r/min and then filtered. The other three non-fumigated portions were extracted simultaneously at the time when fumigation commenced. The total organic carbon (TOC) in the extracts was measured using TOC analyzer (TOC-V_{CPH}, Shimadzu, Kyoto, Japan). The MBC was calculated as follows:

$$MBC = E_{\rm C}/k_{\rm EC},$$

where E_C is TOC extracted from fumigated sediments subtract TOC extracted from non-fumigated soils, and k_{EC} is 0.45. Ninhydrin-reactive N in the extracts was measured using ninhydrin colorimetric method (Wu et al., 2006). The MBN was calculated as follows:

$$MBN = mE_{\rm nin\text{-}N},$$

where $E_{nin\text{-}N}$ is ninhydrin-reactive N extracted from fumigated sediments subtract ninhydrin-reactive N extracted from non-fumigated sediments, and m is 5.0.

2.4 *Quantification of 16S rRNA gene by real-time quantitative polymerase chain reaction (qPCR)*

The genomic DNA was extracted from 0.3 g of sample using the fast genomic DNA isolation kit for soils (Shanghai Sangon Biological Engineering Technologyand Services Company Limited, Shanghai, China) according to the manufacturer's instructions. qPCR was performed on a 7500 real-time PCR system (StepOne™, Applied Biosystems, Foster City, CA, USA) using Green-2-Go qPCR Mastermix. The primer set, F357 and R518 (Muyzer et al., 1993), was used for the amplification of the total bacterial 16S rRNA gene. The 20 μL qPCR mixture contained the following: 10 μL of Green-2-Go qPCR Mastermix (2×), 5 pmol of each primer, and 2 μL of template DNA. All the reactions were performed in 8-strip thin-well PCR tubes with ultraclean cap strips. The qPCR thermocycling parameters were as follows: an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and primer annealing at 60°C for 60 s. The plasmids constructed by cloning the sediment samples were used as standards. The copy number of the standard plasmids carrying the 16S rRNA genes ranged from 1.34×10^3 to 1.34×10^8. The qPCRs of the standards and DNA from each sample were performed in triplicate. The specificity of qPCR amplification was determined from the melting curve. In all the experiments, negative controls without template DNA were subjected to the same qPCR procedure to detect and exclude any possible contamination. The gene abundance was normalized per gram of wet sediment.

2.5 *Bacterial community analysis by denaturing gradient gel electrophoresis (DGGE)*

The community DNA was extracted from 0.6 g of the pooled samples from the three replicate plots collected on September 26 of 2013 using a fast genomic DNA isolation kit for soils (Shanghai Sangon Biological Engineering Technology and Services Company Limited, Shanghai, China) following the manufacturer's instructions. The extracted DNA was amplified by PCR using the bacteria-specific primer set, 357F-GC and R518 (Muyzer et al., 1993), in a 50 μL reaction mixture containing 5 μL of 10× PCR buffer, 3 μL of template, 200 μmol/L of dNTPs, 1.5 mmol/L of $MgCl_2$, 0.4 μmol/L of each primer, and 0.5 U of *Taq* polymerase. After 5 min of initial denaturation at 94°C, the following procedure was applied: 1 min of denaturation at 94°C, 1 min of initial annealing at 65°C, which was decreased by 0.5°C per cycle until a touchdown of 55°C, 1 min of primer extension at 72°C, followed by 10 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of primer extension at 72°C, and a final extension at 72°C for10 min. The amplification products were analyzed by electrophoresis in 1% (w/v) agarose gel containing GoldView.

The Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used for the sequence-specific separation of the PCR products. The PCR samples were directly applied onto 8% (w/v) polyacrylamide gels in a running buffer (1% tris-acetate-EDTA). The gels were prepared with a denaturing gradient from 30% to 60% of urea and formamide with a polyacrylamide and bis-acrylamide ratio of 37.5:1. Electrophoresis was performed at a constant voltage of 70 V and constant temperature of 60°C for 16 h. Subsequently, the gels were stained with Genfinder according to the manufacturer's instructions and photographed under UV transillumination. The DGGE fingerprint patterns were analyzed using the Quantity One Software (Bio-Rad Laboratories). Presence-absence matrices were used to determine differences between the DGGE patterns and dendrogram was constructed using the Dice coefficient and the unweighted pair-group method with arithmetic average algorithms. These analyses were performed in the NTSYS software package (version 2.10e, Exeter Software, Setauket, New York).

The selected DGGE bands were excised from the DGGE gel, eluted, and reamplified with the same primers but without GC-clamp. The PCR products were sequenced by Shanghai Sangon Biological Engineering Technology and Services Company Limited (Shanghai, China). The sequences of the DGGE bands were aligned using the ClustalW program (www.ebi.ac.uk/clustalw) and compared with other bacterial 16S rRNA gene sequences available in the NCBI database of GenBank (http://www.ncbi.nlm.nih.

gov/BLAST). The construction of a neighbor-joining tree was based on the Kimura-2-parameter distances, and a bootstrap analysis of 1 000 resamplings was performed using MEGA 4 (Tamura et al., 2007).

2.6 *Data analysis*

One-way analysis of variance (ANOVA) and Tukey's test were performed to determine whether the microbial biomass and bacterial abundance differed among the treatments. A two-way ANOVA was used to analyze the effects of the amount or form of N and sampling date, as well as their interaction on the microbial biomass and bacterial abundance.

3 Results

3.1 *Effects of N fertilization on MBC and MBN*

Applications of 0.1 and 0.2 g N resulted in significantly higher MBC, when compared with that noted in the control, over 6 months following N application (Fig. 1, P<0.05), while the addition of 0.4 and 0.8 g N significantly increased MBC, when compared with that in the control, across all the sampling dates (Fig. 1, P<0.05), except August. Furthermore, application of 0.2 g N significantly enhanced MBC, when compared with that found in other treatments at all the sampling dates (Fig. 1, P<0.05). However, there were no significant differences in MBC among 0.1, 0.4, and 0.8 g N applications across all the sampling dates. A two-way ANOVA showed that MBC was significantly affected by the amount of N and sampling date (Table 1, P<0.05); however, no interaction was found between the factors (Table 1).

The addition of urea, $(NH_4)_2SO_4$, and NH_4NO_3 significantly increased MBC, when compared with that observed in the control across all the sampling dates (Fig. 2, P<0.05), with the exception of NH_4NO_3 application in July and $(NH_4)_2SO_4$ addition in September. Significant differences in MBC were observed between urea and NH_4NO_3 applications in July (Fig. 2, P<0.05) and between urea and $(NH_4)_2SO_4$ applications across all the sampling dates (Fig. 2, P<0.05), with the exception of November. However, there were no differences in MBC between $(NH_4)_2SO_4$ and NH_4NO_3 applications at all the sampling dates. Thus, the MBC was significantly affected by both the N form and sampling date, and a significant interaction was found between the factors (Table 1, P<0.05).

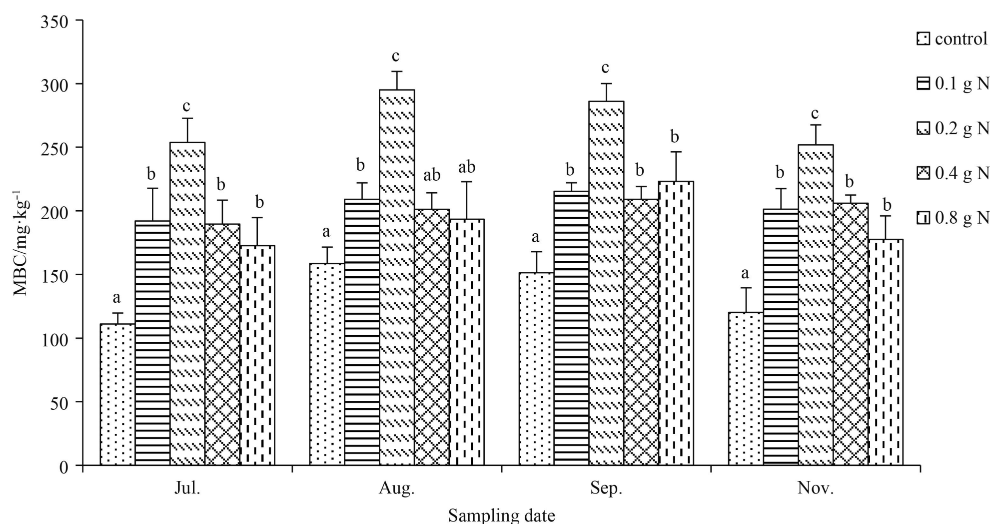

Fig. 1. Effects of nitrogen amounts on microbial biomass carbon. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

Table 1. Probability values of factors influencing microbial biomass carbon, microbial biomass nitrogen and bacterial 16S rRNA gene abundance

Factor	MBC	MBN	16S rRNA gene abundance
N amount	0.000	0.000	0.000
Sampling date	0.000	0.000	0.000
N amount×sampling date	0.332	0.494	0.210
N form	0.000	0.006	0.000
Sampling date	0.000	0.000	0.000
N form×sampling date	0.002	0.133	0.177

The addition of 0.1 and 0.2 g N resulted in significantly higher MBN, when compared with that found in the control, over 6 months following N application (Fig. 3, P<0.05), while the addition of 0.4 g N significantly increased MBN, when compared with the control, across all the sampling dates (Fig. 3, P<0.05), except September. Moreover, application of 0.8 g N merely led to a significantly higher MBN in September and November, when compared with the control (Fig. 3, P<0.05), while 0.2 g N application significantly enhanced MBN, when compared with 0.1 g N addition in August and September (Fig. 3, P<0.05) and 0.4 and 0.8 g N applications across all the sampling dates (Fig. 3, P<0.05). Nevertheless, there were no significant differences in MBN following 0.1, 0.4, and 0.8 g N applications across all the sampling dates. Thus, MBN was significantly affected by the N amount and sampling date (Table 1, P<0.05); however, no interaction was found between the factors (Table 1).

The addition of urea, $(NH_4)_2SO_4$, and NH_4NO_3 significantly increased MBN, when compared with that observed in the control, across all the sampling dates (Fig. 4, P<0.05), with the exception of $(NH_4)_2SO_4$ application in July and September and NH_4NO_3 addition in July. Furthermore, significant differences in MBN were observed only in September following urea and $(NH_4)_2SO_4$ applications (Fig. 4, P<0.05). However, there were no differences in MBN following urea and NH_4NO_3 applications, and between $(NH_4)_2SO_4$ and NH_4NO_3 applications at all the sampling dates. Thus, MBN was significantly affected by both the

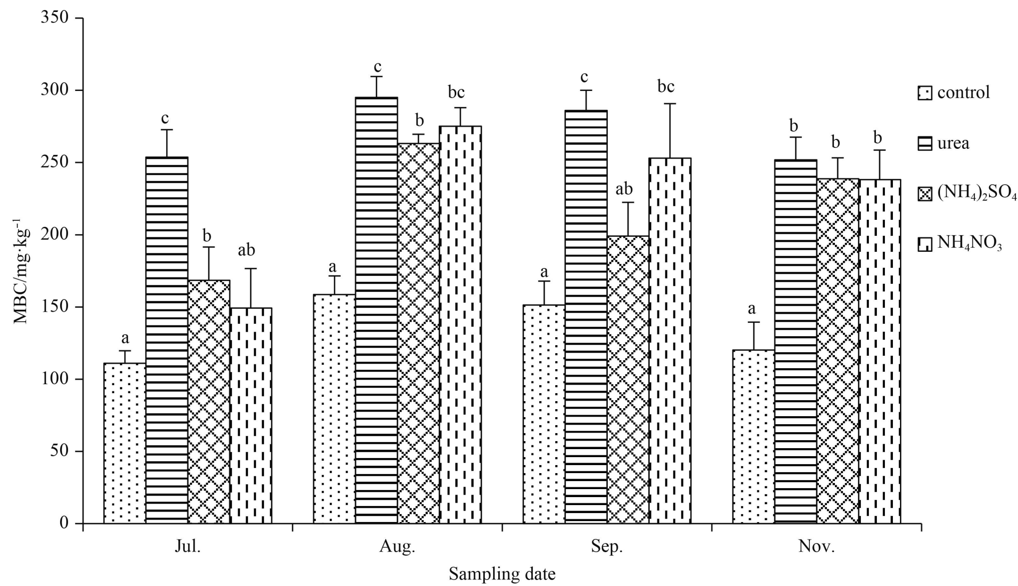

Fig. 2. Effects of nitrogen forms on microbial biomass carbon. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

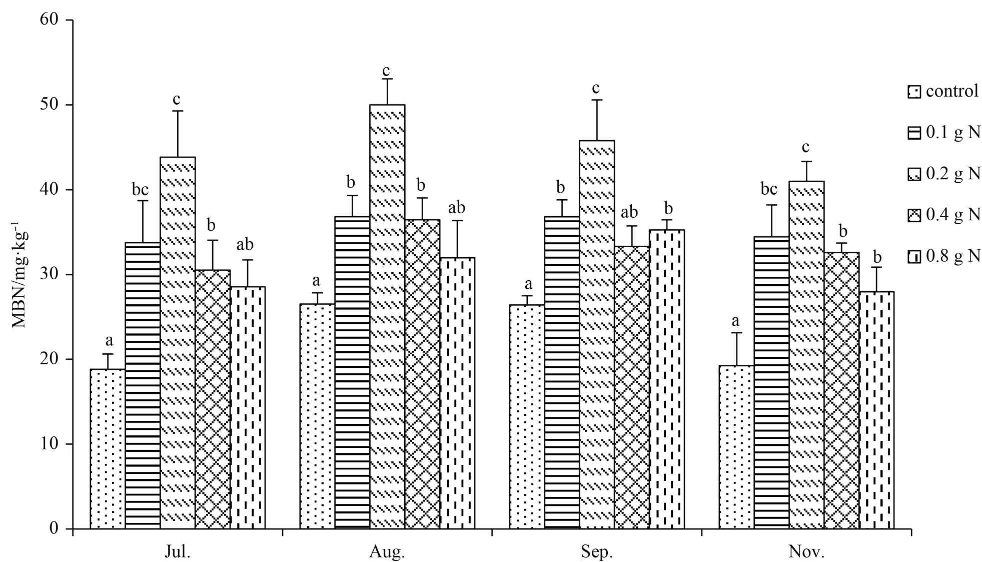

Fig. 3. Effects of nitrogen amounts on microbial biomass nitrogen. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

N form and sampling date (Table 1, P<0.05); however, no significant interaction was found between the factors (Table 1).

3.2 *Effects of N fertilization on bacterial abundance*

The abundance of total bacteria detected with qPCR by targeting universal bacteria-specific 16S rDNA regions showed significant increase across treatments with various amounts of N, when compared with the control at all the sampling dates (Fig. 5, P<0.05), with the exception of 0.8 g N application in August and September. Moreover, the addition of 0.1, 0.2, and 0.4 g N significantly enhanced bacterial 16S rRNA gene abundance, when compared with 0.8 g N application at all the sampling dates (Fig. 5, P<0.05). However, there were no differences in the 16S rRNA gene abundance among treatments with 0.1, 0.2, and 0.4 g N at all the sampling dates. Thus, the bacterial 16S rRNA gene abundance was significantly affected by the N amount and sampling date (Table 1, P<0.05); however, no interaction was found between the factors (Table 1).

The applications of urea, $(NH_4)_2SO_4$, and NH_4NO_3 resulted in significantly higher bacterial abundance, when compared with that noted in the control across all the sampling dates (Fig. 6, P<0.05). Moreover, significant differences in the bacterial abundance were observed between urea and $(NH_4)_2SO_4$ applications in July, August and November (Fig. 6, P<0.05), between urea and NH_4NO_3 applications only in August (Fig. 6, P<0.05), and between $(NH_4)_2SO_4$ and NH_4NO_3 applications in July alone. Thus, bacterial abundance was significantly affected by both the N form and sampling date (Table 1, P<0.05), and no significant interaction was found between the factors (Table 1).

3.3 *Shifts in bacterial community composition*

The DGGE patterns from the sediments were different among

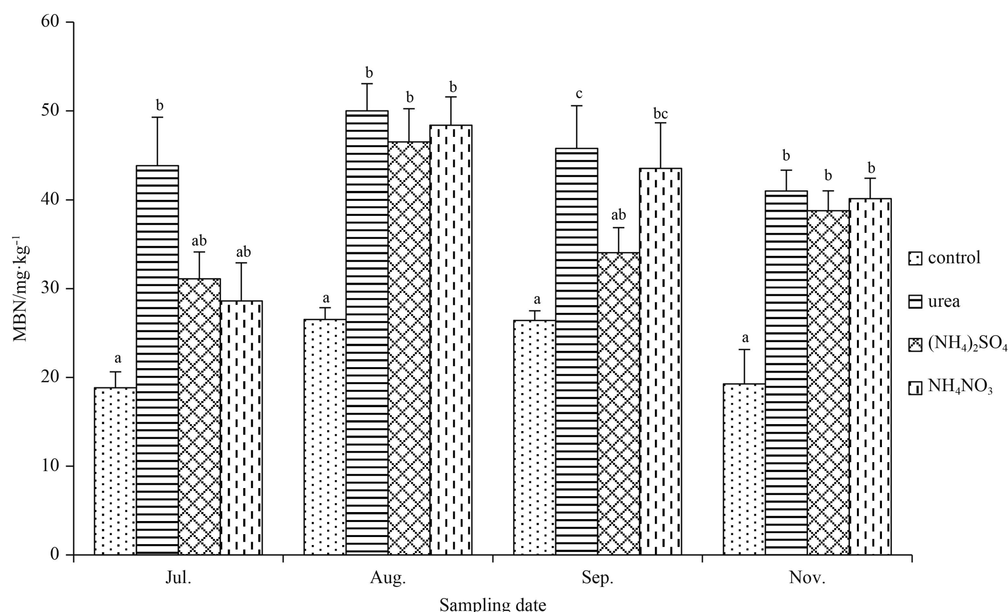

Fig. 4. Effects of nitrogen forms on microbial biomass nitrogen. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

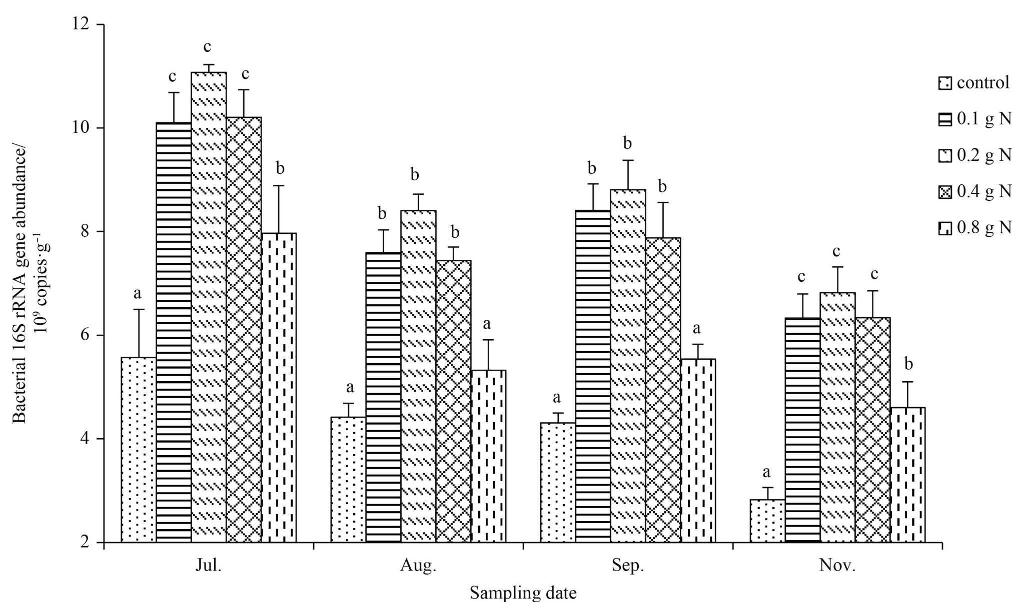

Fig. 5. Effects of nitrogen amounts on 16S rRNA gene abundances. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

the treatments, and numerous discrete DGGE bands, resulting from the differences between the 16S rRNA gene sequence of different bacterial species, were apparent (Fig. 7). Each band represented at least one unique ribotype. A higher number of ribotypes was detected in the control sediment (23 bands), while the least number of ribotypes was detected in the sediment with 0.2 g N application (16 bands) among different concentrations of N treatments, and in the sediment with NH_4NO_3 application (12 bands) among different forms of N treatments (Fig. 7). The different banding patterns among the sediments tested suggested that each sediment contained different bacterial community composition. Cluster analysis of the DGGE profiles revealed that A0 and B2 belonged to one clade, while A1, A3, A4, A2 and B3 formed the other clade. These findings indicated that treatments with 0.1, 0.2, 0.4 and 0.8 g N, and NH_4NO_3 had significant effect on the sediment bacterial community structure; however, there were no significant differences in the community between the control and $(NH_4)_2SO_4$ treatment (Fig. 8). The BLAST analysis of the 16S rRNA gene sequences obtained from the DGGE bands classified the sequences into six classes, namely, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, Bacilli and Flavobacteria, and unclassified bacteria (Table 2, Fig. 9).

4 Discussion

We observed enhanced sediment MBC and MBN in response to N fertilization during the growing season of *S. heteroptera* (Figs

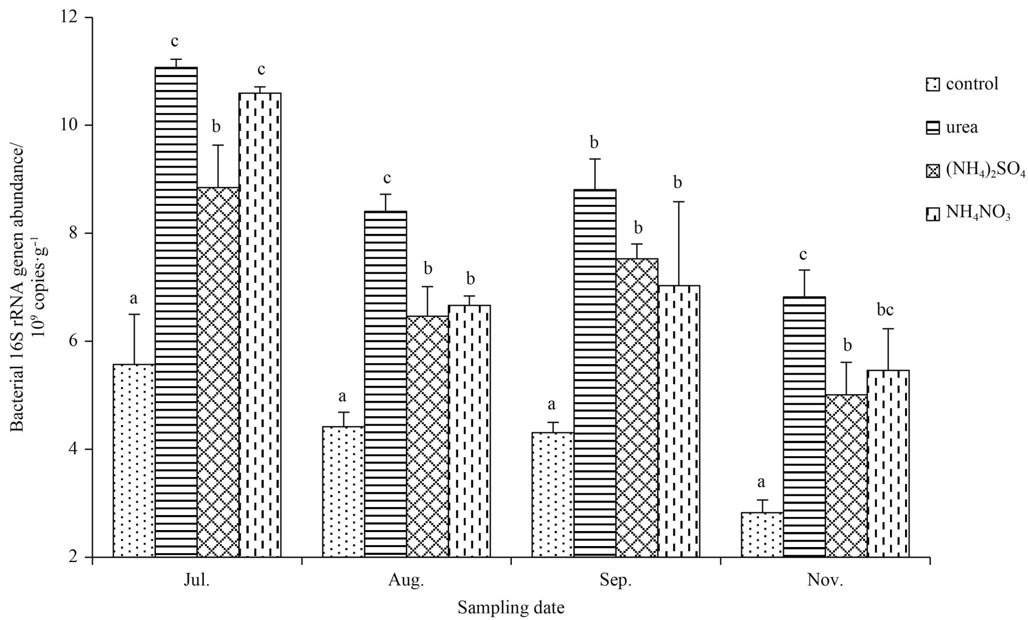

Fig. 6. Effects of nitrogen forms on 16S rRNA gene abundance. Means (n=3) with different letters at same sampling date are significantly different (P<0.05).

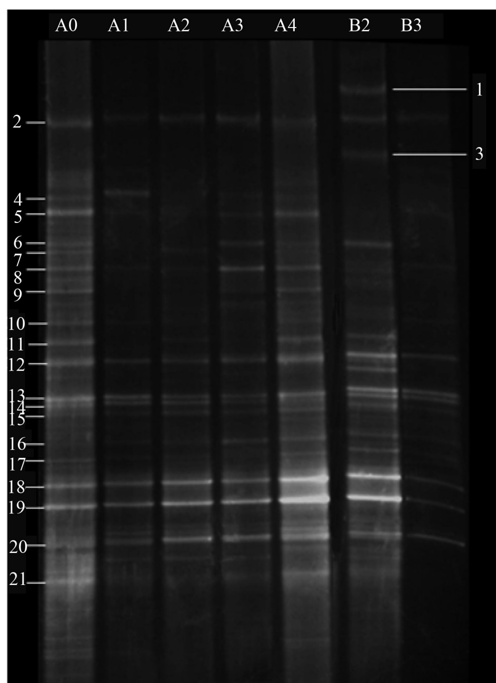

Fig. 7. DGGE profile of PCR-amplified 16S rRNA gene (bands marked were excised and sequenced).

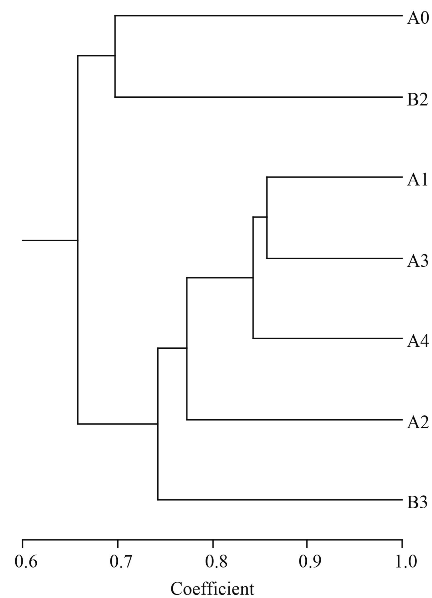

Fig. 8. Dendrogram comparing similarity levels of the bacterial community in different sediment samples.

1–4), similar to that noted in several previous studies on microbial biomass in fertilized soils (Zhang and Zak, 1998; Alon and Steinberger, 1999; Cusack et al., 2011; Zhou et al., 2012). The N addition of 50 g/m^2 was reported to significantly increase MBC over 4 months (Zhang and Zak, 1998), while N amendments (25, 50, and 100 kg/hm^2 calculated by NH_4NO_3) were demonstrated to result in a significant increase in soil MBC in Negev Desert soil over a 1-a study period (Alon and Steinberger, 1999). In addition, Cusack et al. (2011) found that microbial biomass increased in response to long-term (4–6 a) N addition (50 kg/(hm^2·a), calculated by NH_4NO_3-N) in two N-rich tropical rain forests. Furthermore, the addition of N (6 and 24 g/(m^2·a)) as NH_4^+:NO_3^- (2:1, NH_4NO_3 and NH_4Cl) for 1 and 2 a resulted in an increase in MBN in a Gurbantunggut Desert soil (Zhou et al., 2012). Nevertheless, the effect of N fertilization on soil microbial biomass is controversial. Some studies have shown that soil microbial biomass remained unaffected (Zak et al., 2006; Liu et al., 2007) or decreased following N fertilization (Compton et al., 2004; Wallenstein et al., 2006; Treseder, 2008; Li et al., 2010; Ramirez et al., 2012). These contrasting responses may arise from the differences in the duration and amount of N added, the form in which N was applied, and the N demand or N saturation of the sites investigated. In the present study, the increases in the MBC and MBN following N

Table 2. Blast analysis of the 16S rRNA gene sequences derived from DGGE bands

Band	Closest match	Accession No.	Similarity/%
1	Uncultured bacterium clone GBc170	JQ612260	99
2	Uncultured Nitrosomonadales bacterium isolate DGGE gel band AOBseq_3a	HQ455816	100
3	Uncultured bacterium clone LGH02-B-026	HQ916556	100
4	*Aerococcus* sp. bw5	KR263090	100
5	*Pseudomonas* sp. 271	KT034417	100
6	Uncultured bacterium clone HTC91	KJ664620	99
7	*Methylobacterium fujisawaense* strain MECA_19.1.2	KT720195	100
8	Uncultured bacterium clone KS50	KT239336	100
9	Uncultured bacterium clone YU-24-D	AB780331	100
10	Uncultured bacterium isolate DGGE gel band 36	JF504660	92
11	*Shigella flexneri* strain B1.65	KU195386	100
12	*Sphingomonas paucimobilis* strain Ph_03A5.1	KT719969	100
13	Uncultured bacterium clone Intial19	KT900352	99
14	Uncultured bacterium clone Intial19	KT900352	99
15	Uncultured alpha proteobacterium clone MS139A1_G02	EF699761	99
16	Uncultured bacterium clone LNH_12_1_11_Water.117868	KM141506	100
17	Uncultured bacterium, clone MlH4	FN567511	99
18	*Sphingomonas paucimobilis* strain MSL_3038	KT719876	99
19	*Sphingomonas paucimobilis* strain AS14b	KT892648	100
20	*Actinokineospora* sp. SCAU5231	KT182464	100
21	Uncultured *Owenweeksia* sp. clone C146100416	JX530590	100

addition may be owing to the increase in available N (unpublished data), which would have been immobilized by the microorganisms, leading to an increase in soil microbial biomass in the sediment.

In recent times, qPCR has become a powerful technique for the quantitative analysis of bacteria from environmental samples (Dang et al., 2010; Zhang et al., 2011; Tsuboi et al., 2013; Luo et al., 2014). In the present study, qPCR, which is a culture-independent method, was applied to investigate the bacterial abundance in the sediment following treatments with different amounts and forms of N. The sediment bacterial 16S rRNA gene abundance was found to significantly increase following N addition (Figs 5 and 6). Similarly, in a previous study, a positive response of soil bacterial number to short-term (10 months) N addition (NH_4NO_3) was reported in a nursery of Dinghushan Biosphere Reserve, and the available N in the soil was significantly correlated with the number of bacteria (Xue et al., 2007). Moreover, inorganic chemical fertilizer management adoption for more than 100 a significantly increased the abundance of 16S rRNA gene in a semi-arid tropical soil (Chinnadurai et al., 2014). However, Bowen et al. (2011) found that the 16S rRNA gene abundance was unaffected by significant variations in the external nutrient supply in the sediment of the Great Sippewissett Marsh. In addition, long-term (20 and 34 a) application of N fertilizer (urea) was noted to result in a significant decrease in the 16S rRNA gene abundance (Shen et al., 2010; Zhou et al., 2015), which may be explained by the low soil pH (Shen et al., 2010; Zhou et al., 2015) and TOC following N treatment (Shen et al., 2010).

In the present study, we found clear shifts in the bacterial community corresponding to fertilization (Figs 7–9, Table 2), which are in agreement with those reported in previous studies on bacteria in fertilized soil and sediment (Nicol et al., 2004; Bowen et al., 2009; Ramirez et al., 2010, 2012). The soil microcosm experiments performed by Nicol et al. (2004) to examine the effect of NH_4NO_3 application on the bacterial community in unmanaged grassland soil revealed distinct shifts in the bacterial community, as indicated by the DGGE profiles, following NH_4NO_3 application for 30 days. Furthermore, Bowen et al. (2009) studied the effects of fertilization on the bacterial structure in the sediments from five habitats within the marsh. Their results indicated the occurrence of a direct response of the microbial community to the addition of N and phosphorus fertilizers only in one salt marsh habitat, namely, a region of the marsh creek wall heavily colonized by filamentous algae, suggesting that a shift in the carbon supply from the filamentous algae could have led to the differentiation in the microbial community. Nevertheless, deep pyrosequencing of the 16S rRNA gene did not reveal any fertilization effect on the composition of the bacterial community in the salt marsh sediments (Bowen et al., 2011). It must be noted that plant growth was significantly affected by N application in the present study (data not shown). Therefore, further research is necessary to determine whether the bacterial community shifts are a direct result of N addition or an indirect result of carbon supply to the sediment from *S. heteroptera*.

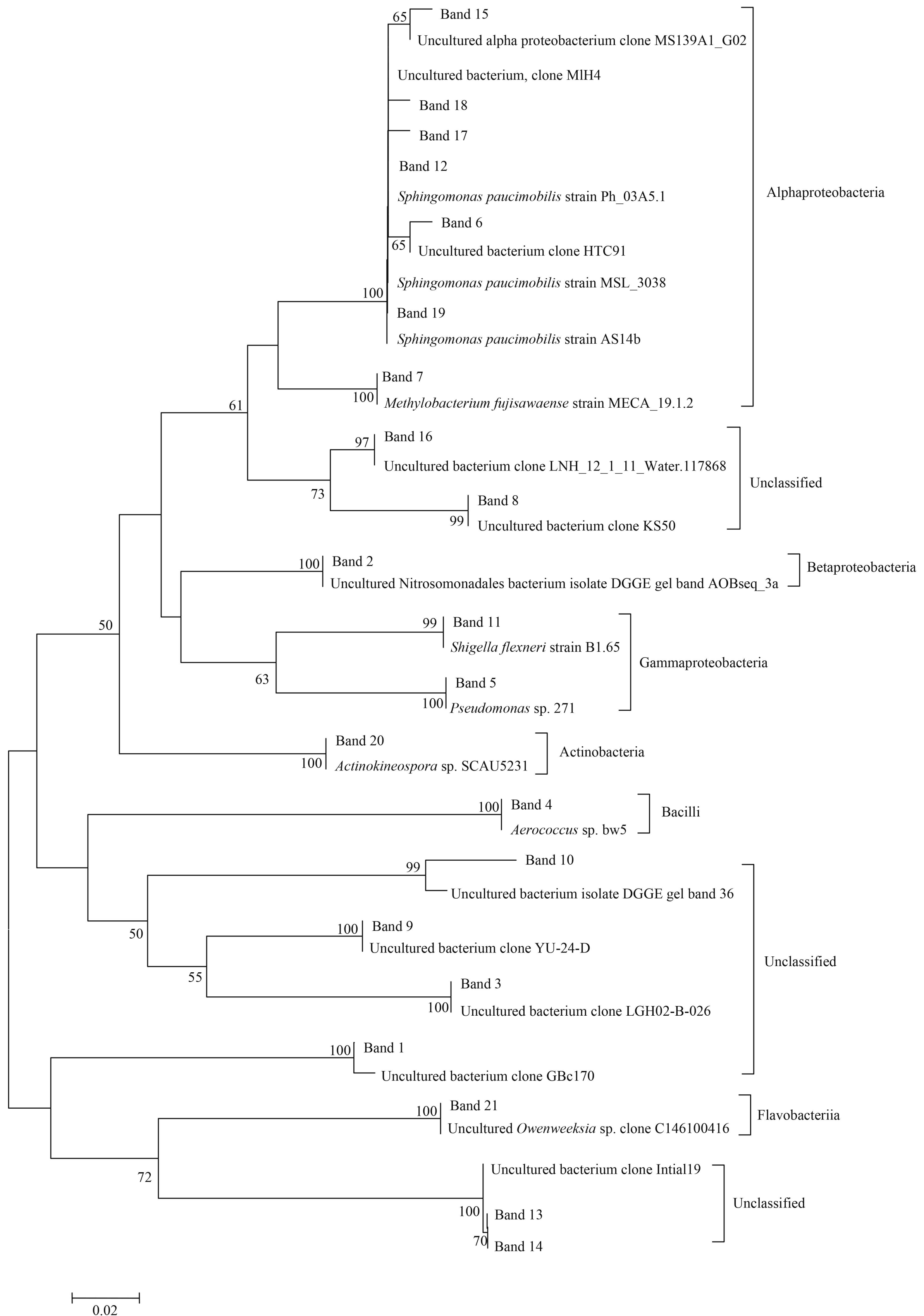

Fig. 9. Phylogenetic relationships among the bacterial 16S rRNA gene nucleotide sequences recovered from the salt marsh plots. The tree construction was based on the 16S rRNA gene sequences of the excised DGGE bands using the neighbor-joining algorithm with the Kimura two-parameter correction. Bootstrap values greater than 50% are shown at the internal nodes. The scale bar represents a 2% estimated difference in nucleotide sequences.

5 Conclusions

Application of N increased MBC, MBN, and bacterial 16S rRNA gene abundance. Moreover, both the amount and form of N affected the above-mentioned microbial parameters and changed the bacterial diversity and community composition. To the best of our knowledge, this is the first study to demonstrate the effect of N application on sediment microbial biomass and bacterial abundance in a salt marsh ecosystem.

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