Elevated atmospheric CO_2 impact on carbon and nitrogen transformations and microbial community in replicated wetland

Dawei Jiang[1], Lifei Chen[1*], Nan Xia[1,2*], Eyram Norgbey[3], Desmond Ato Koomson[3] and
Williams Kweku Darkwah[3*]

Abstract

Background: Elevated atmospheric CO_2 has direct and indirect influences on ecosystem processes. The impact of elevated atmospheric CO_2 concentration on carbon and nitrogen transformations, together with the microbial community, was evaluated with water hyacinth (*Eichhornia crassipes*) in an open-top chamber replicated wetland. The responses of nitrogen and carbon pools in water and wetland soil, and microbial community abundance were studied under ambient CO_2 and elevated CO_2 (ambient + 200 μL L^{-1}).

Results: Total biomass for the whole plant under elevated CO_2 increased by an average of 8% ($p = 0.022$). Wetlands, with water hyacinth, showed a significant increase in total carbon and total organic carbon in water by 7% ($p = 0.001$) and 21% ($p = 0.001$), respectively, under elevated CO_2 compared to that of ambient CO_2. Increase in dissolved carbon in water correlates with the presence of wetland plants since the water hyacinth can directly exchange CO_2 from the atmosphere to water by the upper epidermis of leaves. Also, the enrichment CO_2 showed an increase in total carbon and total organic carbon concentration in wetland soil by 3% ($p = 0.344$) and 6% ($p = 0.008$), respectively. The total nitrogen content in water increased by 26% ($p = 0.0001$), while total nitrogen in wetland soil pool under CO_2 enrichment decreased by 9% ($p = 0.011$) due to increased soil microbial community abundance, extracted by phospholipid fatty acids, which was 25% larger in amount than that of the ambient treatment.

Conclusion: The study revealed that the elevated CO_2 would affect the carbon and nitrogen transformations in wetland plant, water, and soil pool and increase soil microbial community abundance.

Keywords: Elevated CO_2, Carbon transformation, Nitrogen transformation, Microbial community abundance, Phospholipid fatty acids, Open-top chamber

Introduction

Carbon dioxide (CO_2) is the most important greenhouse gas, which has increased by around 2 ppm of the current rate per year. Enriched atmospheric CO_2 concentration can both enhance and improve soil microorganism activities through plant photosynthesis, organic substrate quantity, organism's respiration, and microbial biomass allocation in the ecosystem (Liu et al. 2018). There are many considerable interests in understanding whether the elevated atmospheric CO_2 can regulate the carbon sequestration and nitrogen mineralization in soil, and how these changes respond to the ecological environment (Yu and Chen 2019).

Increase in elevated atmospheric CO_2 causes an increase in nitrogen immobilization (Chang 2019) and a reduction in nitrification in soils by increasing the

* Correspondence: lfchen@jlau.edu.cn; 493362595@qq.com;
williamsdarkwakwaku@yahoo.com
[1]College of Horticulture, Jilin Agricultural University, Changchun 130118,
China
[3]Key Laboratory of Integrated Regulation and Resource Development on
Shallow Lakes, Ministry of Education, Environmental Engi neering
Department, College of Environment, Hohai University, 1 Xikang Road, Gulou,
210098 Nanjing, China
Full list of author information is available at the end of the article

allocation of carbon to roots. The increasing CO_2 is generally associated with the reduction in nitrate reductase activity, which might reduce the availability of electron acceptors for denitrification (Chang 2019). The rising atmospheric CO_2 has direct and indirect influences on nitrogen losses in ecosystems. A study by Conthe and coworkers (2019) revealed that the direct and indirect ways are releasing NO and N_2O into the atmosphere and leaching for NO_3^--N and denitrification, respectively. Other studies revealed that increasing CO_2 (inflow of C to the soil) would change the rates of nitrogen mineralization (Gao et al. 2019). The rate of N mineralization is crucial in the determination of available nutrients for vegetative production (Pang et al. 2019). On the other hand, the status of available nutrients may also indicate N losses or gains from terrestrial ecosystems (Yu and Chen 2019). Alternatively, plants grown under elevated CO_2 showed variations in root responses, depending on species and environmental conditions such as temperature, pH, light, water, and nutrient availability (Gao et al. 2019). Furthermore, Zhang et al. (2019a) showed significant effects of soil pH on soil respiration with microbial activity. Thus, it is important to investigate the effects of elevated CO_2 on the environment.

The increasing atmospheric CO_2 concentrations have the potential alteration of carbon cycling for terrestrial ecosystems (Yu and Chen 2019). Previous works have demonstrated that a rise in atmospheric CO_2 can cause a greater exudation of labile C from plant roots. This promotes the growth of microbes (Pang et al. 2019). A report showed that changes in C availability (organic matter) or substrate quality (lower soil C to N ratio), would promote the growth of Gram-negative bacteria (Laut et al. 2020). High plant growth under elevated CO_2 is likely to provide more organic substrates for microbial metabolism in soil, and fuel more rapid rates of soil carbon cycling (Yang et al. 2019). Bacteria in the soil can recycle carbon (C), nitrogen (N), sulfur (S), and other chemical elements in the decomposition process. Bacteria contribute to nutrient mineralization in soil (Zhang et al. 2019a). Accordingly, the soil microbial biomass is a more sensitive indicator of soil fertility than the soil organic matter because it responds readily to change the soil's physical and chemical environment (Brym et al. 2014; Laut et al. 2020). It must be noted that elevated CO_2 could lead to an increase in microbial community growth and immobilization of N in soil (Yang et al. 2019; Xing et al. 2020).

In recent years, the phospholipid fatty acids (PLFAs) analysis method has been widely employed and recognized to be a robust technique to study soil microbial community (Fan et al. 2017; Li et al. 2018). In this study, this method was used to measure the composition and abundance of the microbial community in wetland soil under two atmospheric CO_2 levels.

Previous studies reported mainly on induced CO_2 response on the plant nutrient cycling and soil microbial community (Fan et al. 2017). Elevated atmospheric CO_2 concentration adversely affects global warming and climate and inversely affects the plant growth responses to photosynthesis, soil carbon sequestrations, and nitrogen cycling. Understanding the effect of elevated CO_2 on various wetland systems is paramount. In fact, plants and microbes directly respond to the ecosystems with the increases of elevated atmospheric CO_2. The objective of this present study is to examine the interconnected processes in wetland ecosystem for correlation of carbon, nitrogen cycle with water hyacinth (*Eichhornia crassipes*) in water, wetland soil, and soil microbial community composition between two concentrations of atmospheric CO_2.

Also, the field observation of CO_2 effect on specific C and N cycling in a natural lake ecosystem is difficult to analyze. This present research contributes to understanding the internal problem of the natural lake ecosystems between two atmospheric CO_2 (elevated and ambient) concentrations. Given the research needs, open-top chamber (OTC) for CO_2 elevation was used as the imitation of field observation of natural lake ecosystems, in which sediment and wetland plant species were used. Therefore, the carbon (C) and nitrogen (N) transformations, total organic carbon (TOC), inorganic carbon (IC), total carbon (TC), total nitrogen (TN), and nitrogen interactions in water and wetland soil were monitored and the microbial community abundance was explored using the method of PLFAs in this experiment.

Materials and methods
Experimental design and treatments
Open-top chamber (OTC)
A sketch of an open-top chamber (OTC) used in this study is shown in Fig. 1. OTC was $13.5 \, m^3$ in volume and the base was made by a brick frame and it was covered by 0.15-cm-thick glass and placed inside a ventilated greenhouse. One OTC was used for ambient CO_2 and another was used for elevated CO_2 (ambient + $200 \, \mu L \, L^{-1}$) without any input of N treatment. Each OTC was separated into four partitions. Among these four partitions, two of them were cultured with water hyacinth and the remaining two were used as control (without plants and soil). The greenhouse was equipped with a fan that continually blew air into the chamber to adjust the daily room temperature (24–35 °C). Elevated CO_2 was pumped into the chamber through a plastic drainage pipe (diameter 0.5 cm), which was laid inside of open-top chamber along the edges. To control CO_2
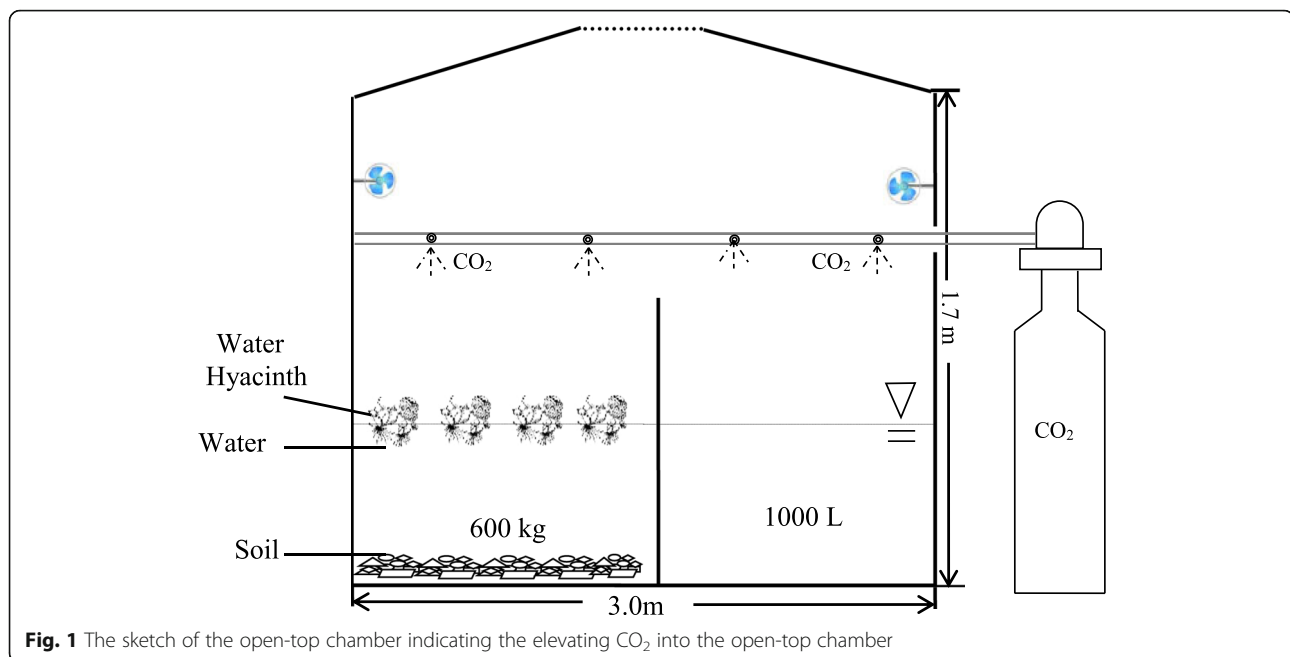
Fig. 1 The sketch of the open-top chamber indicating the elevating CO_2 into the open-top chamber

concentration, TES 1370 NDIR CO_2 meter was used daily to detect the concentration of CO_2 inside two chambers.

Experimental site, sediment, and plant sampling

Sediments used in this experiment were collected from Taihu lake, an area of approximately 36895 km^2 (31° 25.106′ N, 120° 14.758′ E), in the east of China. An equal height, about 14–15 cm (600 kg in weight), of air-dried sediments was placed into duplicated partitions before loading the culture solution into the open-top chamber.

Water hyacinth was obtained from the lake on the campus of Jilin Agricultural University, Changchun, China. After collection, the plants were washed and air-dried in a shady and cool place. Uniform leaves and healthy plants that weighed approximately 200 g were cultured inside the duplicated partitions appropriately. The cultured solution (tap water being used) of 40 cm in depth, which was nearly equal to 1 l, was poured into the chambers.

Chemical analysis for water and soil

The experiment was run for 120 days. The soil samples were dried naturally and grounded. The grounded sample was sieved through a 2-mm sieve. Soil pH was measured in a ratio of 1:2 representing a mixture of soil to deionized water. A measurement of TN for soil was conducted using a UV spectrometry (SHIMADZU UV-2450) as soon as the samples were pre-filtered with a 0.45 μm glass fiber filter. Water samples were collected and pre-treated by the SHIMADZU TOC-CPN. Water

TOC, TC, IC, and TN were determined by using TOC/ TN analyzer (SHIMADZU TOC-CPN) and soil TOC, TC, and IC were analyzed by the SSM analyzer (SHIM ADZU TOC-CPN), respectively. Gas chromatography-mass spectrometry (GC-MS) QP-2010 Plus with AOC-20i verified the phospholipid fatty acids (PLFAs).

Methodology for GC-MS

Fatty acid methyl esters (FAMEs) were analyzed by GC-MS (QP2010 Plus SHIMADZU), with VF-23 ms fused silica (30 m × 0.25 mm × 0.25 μm) (Varian, Inc.). The methodology for GC-2010 was a 1-μl single injection, – 1 split ratio, split-less mode, with gas Helium as carrier together with pressure 57.4 kPa, and the oven temperature: (1) from 50 (held for 2 min) to 100 °C, at 5 °C/min; (2) from 100 to 200 °C, at 4 °C/min; and (3) from 220 °C (held for 4 min) to 240 °C, at 4 °C/min. The column and temperature program details were ion source temperature of 200 °C, interface temperature of 260 °C, and solvent cut time of 5 min, respectively.

Microbial phospholipid fatty acids (PLFAs) profile

The extraction of lipids from fresh sediment in fractionation and quantification was described by Guo et al. (2019). After 5 g of fresh sediment was randomly picked out, visible fine roots were firstly removed and gravimetric water content was then measured at 105 °C, the samples were moved to the glass tube (Teflon-lined screw cap). The samples were fractionated using silicic acid chromatography. Also, a stream of nitrogen gas was used to dry the phospholipid fraction and later stored at – 20 °C. The resultant polar lipid fractions were then

subjected to mild alkaline methanolysis, and the resultant fatty acid methyl esters (FAMEs) were extracted with two 2-ml aliquots of chloroform. The sample was dried under nitrogen gas at room temperature. The resultant FAMEs were re-liquefied in 1 ml hexane containing an internal standard (19:0 FAME at 40 ng/ml) to transfer to GC vials of (GC-MS) for the analysis of microbial community abundance.

The nomenclature for microbial PLFAs Based on Frostegård and Bååth (1996), the designation for the systematic names of FAMEs was A:BωC, where

- *A*—the total number of carbon atoms
- *B*—the number of double bonds
- *ω*—position of the first double bond
- *C*—the number of carbon atoms from the aliphatic end

Suffixes "c" and "t" refer to the "cis" and "trans" conformations, respectively.

Methyl branching, at the "iso and anteiso" positions from the methyl end of the molecule, and methyl branching at the 10th carbon atom from the carboxyl end of the molecule are designated by the prefixes "i", "a", and "10Me" respectively. The prefix "cy" denotes cyclopropane fatty acids.

Plant relative CO_2 treatment effect

The relative CO_2 treatment effects (%) were calculated by Eq. 1:

$$\frac{(\text{elevated} - \text{ambient}) \times 100}{\text{ambient}} \qquad (1)$$

The floating aquatic plant (water hyacinth) was harvested at the end of the growing period, i.e., (treatment for 4 months) to measure the whole-plant biomass under two different CO_2 concentrations.

Statistical and data analyses

Treatment effects (the CO_2 effect) on data analyses were analyzed using one-way ANOVAs and CO_2 effects were also analyzed with paired-samples t test with using SPSS Statistics 17.0 (SPSS Inc., USA), respectively. The relation of samples (R^2) was assessed statistically by the linear regression analysis of SPSS Statistics 17.0. All determinations of chemical analysis for plant, water and soil samples were performed in triplicate analyses with one blank from duplicate treatments in OTCs. The measurement of plant growth was expressed as mean of duplicate treatments in OTCs. The data are presented as mean ± standard error, p, and F value. Statistical significance was accepted at $\alpha = 0.05$.

Results

Effects of elevated CO_2 on plant biomass

After four months of incubation, whole-plant biomass showed an 8% increase ($p = 0.022$) under elevated CO_2 compred to ambient CO_2 (Fig. 2). Water TC with plant and water TC as control (without plant) changed (in time) for elevated chamber and ambient chamber during treatment CO_2 (Fig. 3). The presence of plants influenced the CO_2 effects in the experiment. From the study (Fig. 3), the mean water TC with the plants was increased by 21% in time with 10.52 ± 0.3 mg/L with plants as compared to 8.69 ± 0.2 mg/L without plants (control) under the same CO_2 concentration. However, there were no significant differences ($p > 0.05$) for water TC as control (1%) with 8.69 ± 0.2 mg/L in elevated treatment as compared to 8.57 ± 0.3 mg/L in ambient treatment.

Carbon content in water and soil with plants

There was an increase of 7% ($p = 0.001$, $F = 34.229$) for mean water TC with the plants with 10.52 ± 0.3 mg/L under CO_2 treatment and 9.83 ± 0.2 mg/L under ambient treatment (Fig. 4a). There was a significant increase of 21% ($p = 0.000$, $F = 137.587$) for water TOC with 5.56 ± 0.3 mg/L in the elevated treatment as compared to the 4.58 ± 1.0 mg/L in the ambient treatment (Fig. 4b). But water IC concentration was lower in elevated CO_2 treatment than ambient treatment. There was a decrease in IC concentration with time by 5% ($p = 0.540$, $F = 0.422$). The concentration was 4.96 ± 0.7 mg/L in the elevated treatment as compared to 5.24 ± 0.5 mg/L in the ambient treatment correspondingly (Fig. 4c).

In this experiment, there is a significant difference in the soil carbon changes between the two treatments. In the high CO_2 treatment, soil TC concentration was 3%
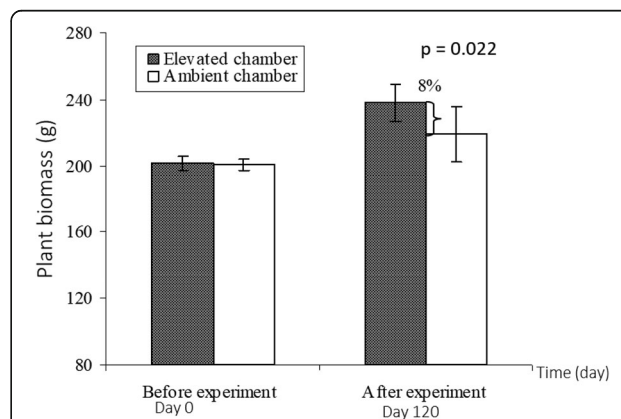
Fig. 2 Effects of elevated CO_2 concentration on whole-plant biomass accumulation. The average percent showed the CO_2 effect. Mean plant biomass ($n = 2$ times observation with duplicates) affected by enrichment CO_2 and S.E. located on the length of value
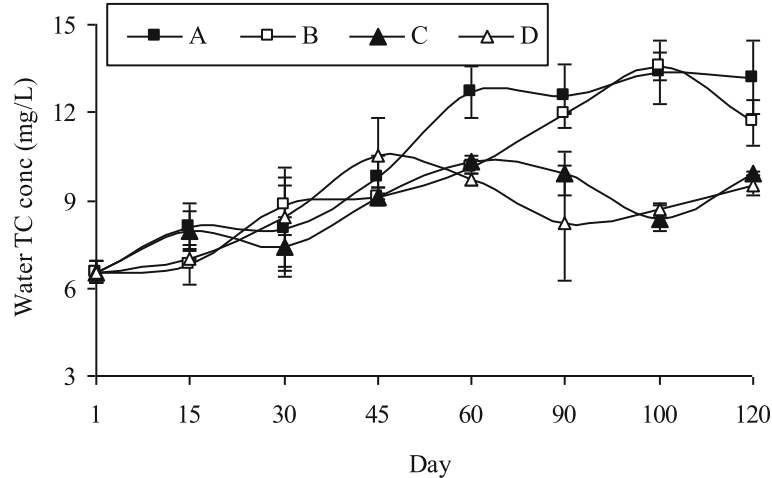
Fig. 3 a Water TC (with plants) under elevated treatment, **b** water TC (without plants) under elevated treatment, **c** water TC (with plants) under ambient treatment, and **d** water TC (without plants) under ambient treatment (in time) during treatment. Each value indicated mean and standard error (S.E.). There were no significant differences between the two treatments ($p \geq 0.05$)

(p = 0.344, F = 1.057) higher relative to the ambient treatment (Fig. 5a), and soil TOC concentration was 6% (p = 0.008, F = 2.573) greater as indicated in Fig. 5b. The mean content of TC, under elevated CO_2, was 13.42 ± 0.34 mg/g against 12.91 ± 0.52 mg/g under ambient CO_2. Similarly, the mean content of TOC, under elevated CO_2, was 10.74 ± 1.21 mg/g against 10.12 ± 0.89 mg/g under ambient CO_2. Soil IC declined by 3% (p = 0.257, F = 1.570) (Fig. 5c). The mean content of IC, under elevated CO_2, was 2.68 ± 0.23 mg/g against 2.79 ± 0.25 mg/g under ambient CO_2.

Total nitrogen in water and soil with plants

There was a substantial increase of 26% (p = 0.000, F = 245.281) in the mean water TN concentration with 1.39 ± 0.3 mg/L in the elevated treatment as compared to 1.10 ± 0.07 mg/L in the ambient treatment at the end of the experiment (Fig. 6a). The mean N concentration for wetland soil under CO_2 enrichment was 9% decrease (p = 0.011, F = 13.405), which was lower than in ambient treatment with 0.55 ± 0.36 mg/g dried soil for higher CO_2 treatment and 0.60 ± 0.07 mg/g dried soil for ambient treatment from initial to final extraction time, respectively (Fig. 6b). The total N loss of the water and soil pool under elevated chamber was 9% with 110 ± 3.3 mg/g dried soil CO_2 under elevated chamber paralleled to 121 ± 4.7 mg/g dried soil CO_2 under ambient chamber (Fig. 7). This result was consistently maintained throughout the experimental process.

Soil microbial community abundance
General background for taxonomic and functional biomarkers

Microbes can be distinguished into four groups: one with a high proportion of methyl branched-chain or monounsaturated FAME's, i.e., Gram-positive bacteria; the second, with a large proportion of cyclopropane fatty acids, i.e., Gram-negative bacteria; the third, with fungal indicators of PLFAs: C18:1ω9t (saprotrophic fungi), C18:3ω3c and C18:2ω6c and C16:1ω5c (mycorrhizal (AM) fungi); and lastly, C20:4ω6c was a species of protozoa as shown in Table 1 (Zhang et al. 2019b; Tzanakakis et al. 2019; Gorman-Lewis et al. 2019).

In this study, Gram-positive bacterial PLFAs or nitrifiers (ammonia-oxidizing bacteria) were iC14:0, C16:0, C17:0, C18:0, and C20:0 as indicated in Table 1. Among them, methane utilizing bacteria were C16:0 and C18:0. Gram-negative bacteria or microeukaryotes PLFAs were cyc17:0 (sulfate-reducing bacteria), 10meC16:0 (actinomycetes), C16:1ω5c, C16:1ω7c, and C18:1ω9t. Moreover, C18:3ω3c and C18:2ω6c were fungi.

Reaction of microbial PLFAs during CO_2 run

Figure 8 showed each and the total content of microbial PLFAs for sediments under elevated CO_2 and ambient CO_2. In CO_2 treatment, total microbial community abundance was 45.4 nmol/g (p = 0.023) whereas 36.2 nmol/g in ambient conditions. These microbial PLFAs showed a 25% significant difference between elevated and ambient conditions during the course of the CO_2 enrichment.

The Gram-negative and -positive bacterial PLFAs contents could be observed significantly in CO_2 enrichment conditions. The total Gram-positive bacteria were 3.26% (F = 8.261, p = 0.045), Gram-negative bacteria were 136.27% (F = 22.261, p = 0.0001), total fungi were 46.23% (F = 5.855, p = 0.137), and protozoa were 21.00% than ambient conditions, respectively (Table 1).
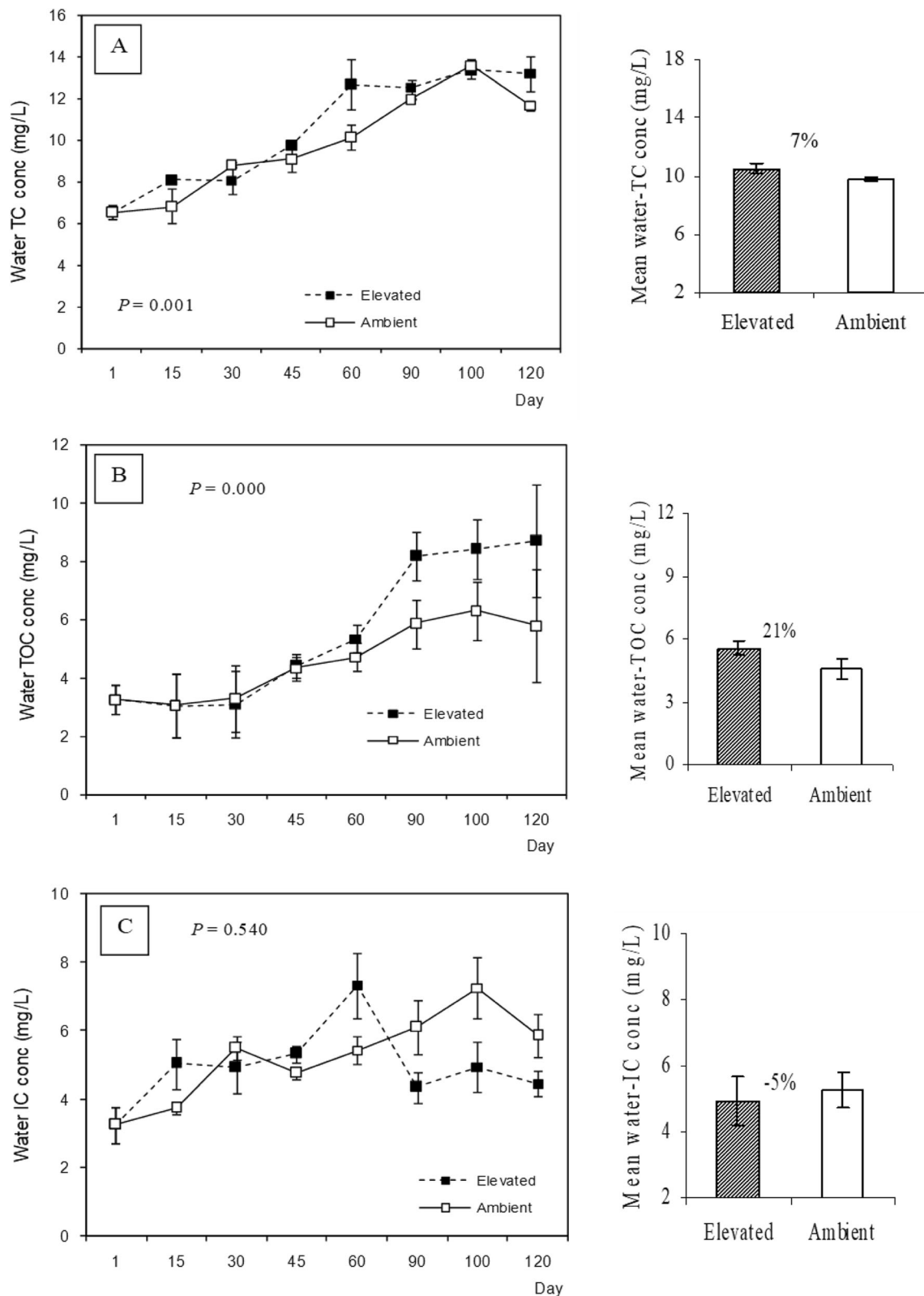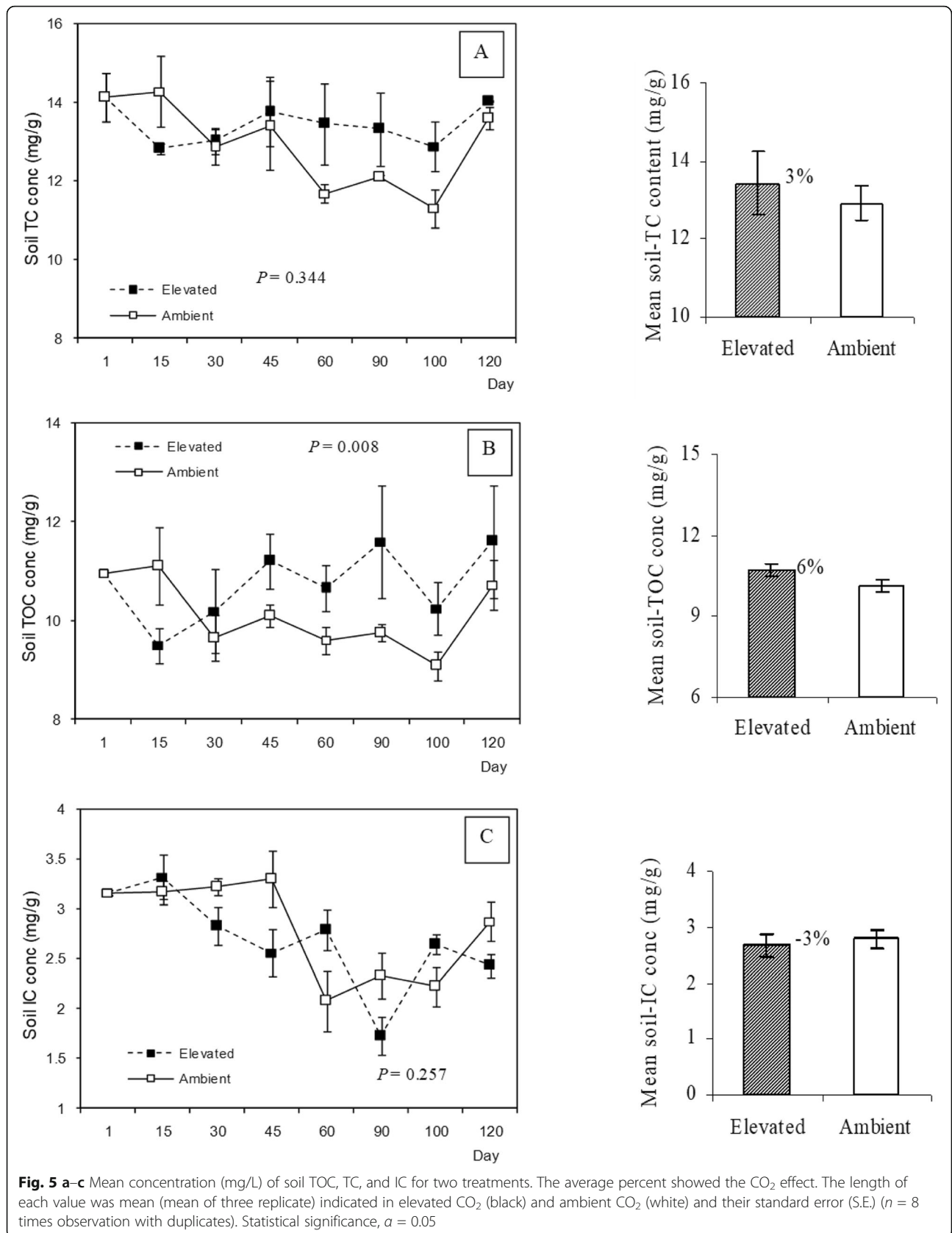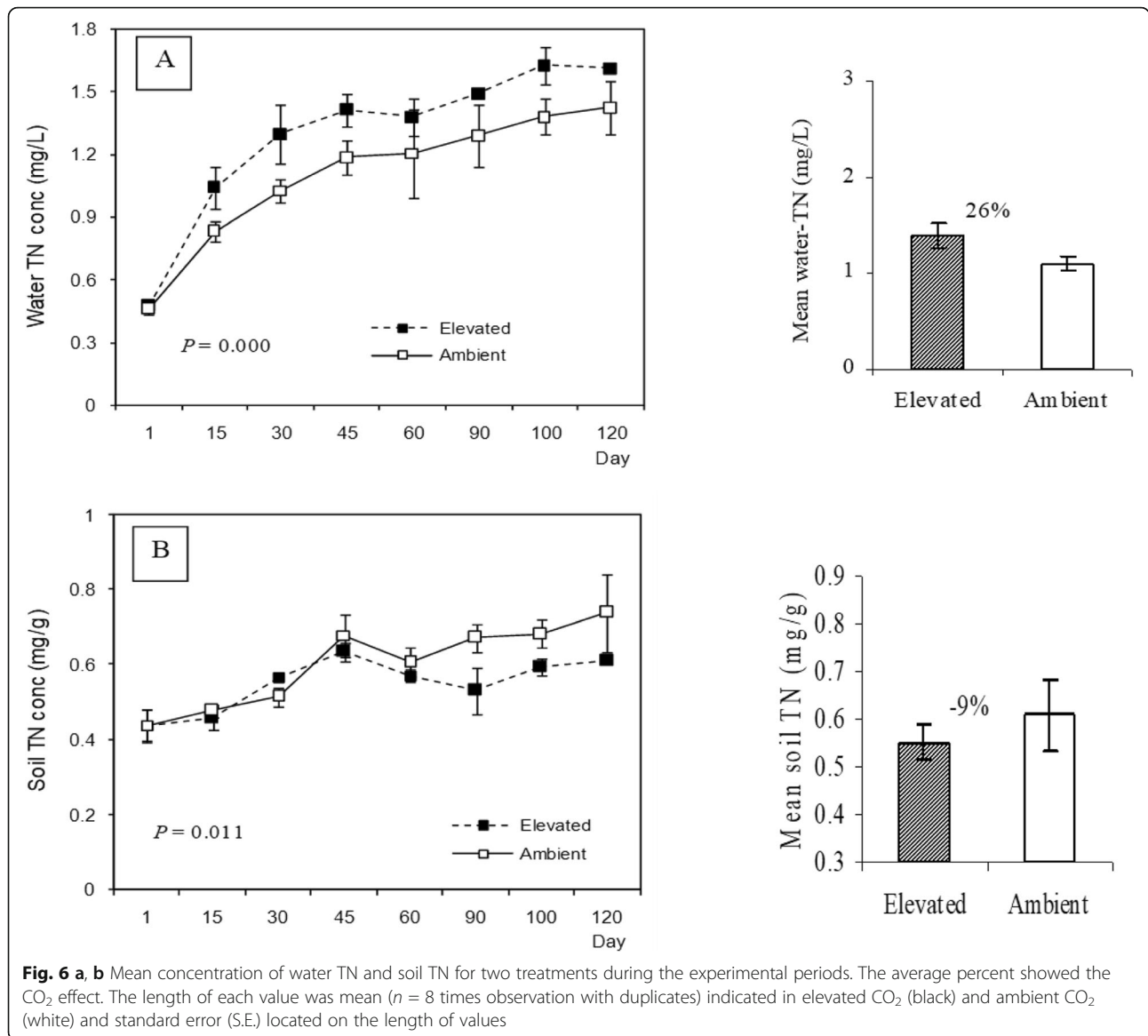
Fig. 4 a–c Mean concentration (mg/L) of water TOC, TC, and IC. The average percent showed the CO_2 effect. The length of each value was mean (mean of three replicate) indicated in elevated CO_2 (black) and ambient CO_2 (white) and their standard error (S.E.) (n = 8 times observation with duplicates). Statistical significance, a = 0.05

Fig. 5 a–c Mean concentration (mg/L) of soil TOC, TC, and IC for two treatments. The average percent showed the CO_2 effect. The length of each value was mean (mean of three replicate) indicated in elevated CO_2 (black) and ambient CO_2 (white) and their standard error (S.E.) ($n = 8$ times observation with duplicates). Statistical significance, $a = 0.05$
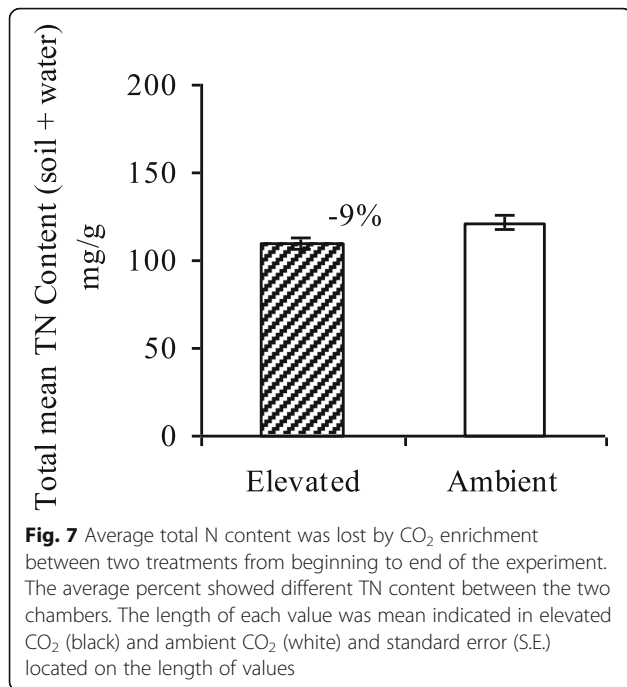
Fig. 6 a, **b** Mean concentration of water TN and soil TN for two treatments during the experimental periods. The average percent showed the CO_2 effect. The length of each value was mean ($n = 8$ times observation with duplicates) indicated in elevated CO_2 (black) and ambient CO_2 (white) and standard error (S.E.) located on the length of values

Discussion

Effects of elevated atmospheric CO_2 concentration on plant biomass and C content in soil and water

Numerous studies approved that elevated CO_2 could increase the biomass of plants. In this study, we showed an increase in plant biomass of water hyacinth by 8% ($p = 0.022$). Song et al. (2009) suggested that increased atmospheric CO_2 consistently enhanced the growth of water hyacinth. Water TC increased by 7% ($p = 0.001$) and water TOC increased by 21% ($p = 0.000$) by CO_2 effect. The increasing of water TOC may depend on plant that absorbs CO_2 from the atmosphere to water and convert inorganic CO_2 to labile organic C, TOC in water because the free-floating aquatic plants can directly exchange CO_2 with the atmosphere through the upper epidermis of leaves (Satake and Shimura 1983). Soil TC

increased by 3% ($p = 0.344$) and soil TOC increased by 6% ($p = 0.008$) by CO_2 effect. According to Walter and Heiman (2000), the transport pathway of C from water to sediment increased through the vascular tissue of the plant below the water tables; thus, labile organic C in soil increased with the increasing CO_2. Moreover, concentrations of water and soil IC decreased by 5% ($p = 0.540$) and 3% ($p = 0.257$), respectively. The plant may have used IC for plant respiration and photosynthesis effects on its growth and many aspects of plant physiology. Another explanation may be the severely reduced supply of IC due to slower CO_2 diffusion rates through underwater photosynthesis (Madsen and Sandjensen 1994).

Generally, an increased TOC concentration in water and soil was due to the plant biomass enhancement

Fig. 7 Average total N content was lost by CO_2 enrichment between two treatments from beginning to end of the experiment. The average percent showed different TN content between the two chambers. The length of each value was mean indicated in elevated CO_2 (black) and ambient CO_2 (white) and standard error (S.E.) located on the length of values

exposed to ambient CO_2 and it was statistically significant. Water TOC concentration with no plant under high CO_2 levels decreased by 1.1% ($p = 0.011$) and water IC increased by 4.1% ($p = 0.173$), respectively. Absorption of CO_2 from the atmosphere to water may depend on CO_2 exchange in plants. According to Maltais-Landry et al. (2009), planted wetlands may sequester 15 times more C than unplanted wetland systems. Thus, the increase in C content in wetlands can be attributed to the increment in plant growth.

In short, wetland plants play an important role in nutrients cycling due to nitrogen uptake, storage, and release processes in the wetland ecosystem (Wang et al. 2013). Wetland plants that will be affected by future CO_2 concentrations will likely follow a common pattern of carbon storage in water and soil as well. Commonly, elevated CO_2 induces growth which increases wetland plants relative to the equal measurement of ambient CO_2 and the following results synchronize with nitrogen dynamics and changes in microbial activity (Yu and Chen 2019; Kelly et al. 2013). The wetland ecosystem can direct the net balance between carbon gains and losses. The C storage occurred due to the combined effects of the increased plant productivity resulting from increased CO_2 and drastic changes in plant residues. The comparison of water TC, with plants, to water TC as a control (without plants), in the elevated chamber, showed that the water TC with plants increased in time (21%) than that of the control. By contrast, exposure of

because water TC without plant did not significantly increase even under elevated CO_2 concentration. CO_2 did not viscously dissolve in water when the pool did not have plants under elevated CO_2. Water TC concentration without plant exposed to high CO_2 levels slightly increased by 1.4% ($p = 0.046$) compared with that

Table 1 Extractable quantity of FAME biomarkers representing bacteria, fungi, and protozoa (nmol/g)

| Biomarker	Elevated chamber		Ambient chamber		Average % larger than ambient, *p* value
	nmol/g	S.E.	nmol/g	S.E.	
Gram-positive bacteria					
iC14:0	1.07	0.05	1.21	0.05	3.26%, $p = 0.045$
C16:0	11.62	3.20	10.67	2.40	
C17:0	4.99	0.10	0.43	0.20	
C18:0	3.12	1.40	5.77	1.50	
C20:0	0.51	0.01	1.23	0.30	
Gram-negative bacteria					
10meC16:0	0.55	0.02	1.87	0.09	136.27%, $p = 0.0001$
C16:1ω5c	8.83	2.10	4.24	1.90	
C16:1ω7c	0.81	0.30	0.99	0.30	
cycC17:0	3.18	1.90	0.70	0.90	
C18:1ω9t	7.66	1.30	6.73	1.70	
Fungi					
C18:2ω6c	2.54	1.00	1.36	1.33	46.23%, $p = 0.137$
C18:3ω3c	0.43	0.20	0.98	0.23	
Protozoa					
C20:4ω6c	0.10	0.03	0.05	0.01	21.00%, ns
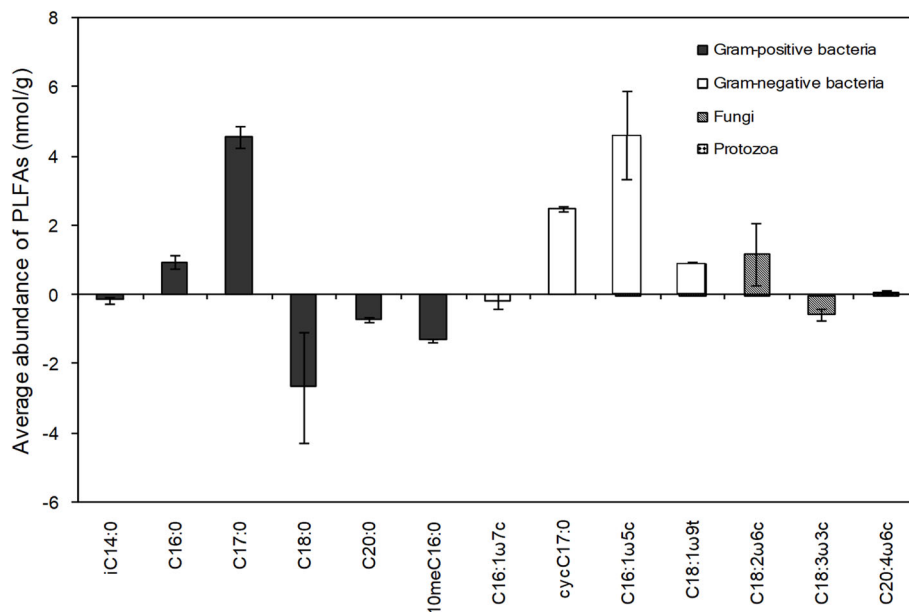
Fig. 8 Comparison of concentrations (nmol/g) of different microbial PLFAs. The length of each value indicated less and greater quantitative result was mean than ambient treatment for during enrichment CO_2 (white) and S.E. There were significant differences between two treatments at ($p \leq$ 0.05). Values are means ± S.E

water TC without plants to high CO_2 had no significant effect as compared to ambient treatment of water TC without plants, which increased by 1%.

Effects of elevated atmospheric CO_2 concentration on N cycling (soil/water) and microbial community abundance

Several research works have elucidated that effect of elevated CO_2 on dissolved organic C and its relationship with soil microorganisms (Zhao et al. 2009; Jinbo et al. 2007; Hungate 1999). Soil organic C is an important labile C fraction because it is the main energy source for microorganisms to increase N mineralization rate in freshwater marshes (Zhao et al. 2009; Jinbo et al. 2007; Hungate 1999). Dissolved organic C has also been proposed as an indicator of the C available to soil microorganisms (Mikan et al. 2000; Kang et al. 2005; Smolander and Kitunen 2002; Sowerby et al. 2000). In this study, under elevated CO_2, TN in water increased by 26% (p = 0.0001) compared to ambient CO_2. The increase may be attributed to the different microbial populations and functions since soil microbial community could substantially transform N into gasses (called nitrous oxide and nitrogen gas) in wet soil which dissolve in water. Moreover, sediment is a net sink of N, which is the net transfer of N from the water column to sediment or from sediment to water.

Similarly, decreasing in soil TN concentrations (9%, p = 0.011) may also depend on the soil microbial community abundance because increasing C input to

the soil under elevated CO_2 could lead to increased microbial growth, immobilization of N, and thereby reduction of dissolved N in soil (Nord et al. 2015; Niklaus et al. 1998). Increased N demand by heterotrophic microorganisms (ammonium used bacteria) would decrease ammonium concentrations decreasing nitrate (oxidation from ammonium) availability for nitrifiers (nitrate used bacteria) and this may follow decrease in soil nitrate concentrations (Hungate 1999). The ammonia-oxidizing bacterial community in the sediment surface can impact the transport of nitrogen to overlying water (Satoh et al. 2007). Sediments are a major site of the nitrification-denitrification by main microbes that can remove the labile N from the sediments to overlying water bodies and thereby reduction in N concentration in soil (Satoh et al. 2007).

In this study, as C17:0, ammonia-oxidizing bacteria became dominant in elevated CO_2 soil, its populations and functions also stimulate the N cycle (Satoh et al. 2007). Ammonia-oxidizing bacteria convert ammonia into nitrous oxide, thereby supporting higher rates of denitrification and the main removal pathway for N to water. When fungi are inhibited in soil, denitrifiers or ammonia-oxidizing bacteria produced a substantially high amount of nitrous oxide and increased nitrate availability in soil (Hu et al. 2001; Balser and Firestone 2005). Anaerobic sulfate-reducing bacteria (cycC17:0) is also associated with N_2O production. C16:1ω5c, a mycorrhizal fungi biomarker, dominated

elevated CO_2 soil in the present study. Mycorrhizal fungi increased the community metabolic efficiency and enhanced C storage as well as increases fungal PLFAs, and C18:2ω6c which may be connected with the decreased total and available soil N, and hence, denitrification occurs (Hu et al. 2001; Balser and Firestone 2005). N_2O production by denitrification is especially enhanced at high C content due to the energy source for microbes to enhance the heterotrophic nitrification process (Hu et al. 2001; Balser and Firestone 2005).

Moreover, the increased soil labile TOC at elevated CO_2, when it occurs, enhances higher microorganisms resulting in decreased soil N concentrations (Barnard et al. 2005). Gram-negative bacteria, which lead to further C and N recycling, were higher in abundance (by 136.27%, $p = 0.011$) under elevated treatment in this study and contributed to soil C storage as well as lead to more available N in the soil (Balser and Firestone 2005; Fraterrigo et al. 2006). Lipson and Näsholm (2001) mentioned that dominant Gram-negative bacteria are associated with nitrous oxide production (denitrification). Soil C sufficiently supports denitrifier populations resulting in denitrification (Wrage et al. 2001).

The results from this study also agreed with other studies (Wrage et al. 2001; Jensen and Andersen 1992; James et al. 1997). The diffusive NH_4^+ transformation by ammonia used bacteria from aquatic sediments contribute a significant amount of N to the water column. Although excessive external nutrient loads have reduced, internal nutrient loads can have significant influence on the water quality of shallow lakes (Wrage et al. 2001; Jensen and Andersen 1992; James et al. 1997). Since soil N concentration was decreased (9%, $p = 0.011$) by microbial activity, such decreased soil N reflected the increased overlying water concentration of N (26%, $p = 0.0001$). Therefore, the contribution of biochemical pathways of internal cycles of C and N to water is governed by the compositions of the bacterial populations and the functions of the resident soil. The results showed that under higher CO_2 this is a significant effect on the microbial community composition and cascading impact on the wetland ecosystem of plant, water, and soil of C and N cycling during the growing season.

Conclusions

The study showed conclusively that the effect of CO_2 enrichment is likely to increase plant biomass, and positively affect the microbial community abundance in the soil of wetlands. Besides, C availability is one of the main driving forces to increase dissolved inorganic nitrogen in water which would closely relate to soil mineralization and microbial uptake of C by a significant increase of the microbial community abundance under elevated CO_2. The concentration of TOC concentrations in water with plants increased significantly under elevated CO_2 that consequently indicated the significant increase in concentrations of TOC in the soil. This suggests that the upper epidermis of leaves and roots of plants can directly exchange CO_2 (IC) for labile TOC from the atmosphere to water and soil. Under elevated CO_2 with plants, significant increase in the TC and TOC concentrations to soil may increases microbial-C availability thereby stimulating increased demand for N, increasing microbial NH_4^+ immobilization (consumption) and decreasing net NH_4^+ mineralization (production) in soil. The information garnered from this study complements past studies and thereby provides valuable information to stakeholders and researchers on understanding the impact of CO_2 elevation on the ecosystem.

Authors' contributions
Conceptualization: D.J., L.C., and N.X. Data collection and analysis: D.J., D.A.K., N.X., E.N., and W.K.D. Writing—original draft preparation: D.J., D.A.K., N.X., E.N., and W.K.D. Writing—review and editing: D.A.K., L.C., W.K.D., and E.N. Supervision: L.C. and W.K.D. Funding acquisition: L.C. All authors have read and agreed to the published version of the manuscript.

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Author details
[1]College of Horticulture, Jilin Agricultural University, Changchun 130118, China. [2]Jilin Tiandiren Landscape Architecture Design Engineering Co. Ltd., Changchun 130117, China. [3]Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Environmental Engi neering Department, College of Environment, Hohai University, 1 Xikang Road, Gulou, 210098 Nanjing, China.

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