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ENVIRONMENTAL BIOTECHNOLOGY

Impact of elevated CO_2 and N addition on bacteria, fungi, and archaea in a marsh ecosystem with various types of plants

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Abstract The individual effects of either elevated CO_2 or N deposition on soil microbial communities have been widely studied, but limited information is available regarding the responses of the bacteria, fungi, and archaea communities to both elevated CO_2 and N in wetland ecosystems with different types of plants. Using a terminal restriction fragment length polymorphism (T-RFLP) analysis and real-time quantitative PCR (RT-Q-PCR), we compared communities of bacteria, fungi, and archaea in a marsh microcosm with one of seven macrophytes, *Typha latifolia*, *Phragmites japonica*, *Miscanthus sacchariflorus*, *Scirpus lacustris*, *Juncus effusus*, *Phragmites australis*, or *Zizania latifolia*, after exposing them to eCO_2 and/or amended N for 110 days. Overall, our results showed that the elevated CO_2 and N may affect the bacterial and archaeal communities, while they may not affect the fungal community in terms of both diversity and abundance. The effects of elevated CO_2 and N on microbial community vary depending on the plant types, and each microbial community shows different responses to the elevated CO_2 and N. In particular, elevated CO_2 might force a shift in the archaeal community irrespective of the plant type, and the effect of elevated CO_2 was enhanced when combined with the N effect. This study indicates that elevated CO_2 and N addition could lead to changes in the community structures of bacteria and archaea. Our results also suggest that the fungal group is less sensitive to external changes, while the bacterial and archaeal groups are more sensitive to them. Finally, the characteristics of the plant type and relevant physicochemical factors induced by the elevated CO_2 and N may be important key factors structuring the microbial community's response to environmental change, which implies the need for a more comprehensive approach to understanding the pattern of the wetland response to climate change.

Keywords Elevated CO_2 · N deposition · Bacteria · Fungi · Archaea · Community composition · Wetland

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Introduction

Atmospheric CO_2 concentrations have been increasing dramatically as a result of anthropogenic activities, and future estimates of the atmospheric CO_2 concentration for the year 2050 range between 450 and 600 ppm (IPCC 2007). Ever-growing fertilizer use and atmospheric N deposition are regarded as key factors affecting the nutrient cycle of wetland ecosystems. As elevated CO_2 and N deposition increases the supply of carbon to the soil ecosystem and lessen the N limitation of the soil, this could affect the nutrient cycle of soil ecosystems substantially (Bragazza et al. 2012). Wetlands are known to play a key role in the global biogeochemical cycle, including the flux of trace gas (Schimel and Gulledge 1998; Limpens et al. 2008), which is essentially mediated by microorganisms. Many studies about the effects of global climate changes on microbial communities have been conducted in various types of ecosystems, including

wetlands (Kim et al. 2008; Toberman et al. 2008; Drigo et al. 2009; Blagodatskaya et al. 2010; Castro et al. 2010; Edwards and Zak 2011; Weber et al. 2011; Deng et al. 2012; Dunbar et al. 2012; Eisenhauer et al. 2012; Gutknecht et al. 2012; He et al. 2012; Kim et al. 2012; Ramirez et al. 2012; Eisenlord et al. 2013; Andresen et al. 2014).

However, the majority of these studies have focused mainly on the responses of bacteria and fungi, and little attention has been paid to the effects of elevated CO_2 or N on the total archaea community (Nelson et al. 2010; Hayden et al. 2012; Long et al. 2012; Peng et al. 2013), even though the roles of archaea in C and N cycling have been shown to be much more important than previously reported (Offere et al. 2013). Additionally, most of these studies were performed focusing on the individual effect of a single one of those factors in terrestrial ecosystems such as grasslands or forests.

Considering the importance of wetland ecosystems, it is therefore necessary to determine how microbial communities in wetland ecosystems respond to elevated CO_2 and N in order to gain a better understanding of the overall effects of elevated CO_2 and N on natural ecosystems.

The plant type is known to be an important factor determining microbial community structures, and many studies have identified the rhizosphere community composition as specific to the plant species (Marschner et al. 2001; Smalla et al. 2001; Chung et al. 2007; Bomberg and Timonen 2009; Wang and Gu 2013). As such, the responses of bacteria, fungi, and archaea to elevated CO_2 and/or N are expected to differ according to the plant type.

In this study, we examined the effects of elevated CO_2 and/or N addition on the dynamics of a community structure of total bacteria, fungi, and archaea with various plant types using a temperate marsh microcosm. We hypothesized that i) elevated CO_2 or N addition causes a change in the microbial community structure; ii) elevated CO_2 has positive effects on three types of microbial biomasses, but N addition has a negative effect on the fungal biomass; and iii) the effect of elevated CO_2 and N on the microbial community is different according to the plant types, especially between C3 and C4 plants. For the analysis of the microbial community structure, we employed molecular methods, including T-restriction fragment length polymorphism (T-RFLP) and real-time quantitative PCR (RT-Q-PCR) targeting the bacterial and archaeal 16S ribosomal RNA (rRNA) gene and the fungal ITS gene.

Materials and methods

Experimental setting

Among emergent macrophytes commonly found in natural wetlands of temperate regions, *Typha latifolia*, *Phragmites japonica*, *Miscanthus sacchariflorus*, *Scirpus lacustris*, *Juncus effusus*, *Phragmites australis*, and *Zizania latifolia* were selected for this study. These species are widely naturalized in many rivers and streams in temperate regions of Asia, including Korea (Yang and Kim 2007). After 4 week of incubations of vegetative propagules (1 year old) in shallow indoor containers, propagules showing new shoots were selected and then planted into cores (10-cm diameter×20-cm deep) packed with sandy soil (organic matter content <0.1 %) (Kim and Kang 2008). Before planting, roots were washed thoroughly with tap water and then weighed. Four plant-soil cores of each of the seven plant species were randomly assigned to one of four containers (70×70×25 cm^3) having two different N concentrations (0 mg N/L and 8.8 mg N/L). Two containers with same N concentration was assigned to one of two growth chambers (140×74×1200 cm^3, Dasol Science, Korea), simulating ambient (380±21.4 ppm) or elevated (757±20.6 ppm) atmospheric CO_2 conditions. Growth chambers simulated temperate diurnal temperature gradients typical of the growing season (18–23 °C). Photoperiod radiation (11,000 lux) exposure lasted 12 h, and the skotoperiod was 10 h, with 1-h dawn and dusk transitions. Humidity was maintained at 70 % during incubation. Water levels in the containers were checked weekly and were adjusted with distilled water to compensate for evapotranspiration losses. Soil cores within each container were rotated twice a month to minimize possible chamber effects, if any, and were incubated for 110 days.

Measurement of plant growth and biogeochemical factors

After incubation, shoots were cut out at the soil surface and their base washed to remove any adhering sediments. Each core was then excavated and wet sieved (2-mm mesh) to recover below-ground plant materials. All above- and below-ground tissue samples were then weighed after drying to constant weight in an oven at 75 °C. Soils at the depth of 0–10 cm were removed to measure various biogeochemical parameters such as pH, extracellular enzyme activities, and root-derived C including dissolved organic carbon (DOC) and phenolics. pH of soil samples was analyzed using a pH meter. Extracellular enzyme activities of β-glucosidase, N-acetylglucosaminidase, phosphatase, and arylsulfatase were determined to assess general microbial activities in samples. Those enzymes are involved in carbon, nitrogen, phosphorus, and sulfur mineralization, respectively. Four types of enzyme activity were measured using methylumbelliferyl compounds as a model substrate (Freeman et al. 1995). Soil (1 cm^3) was gently homogenized with 9 ml of substrate solution for 1 h. After centrifuging at 10,000 rpm for 5 min, fluorescence of the supernatant aliquot was determined with a

fluorometer at 450-nm emission and at 320-nm excitation wavelength (TD-700, Turner Designs, USA). For every treatment, a quench standard was made with standard solution (10 μM 4-methylumbelliferone) plus soil sample to correct interference of phenolics. Samples for the measurement of extractable DOC were prepared by adding deionized water (9 ml) to soil (1 cm^3) and shaking for 10 min. After centrifuging at 10,000 rpm for 5 min, samples were passed through a 0.45-μm filter and frozen until analyses. DOC was measured by a TOC analyzer (Shimadzu, Model TOC-5000, Japan). Phenolic contents were determined using a Folin-Ciocalteu phenol reagent (Box 1983).

Molecular analysis of microbial communities in soil

Soil samples collected for measurement of biogeochemical parameters were also used for DNA extraction. From about 0.5 g of each soil sample, DNA was isolated using an UltraClean Soil DNA Isolation Kit (MoBio, USA), as specified by the manufacturer. DNA samples were amplified by PCR using the fluorescently labeled forward primer 27 F (5′-[6FAM]-AGAGTTTGATCCTGGCTCAG-3′) and the unlabeled reverse primer 927R (5′-CCGTCAATTCCTTTRAGTTT-3′), which target bacterial 16S rRNA genes (Lane 1991). For the fungal community, PCR was performed using the fluorescently labeled forward primer ITS1F (5′-[HEX]-CTTGGTCATTTAGAGGAAGTAA-3′) and the unlabeled reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), which target the fungal internal transcribed spacer (ITS) region of the rRNA gene (White et al. 1990; Gardes and Bruns 1993). For the archaeal community, PCR was performed using the unlabeled forward primer Arch109F (5′-ACKGCTCAGTAACACGT-3′) and the fluorescently labeled reverse primer Arch915R (5′-[NED]-GTGCTCCCCCGCCAATTCCT-3′), which target the archaeal 16S rRNA gene (Stahl and Amann 1991; Großkopf et al. 1998). Each PCR reaction was performed with a total volume of 50-μl reaction mixture containing approximately 100 ng of template DNA, PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 20 mM Tris–HCl [pH 8.4], 0.1 % Triton X-100), 200 μM of dNTPs, 2.0 U of *Taq* polymerase (Promega, USA), 4 μg of bovine serum albumin (Sigma, USA), and 25 pmol of each primer. All PCR was performed using a MJ Research thermal cycler PTC 100 (MJ Research, Waltham, MA) with the following program: 5 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 50 °C (for *Bacteria* and *Fungi*) or 52 °C (for *Archaea*), and 72 °C for 1 min 30 s, and a final extension step at 72 °C for 10 min. To minimize the error due to unequivocal distribution of DNA and PCR bias, two replicate PCR reactions were performed and pooled for each sample. For T-RFLP analysis, the pooled PCR products were purified using a NucleoSpin® Extract II PCR clean-up Gel extraction kit (MACHERY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions. Approximate 300 ng of purified PCR product were added to a reaction mixture (final volume 25 μl) containing 10 U of restriction endonuclease *Hha*I (Promega, Madison, WI) (for bacterial 16S rRNA and fungal ITS) or *Taq*I (for archaeal 16S rRNA gene) and incubated at 37 °C except archaea (65 °C) for 4 h. The digests were desalted using SigmaSpin Post-Reaction Purification Column (Sigma, USA), and aliquots (1 μl) were used for T-RFLP analysis. The terminal fragment size analysis was performed using an ABI 3730 DNA Analyzer (Applied Biosystems) in conjunction with GeneScan software (Applied Biosystems). Terminal reaction fragments (T-RFs) were quantified via peak area integration using a minimum peak height threshold of 50 relative fluorescent units. We excluded T-RFs with a size less than 35 bases and calculated the proportion of each T-RF in each sample. T-RFs having a proportion less than 0.1 % were excluded from subsequent analyses.

Real-time quantitative PCR

To estimate the bacterial, fungal, and archaeal biomass, we performed q-PCR using CFX96 (Bio-Rad, Hercules, CA) and SYBR Green as a detection system (Bio-Rad, USA). Each reaction in 20 μl contained the specific primer set for each group for Bacteria: 341 F (CCTACGGGAGGCAGCAG)-515R (ATTCCGCGGCTGGCA) (Lane 1991); for Fungi: the ITS1F-ITS4 primer pair; and for Archaea: Arch349F (5′-GYGCASCAGKCGMGAAW-3′)-Arch806R (5′-GGACTACVSGGGTATCTAAT-3′) (Takai and Horikoshi 2000). The amplification followed a three-step PCR for all targeted genes: 40 cycles with denaturation at 94 °C for 25 s, primer annealing at 50 °C (for bacteria and fungi) or 52 °C (for archaea) for 25 s, and extension at 72 °C for 25 s. Two independent real-time PCR assays were performed on each soil DNA extract. The standard curves were created using 10-fold dilution series of plasmids containing the bacterial 16S rRNA gene, fungal ITS region, and archaeal 16S rRNA gene from environmental samples for bacterial, fungal, and archaeal communities, respectively.

Statistical analyses

The biogeochemical factors investigated in each sample were analyzed with a two-way ANOVA and correlation analysis using SPSS version 18.00 (SPSS Inc., USA). Shannon diversity index was calculated using the T-RFLP data as representations of different phylotypes. We also analyzed T-RFLP profiles using nonmetric multidimensional scaling (NMS) method and multi-response permutation procedures (MRPP) by PC-ORD 6.0 (MjM Software, USA) in order to investigate the similarities in community structure among the samples.

Results

Effects of elevated CO_2 and/ or N on the microbial community structures

In the bacterial community, the top 20 dominant T-RFs comprised 80 % of the total T-RFs. Among the dominant T-RFs, one type and four types of T-RF showed a higher frequency than in other samples in ambient CO_2 with N samples and in elevated CO_2 with N samples, respectively. In the fungal community, the top 10 dominant T-RFs comprised 85 % of the total T-RFs. There were no indicators T-RFs in the fungal T-RF profile. In the archaeal community, the 10 dominant T-RFs comprised 90 % of the total fragments. Three types and one type of T-RF out of the dominant T-RFs were higher in ambient CO_2 samples and in elevated CO_2 samples than in other samples, respectively.

An NMS multivariate analysis was applied to reveal the underlying structures of the T-RFLP profiles of the microbial communities according to treatment (elevated CO_2 and/or N amendment) and plant types. The NMS results showed that the archaeal and bacterial communities were shifted by elevated CO_2 or the combination of elevated CO_2 and N. However, the fungal community was not shifted by either elevated CO_2 or N (Fig. 1). This result was further confirmed with an MRPP analysis (Table 1). The results of the MRPP showed that all types of microbial communities were significantly different depending on the plant types (Table 2). The effect of elevated CO_2 and N on each microbial community also varied according to the plant types (Table 3).

Effects of elevated CO_2 and/ or N on the diversity of microbial community

The response pattern of the microbial diversity to elevated CO_2 and N was similar to that of the community structure. The results from the effects of elevated CO_2 and N on the diversity of each microbial community also revealed differences among the microbial types (Fig. 2a). The diversity of the bacterial community was decreased by elevated CO_2 or N alone, while the combination of elevated CO_2 and N led to an increase in the bacterial community diversity (Fig. 2a). The fungal community was not significantly affected by either elevated CO_2 or N. The effect on the diversity also varied depending on the plant types (Fig. 2b). The archaeal community diversity was increased by elevated CO_2 and elevated CO_2 with N, while changes in N did not affect the archaeal community diversity (Fig. 2c). The impact of elevated CO_2 and/or N on the diversity of each microbial community varied depending on the plant types (Fig. 3).

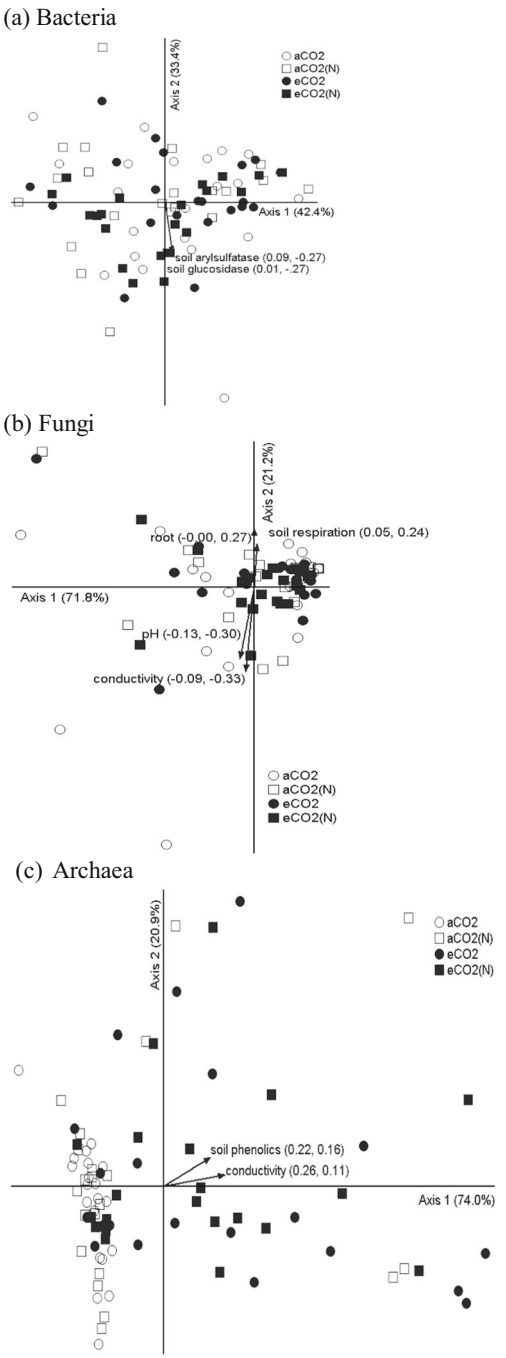

Fig. 1 Nonmetric multidimensional scaling (NMS) graph of microbial gene T-RFP profile in soil samples. **a** bacterial 16S rRNA gene, **b** fungal ITS gene, **c** archaeal 16S rRNA gene

Effects of elevated CO_2 and/ or N on the microbial abundances

We conducted real-time PCR for the bacterial and archaeal 16S rRNA and fungal ITS genes in order to estimate the quantity of each gene-containing community (Fig. 4). The results showed that the copy numbers of the bacterial 16Sr RNA gene ranged from 5.0×10^9 to 2.0×10^{10}/soil g and were increased

Table 1 Results of MRPP (A statistics) testing between different groups from T-RFLP profiles (treatment)

Test and treatments	A statistics		
	Bacteria	Fungi	Archaea
aCO_2 vs eCO_2	ns	ns	0.117**
aCO_2 vs aCO_2(N)	ns	ns	ns
aCO_2 vs eCO_2(N)	0.022*	ns	0.061*
eCO_2 vs aCO_2(N)	ns	ns	ns
eCO_2 vs eCO_2(N)	ns	ns	ns
aCO_2(N) vs eCO_2(N)	ns	ns	ns

ns not significant

*$p<0.01$, **$p<0.001$

Table 3 Results of MRPP (A statistics) testing between different groups from T-RFLP profiles (treatment × plant). Pairs of treatment showing significant differences are presented

	Bacteria	Fungi	Archaea
T. latifolia	aCO_2/eCO_2(N)	–	–
P. japonica	aCO_2/eCO_2(N), aCO_2(N)/eCO_2(N)	–	aCO_2/eCO_2
M. sacchariflorus	aCO_2/aCO_2(N), aCO_2/eCO_2(N)	–	aCO_2/eCO_2, aCO_2/eCO_2(N)
S. lacustris	–	–	aCO_2/eCO_2
J. effusus	–	aCO_2(N)/ eCO_2(N)	aCO_2/eCO_2
P. australis	aCO_2/eCO_2, aCO_2/ eCO_2(N)	–	aCO_2/eCO_2
Z. latifolia	–	–	–

– absence of pairs showing significant difference

by the combination of elevated CO_2 and N (Fig. 4a). The copy numbers of the fungal ITS gene ranged from 2.01×10^6 to 3.48×10^7/soil g. Unlike the bacterial gene copy numbers, the fungal gene copy numbers were not affected significantly by the elevated CO_2 and/or N deposition (Fig. 4b). The copy numbers of the archaeal 16S rRNA gene ranged from 9.13×10^7 to 2.23×10^8/soil g and were significantly increased by N deposition, while they were decreased by elevated CO_2, and the effects of elevated CO_2 and N deposition counteracted one another in the samples combining both (Fig. 4c). An analysis of the results according to the plant types revealed that the effects of elevated CO_2 and/or N on the microbial community structure varied depending on that factor (Fig. 5).

Table 2 Results of MRPP (A statistics) testing between different groups from T-RFLP profiles (plant)

		T.latifolia	*P.japonica*	*M.sacchariflorus*	*S.lacustris*	*J.effusus*	*P.communis*
Bacteria	*T. latifolia*						
	P. japonica	0.07**					
	M. sacchariflorus	0.04**	0.04**				
	S. lacustris	0.03**	0.09**	ns			
	J. effusus	0.03*	0.04**	ns	ns		
	P. australis	0.06**	0.03*	ns	0.04*	ns	
	Z. latifolia	0.02*	0.06**	0.04*	0.04*	ns	0.06**
Fungi	*T. latifolia*						
	P. japonica	0.14*					
	M. sacchariflorus	0.10**	0.09*				
	S. lacustris	0.19**	ns	0.07*			
	J. effusus	0.08*	ns	ns	0.08*		
	P. australis	0.14**	ns	0.05*	0.05*	0.06*	
	Z. latifolia	0.05*	0.11*	0.09**	0.15**	0.07*	0.10*
Archaea	*T. latifolia*						
	P. japonica	0.05*					
	M. sacchariflorus	0.15**	ns				
	S. lacustris	0.14**	0.12**	0.12**			
	J. effusus	0.12**	ns	ns	0.06*		
	P. australis	0.11**	ns	ns	0.07*	ns	
	Z. latifolia	0.07*	0.09**	0.13*	0.10*	0.08*	0.09**

ns not significant

*$p<0.05$; **$p<0.01$

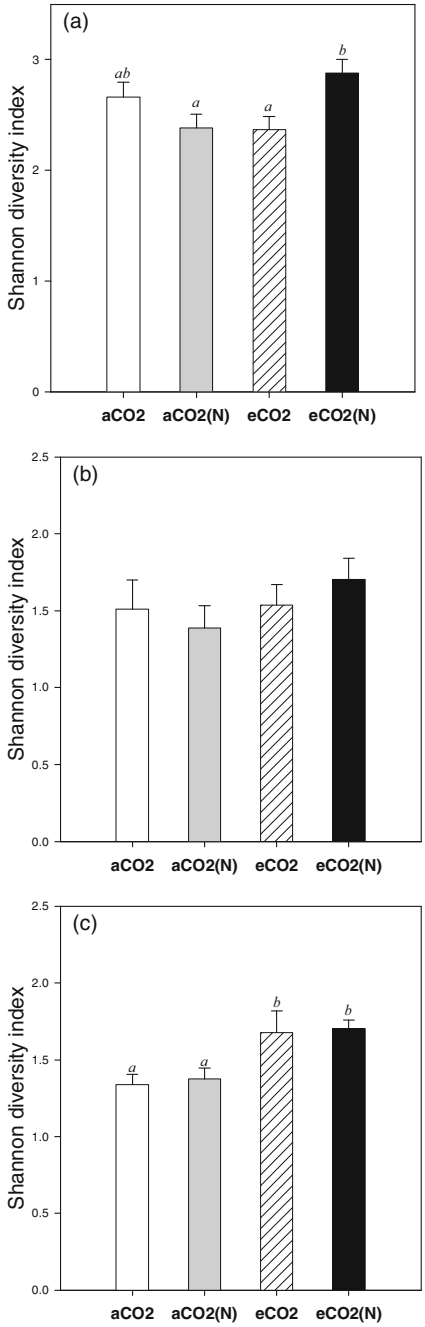

Fig. 2 Microbial diversity in soil samples exposed to different treatments. **a** bacterial 16S rRNA gene, **b** fungal ITS gene, **c** archaeal 16S rRNA gene. Values labelled with different letters are significantly different at P<0.05

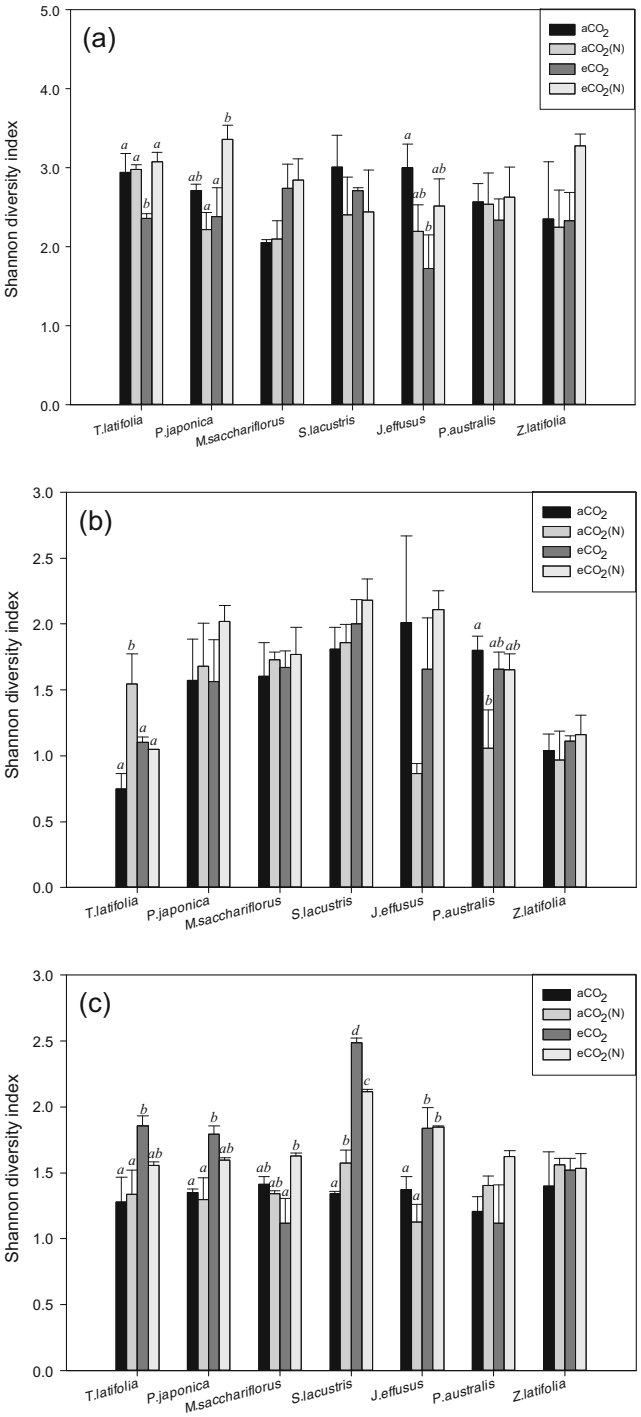

Fig. 3 Microbial diversity in soil samples with different plant types. **a** bacterial 16S rRNA gene, **b** fungal ITS gene, **c** archaeal 16S rRNA gene. Values labelled with different letters are significantly different at P<0.05

Correlation between physicochemical and biological parameters

The result of the correlation analysis showed that the bacterial abundance was correlated with soil arylsulphatase (Table 4). The fungal abundance showed a positive and negative correlation with N-acetyl-glucosaminidase and soil phenolics, respectively. The results demonstrated a negative correlation between N-acetyl-glucosaminidase and the fungal diversity (r=−0.410, p<0.01). The archaeal diversity showed a negative correlation with the archaeal abundance (r=−0.319, p<0.05). The archaeal abundance showed a positive correlation with conductivity, while showing a negative correlation with soil respiration.

A multivariate analysis of the correlations between the physicochemical factors and microbial community structures showed that β-glucosidase and arylsulphatase were mostly

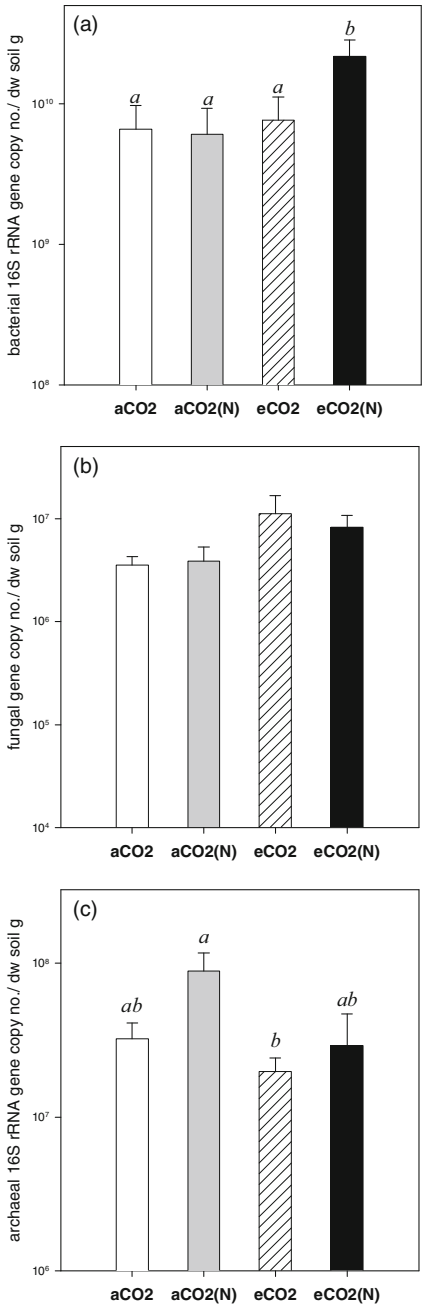

Fig. 4 Microbial gene abundance in soil samples exposed to different treatments. **a** bacterial 16S rRNA gene, **b** fungal ITS gene, **c** archaeal 16S rRNA gene. Values labelled with different letters are significantly different at P<0.05

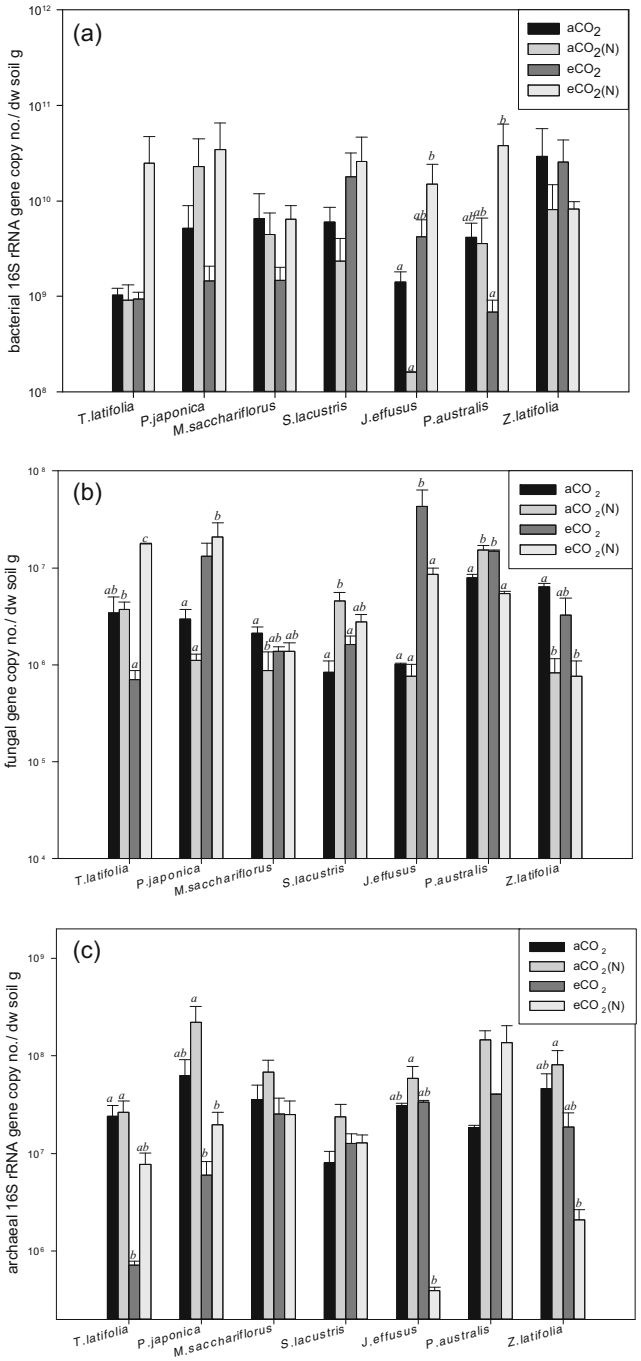

Fig. 5 Microbial gene abundance in soil samples with different plant types. **a** bacterial 16S rRNA gene, **b** fungal ITS gene, **c** archaeal 16S rRNA gene. Values labelled with different letters are significantly different at P<0.05

correlated with the bacterial community structure, while soil respiration and conductivity were correlated with the fungal and archaeal community, respectively (Fig. 1).

Discussion

The overall bacterial community was shifted by elevated CO_2 with N, while the archaeal community was shifted by elevated CO_2 and the combination of elevated CO_2 and N. However, no significant change was found in the fungal community.

The absence of significant effects from the individual elevated CO_2 and N addition on the bacterial community may be due to the variance in other biogeochemical parameters induced by the elevated CO_2 or N. As the plant types play a key role in determining these parameters (Chung et al. 2007; Bomberg and Timonen 2009), the variance of the response to

Table 4 Results of correlation analysis between physicochemical factors and microbial abundances in total samples

	Conductivity	Phenolics soil	Soil_R	Sulf_soil	NG_soil
Bacteria				0.217*	
Fungi		−0.361**			0.533***
Archaea	0.301*		−0.425**		

Soil_R oil respiration, *Sulf_soil* arylsulphatase soil, *NG_soil* N-acetyl-*β*-glucosaminidase soil

*$p<0.05$; **$p<0.01$; ***$p<0.001$

the individual effect between the different planted samples may be one of the reasons for the lack of effect from elevated CO_2 and N on the overall bacterial community. Ge et al. (2010) also reported that spatial factors, which are caused by various parameters including the plant type, have a greater impact in determining the bacterial community than individual elevated CO_2 or N.

However, we observed a decrease in the bacterial diversity from the elevated CO_2 or N, which could be due to the dominance of specific groups. This suggests certain effects of the elevated CO_2 or N on the bacterial community, even though their effects were not apparent in terms of community structure due to the aforementioned variance between the samples. The increase in diversity and abundance from the elevated CO_2 with N may be due to the release from the limitations of C and N, which may lead to a stable bacterial community structure. This also suggests that C supply alone would not increase the bacterial abundance and that N could be a limiting factor for bacterial growth under elevated CO_2 conditions.

An individual effect from elevated CO_2 was observed in the archaeal community, a result consistent with other studies reporting the effect of elevated CO_2 on the archaeal community (Lesaulnier et al. 2008; Hayden et al. 2012). In addition, we also observed an increase in the archaeal diversity with elevated CO_2 and with elevated CO_2 and N, but not with N only, which indicates the significant effect of elevated CO_2 on the archaeal diversity. Combining this result with our observation of the decrease in archaeal abundance from elevated CO_2, we can infer that elevated CO_2 has a large impact on the archaeal community in the marsh ecosystem.

Our results may be due to the presence of methanogenic archaea capable of utilizing CO_2 directly, lowering the degree of variance originating from the difference in the plant types. This is supported by our results showing the effect of elevated CO_2 on the archaeal community in most of the samples, regardless of the plant types. In addition, our previous studies also confirmed the effect of elevated CO_2 on the methanogen community (Lee et al. 2012). In terms of diversity, it has been generally considered that there is a relationship between the diversity and stability or activity of the microbial community despite the controversy on this issue (McCann 2000; Brussaarda et al. 2007). The significant shift observed in the archaeal community suggests the low stability and dynamic response of the archaeal community to external change.

With regard to the abundance, other studies have also reported the increasing effect of elevated CO_2 on the archaeal abundance (Lesaulnier et al. 2008; Dunbar et al. 2012). The increase in archaeal abundance by N but decrease by elevated CO_2 may be due to the competitive interaction between archaea and bacteria. The archaeal abundance decreased in high labile-C (high concentration of DOC or substrate) from root exudate and showed a negative correlation with the bacterial abundance (Rasche et al. 2011; Karlsson et al. 2012). Although not significant, the bacterial abundance was slightly increased by elevated CO_2, but not by N. This would be due to the increase in DOC from root exudate by elevated CO_2 (Freeman et al. 2004a, b; Fenner et al. 2007). The absence of an N effect on bacteria may be related to the significant effect of N on the archaeal abundance. It was reported that ammonia oxidizing archaea (AOA) would be less competitive than plant under N-limited condition subtropical wetland (Wang et al. 2013), while AOA was abundant in macrophyte root surface under high N concentrations and that there was a selective effect of the root on the composition of the archaeal community (Lliros et al. 2014). Ke et al. (2014) also reported a response of AOA to N fertilization without a response from methanogenic archaea in a rice field. As we used soil samples including a rhizospheric soil for analysis, the increasing effect of N on the archaeal abundance in our study is likely to have resulted from the increase in AOA groups rather than methanogens.

The absence of response of the fungal community to elevated CO_2 and N may have been due to the fact that the fungal group in our study mainly consisted of slow-growing fungi which do not respond to increases in labile-C (Chigineva et al. 2009). Another possible explanation may be that the amount of labile-C was not enough to induce a shift in the fungal community (de Graaff et al. 2010).

The response of the microbial group to external change varied with the analyzed parameters, such as the abundance and community structure. A shift of the community with an absence of change in the abundance suggests a shift of the dominant group and the redundancy and resistance of the microbial community to external environmental changes. A change of abundance with no shift in the community structure suggests the stability of the community composition (Bowen et al. 2011).

The effect of elevated CO_2 and N varied depending on the plant type, which showed that the plant type is an important factor structuring the microbial community. Distinctive microbial communities were found in different vegetation types, regardless of the CO_2/N treatments or types of microbes.

Both the quantity and quality of root exudates are key factors determining the microbial community structure (Broeckling et al. 2008; Chigineva et al. 2009; de Graaff et al. 2010; Karlsson et al. 2012; Koranda et al. 2014). This explains the differences in microbial community structures between different planted samples. The variation in the elevated CO_2 or N effects on the microbial groups according to the plant types is related to the characteristics of the plants (Vale et al. 2005). It was suggested that C3 plants were more affected by elevated CO_2 than C4 plants (Freeman et al. 2004a, b; Limpens et al. 2008), while C4 plants were more affected by N than C3 plants (Langley and Megonigal 2010). In our study, the addition of N led to shifts in the bacterial communities of samples with the C4 plant *M. sacchariflorus*, and an individual effect from elevated CO_2 was observed only in samples with *P. australis*, known as an intermediate C3/C4 plant, which indicates a different impact from C3 and C4 plants on microbial communities in the climate change context. The different effects between C4 and C3 plants may be explained by the difference in the quality of DOC between C4 and C3 plants (Kjøller and Struew 2002). In our results, the samples with *M. sacchariflorus* and *P. australis* showed a positive correlation between the bacterial abundance and soil DOC ($r=0.86$, $p<0.01$ in *M. sacchariflorus* and $r=0.63$, $p<0.05$ in *P. australis*), indicating that the DOC from C4 plants may enhance the growth of soil bacteria.

We also analyzed the relationship between the microbiological and physicochemical parameters in order to unravel the underlying reasons for the results of the elevated CO_2 or N effects. The results of the correlation analysis showed that the bacterial abundance was correlated with soil arylsulphatase (Table 4), which suggests that the majority of bacteria may be closely involved in the S cycle. The multivariate analysis also revealed a relationship between arylsulphatase and the bacterial community structure (Fig. 1). Although we could not observe direct effects from elevated CO_2 or N on the fungal community, it was noted through the correlation analysis that the fungal community was significantly related to several parameters, indicating that active fungal groups may have been playing a role in the nutrient cycle in our system. For example, a negative correlation between the fungal abundance and phenolics was observed, possibly due to the fact that the phenolic compounds were decomposed by the fungal community. A lower fungal abundance would lead to a lower decomposition of phenolics, resulting in an accumulation of phenolics in the soil. As the phenolics could inhibit the other hydrolase activity, hydrolase activity would be higher in the low concentration of phenolics (Freeman et al. 2001). Although we did not observe the significant direct correlation between the phenolics and hydrolase activity, there was a positive correlation between the fungal abundance and N-acetyl-glucosaminidase which is produced mainly by fungi (Kuzyakov 2002). The multivariate analysis showed a significant correlation between the soil respiration and the fungal community, which again points to the significant role of the fungal group in the C cycle.

We also observed a negative correlation between the archaeal diversity and archaeal abundance, which indicates that there may have been a consistently dominant archaeal group in our system and that this group may have been primarily affected by the elevated CO_2 or N addition. The positive correlation between the archaeal abundance and conductivity may be due to the fact that greater archaeal abundance was observed in the samples with high conductivity (Edwards et al. 1999). The correlation of the conductivity with the archaeal community was observed in the multivariate analysis (Fig. 1). The negative correlation between the soil respiration and archaeal abundance indicates a low metabolic rate or other metabolism, such as anaerobic respiration, of the archaea. Most of the respiration would be performed by the bacteria and fungi, as was observed in the multivariate analysis of the fungal community, while the archaea may be more directly involved in anaerobic respiration (Offere et al. 2013).

Although we did not analyze the specific groups in each microbial community, the majority of the archaeal communities were assumed to be methanogenic archaea based on our in silico analysis of the T-RFLP profiles, which suggested a high potential CH_4 emission from this marsh system. For example, the dominant T-RF in the archaeal community was T-RF 185, which is assumed to be affiliated with acetoclastic *Methanosarcinaceae*. The frequency of this T-RF was higher in the ambient CO_2 samples than in the other samples. The second most dominant T-RF was T-RF 89, which is affiliated with hydrogenotrophic *Microbiales*. The elevated CO_2 affected the shift of the dominant group from acetoclastic to hydrogenotroph, which was in accordance with our previous studies (Lee et al. 2012).

Our results were also explained in terms of interactions between each microbial group. There have been reports that the bacterial and archaeal communities demonstrate a competitive or neutralistic interaction (Swan et al. 2010), while the fungal and archaeal communities have a synergistic interaction (Karlsson et al. 2012). Considering those reports, the competition between the archaea and bacteria could have caused the change in the archaeal community in our system, and the absence of competition between the fungal and bacterial group would have caused the lack of response of the fungal community.

Our results can be summarized as follows: Elevated CO_2 and N may affect the bacterial and archaeal communities, while they may not affect the fungal community in terms of either diversity or abundance. The effects of elevated CO_2 and N on microbial community vary depending on the plant types, and each microbial community shows different responses to

the elevated CO_2 and N. In particular, elevated CO_2 might force a shift in the archaeal community irrespective of the plant type, and the effect of elevated CO_2 was enhanced when combined with the N effect.

The results of this study indicate that elevated CO_2 and N addition could lead to changes in the community structures of bacteria and archaea, at least temporarily. Our results also suggest that the fungal group was resistant to external changes, while the bacteria and archaea groups were more easily affected by them. Finally, the characteristics of the plant types and relevant physicochemical factors induced by the elevated CO_2 and N may be important key factors structuring the microbial community's response to environmental change.

To better understand the impacts of global climate change on microbial communities and the biological feedback in wetland ecosystems, further studies involving in situ long-term monitoring are necessary. In addition, alternative approaches using stable isotope-probing methods are also desirable to understand the functionality of active microbial communities.

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