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ORIGINAL PAPER

Contrasting effects of ammonium and nitrate inputs on soil CO_2 emission in a subtropical coniferous plantation of southern China

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Abstract Increased nitrogen (N) deposition has been found controversial affecting soil CO_2 emission in terrestrial ecosystems, which leads to serious debate on the efficiency of estimated C sequestration induced by N enrichment. The forms of input N might be responsible for this controversy. This study aims to explore the effects of NH_4^+ (reduced N) and NO_3^- (oxidized N) on soil CO_2 flux and the underlying microbial mechanisms. An N addition experiment, two N fertilizers (NH_4Cl and $NaNO_3$) and two rates (40 and 120 kg N ha^{-1} $year^{-1}$), was carried out in a slash pine plantation of southern China. Soil-atmospheric CO_2 exchange, soil microbial biomass, and community composition were measured using static chamber-gas chromatography and phospholipid fatty acid (PLFA) analyses in the active growing and nonactive growing seasons, respectively. Low level of $NaNO_3$ addition significantly increased soil CO_2 flux in the active growing season, whereas other N treatments did not change soil CO_2 flux. High level of NH_4Cl addition significantly reduced soil fungal biomass (fungal PLFA) and changed microbial community composition (ratio of fungal to bacterial (F/B) PLFAs). The positive relationships between the change in soil CO_2 flux and the change in fungal biomass, as well as between the change in soil CO_2 flux and the change in community composition, were observed in the nonactive growing season. The N forms as NO_3^- or NH_4^+ are important factors affecting C cycles in the subtropical coniferous plantation. These results suggested that the variations of soil CO_2 emission and microbial biomass and community composition in the subtropical plantation depended on the seasons and the levels and forms of N addition.

Keywords Ammonium · Nitrate · Nitrogen deposition · Soil CO_2 flux · Soil microbial community · Subtropical coniferous plantation

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Introduction

Carbon (C) and nitrogen (N) are dynamically distributed among various pools in terrestrial ecosystems, and the biogeochemical cycles of C and N are closely coupled together in a relatively stable equilibrium over a long time period (Thornton and Rosenbloom 2005). Exogenous N inputs into terrestrial ecosystems break this equilibrium, altering the nutrient cycle, changing microbial functions, and providing more nutrients to vegetation growth, and finally change the structure and function at ecosystem level (Chapin et al. 2011). In the past 150 years, human activities have increased the amount of reactive N supplied to the biosphere by 2.5-fold (Galloway et al. 2008), which in turn accelerates N transformation rates (Lu et al. 2011a), reduces biodiversity (Bobbink et al. 2010; Lu et al. 2010), and increases vegetation productivity and ecosystem C sequestration (Magnani et al. 2007; Lu et al. 2011b). However, there are still debates on the efficiency of C sequestration in terrestrial ecosystems caused by N deposition, ranging from 30 to 200 kg C kg^{-1} N (Högberg 2007;

Magnani et al. 2007). Therefore, more process-leveling understandings are needed to reduce the uncertainties.

Root respiration (and associated mycorrhizal fungi) and microbial respiration constitute soil-atmospheric exchange of CO_2, they are the most important C efflux in the ecosystem, and both of them are regulated by soil N availability (Fang et al. 2010, 2014a). However, the effects of increased N deposition on soil CO_2 flux are high variable, including promotion (Cleveland and Townsend 2006; Contosta et al. 2011), inhibition (Mo et al. 2008; Janssens et al. 2010), and no change (Allison et al. 2008), depending on the level and the form of N addition (Fang et al. 2012), on the duration of N addition (Hasselquist and Högberg 2014), and on distinct stages of N saturation (Sutton et al. 2011). In addition, these studies have been conducted using a type of N fertilizer (e.g., NH_4NO_3 or urea) (Mo et al. 2008; Phillips and Podrebarac 2009), and the contrasting effects of oxidized NO_3^- and reduced NH_4^+ have rarely been investigated (Lu et al. 2011a). Recently studies found that the NO_3^- and NH_4^+ might have totally different impacts on ecosystem processes due to plant's preferable uptake, competition between plant and microbial communities, and different action in soils (Gavrichkova and Kuzyakov 2008; Fang et al. 2012).

Bacteria and fungi decay organic matter and release CO_2, which dominates soil heterotrophic respiration (Kuzyakov 2006). N input can promote or inhibit the rate and extent of soil organic matter decomposition through changing the activity and composition of microbial community (DeForest et al. 2004), as well as the chemical property of organic matter (e.g., C/N, lignin/N, alkyl C/O-alkyl C) (Whittinghill et al. 2012). Along with the continuous N input, NO_3^- and NH_4^+ ions are rapidly assimilated by plants and soil microbes; subsequently, the N input stimulates soil microbial activity and decreases litter's C/N ratio leading to increases in litter decay and soil CO_2 emission (Knorr et al. 2005). At the later stage of N saturation, inorganic N accumulation in soils inhibits the activity of white rot fungi and extracellular enzyme synthesis, thereby reduces litter decay and soil CO_2 emission (Zak et al. 2011). The N input rate at the transition point when N input stimulates soil organic matter decomposition changes to suppress soil organic matter decomposition is defined as N critical load (Fang et al. 2014c). For a special ecosystem, however, the critical load of N deposition for the alteration of soil CO_2 emission is not well estimated. Moreover, little information is available on the linkage between soil CO_2 flux and microbial community activity and composition in the field under N enrichment.

Forest plantations in the world total approximately 130×10^6 ha, and annual rate of establishment is about 10.5×10^6 ha. Total C storage in forest plantations is approximately 11.8 Pg C with an annual increase of 0.18 Pg C $year^{-1}$, playing an important role in C sequestration in terrestrial ecosystems (Winjum and Schroeder 1997). The total plantation area in China is 6.26×10^6 ha, accounting for 31.8 % of China's forest area and ranking first in the world (Department of Forest Resources Management 2010). Approximately 63 % of plantations in China distribute in the subtropical climate region where N deposition rate is the highest (>30 kg N ha^{-1} $year^{-1}$) (Jia et al. 2014).

In theory, plant competition for soil N is greater than soil microbes in the N-limiting ecosystems (Kuzyakov and Xu 2013), but plant-microbial competition for N is uncoupled and opposite in the N saturation ecosystems (Kaye and Hart 1997; Månsson et al. 2009). In some grassland and forest ecosystems, low level of N addition increases litter return and stimulates soil microbial activity because it satisfies the N demand of soil microorganisms to decompose the relatively small pool of available labile C substrates with low N contents (Fang et al. 2014b); however, high level of inorganic N inputs suppresses the synthesis of lignolytic enzymes by some fungi due to the decrease in organic matter decay extent (Cusack et al. 2011; Whittinghill et al. 2012). Also, because NO_3^- assimilated by plants and microbes has to be firstly reduced to NH_4^+, this energy-intensive process consumes more C fixed by photosynthesis and releases additional CO_2 (Gavrichkova and Kuzyakov 2008; Tischner 2000). Therefore, we hypothesized that low N promoted soil CO_2 emission and microbial activity, whereas high N reduced them; moreover, NO_3^--N fertilizer addition effects on soil CO_2 flux and microbial activity could be greater than NH_4^+-N fertilizer addition.

To test these hypotheses, we conducted an N addition experiment in a forest plantation of subtropical China (1) to examine the different effects of NH_4^+ and NO_3^- on soil CO_2 flux in terms of root respiration and microbial respiration and (2) to investigate the underlying microbial mechanisms for this difference.

Materials and methods

Site description

This experiment was conducted in a subtropical slash pine plantation at the Qianyanzhou Ecological Station, Chinese Academy of Sciences (26° 44′ 39″ N, 115° 03′ 33″ E), which is located in Jiangxi province, southern China. The Qianyanzhou site is characterized by a subtropical monsoon climate. The mean annual air temperature and mean annual precipitation during 1989–2008 are 17.9 °C and 1505 mm, respectively. The N deposition rate in this area could reach 40 kg N ha^{-1} $year^{-1}$ in 2000s (Jia et al. 2014). The native vegetation, subtropical evergreen broadleaf forest, has almost been wiped out due to long-term deforestation and land use conversion to agriculture. *Slash* pine, *masson* pine, and *Chinese fir* plantations were established since 1985, and the

total area of plantations was 122.73 ha in 1997 (Wang et al. 2004). Slash pine plantation accounts for 33 % of the total area of plantations with total living biomass of 104.13 t ha^{-1} (Ma et al. 2014). Soils are typical red soils, classified as Cambosols, which were derived from sandstone and sandy conglomerate (IUSS Working Group 2006). For the topsoil (0–20 cm), organic matter is 20.44 g kg^{-1}, total N is 1.10 g kg^{-1}, total phosphorus is 1.12 mg kg^{-1}, pH is 4.26, and soil bulk density is 1.54 g cm^{-3}.

Experimental design

On May 1, 2012, the N addition experiment was initialized using a random block design. To evaluate the effects of NH_4^+ and NO_3^- inputs on soil CO_2 flux and microbial community composition, NH_4Cl and $NaNO_3$ were added at two rates: 40 and 120 kg N ha^{-1} $year^{-1}$ to simulate a future increase in the atmospheric N deposition by 1- and 3-fold. A control plot was set up as well. Five treatments were hereafter referred to as control, low NH_4Cl, low $NaNO_3$, high NH_4Cl, and high $NaNO_3$, respectively. A total of 15 plots (20 m×20 m) were established and surrounded by a 10-m-wide buffer strip. N fertilizer solutions were sprayed at the first week of every month by spraying below the canopy, and the control plots received equivalent water (Wang et al. 2014).

Soil CO_2 flux measurement

Soil CO_2 flux was measured using a static opaque chamber and gas chromatography techniques (Wang et al. 2014). The static chambers were made of stainless steel and consisted of two separate parts: a square base frame (length×width×height=50 cm×50 cm×10 cm) and a removable top (length×width×height=50 cm×50 cm×15 cm). The collar was fully inserted into soil and remained intact during the entire observation period. Chambers were temporarily mounted onto the frames for gas flux measurements. The soil CO_2 flux was measured twice a week and conducted between 9:00 and 11:00 a.m. (China Standard Time, CST) since May 1, 2012. The soil CO_2 fluxes of four periods (August 2012, December 2012, March 2013, and May 2013) were selected to correspond the soil microbial biomass. Gas samples were collected five times within 40 min using plastic syringes. The CO_2 concentration of all gas samples was determined by the gas chromatography (Agilent 7890A, Santa Clara, California, USA) within 24 h after gas samples were taken. The soil CO_2 fluxes were calculated based on the slope of linear or nonlinear regression between concentration and time (Zheng et al. 2008). All the coefficients of determination (r^2) of the regression were greater than 0.95 in our study.

Soil sampling and PLFA measurement

There is no rigid division between growing season and non-growing seasons in the subtropical plantation forest. Therefore, we divided the whole year into active growing season (from May to August) and nonactive growing season (from September to April) (Yu et al. 2008). Soil samples were collected four times in active growing season (August 16, 2012, and May 13, 2013) and nonactive growing season (December 23, 2012, and March 12, 2013), respectively. At each plot, the litter layer was removed and mineral soil at depths of 0–15 cm was collected. Five soils from two diagonal lines through each plot were collected and pooled to one composite sample (Fang et al. 2014c). Soils were sieved to 2-mm-mesh size and then transported to the laboratory in a chilled polystyrene box.

Soil microbial community dynamics were assessed using the phospholipid fatty acid (PLFA) method. PLFAs in soils indicate microbial biomass and fingerprint microbial community composition (Frostegard and Bååth 1996). Although the PLFA method relying on extraction and analysis of specific cell components cannot provide information about the physiological status or diversity of the microbial community (Roslev et al. 1998), it is widely applied to investigate the effects of experimental N deposition in various forest and grassland ecosystems (Waldrop and Firestone 2004; van Diepen et al. 2010). Briefly, 8 g of lyophilized soil was extracted using a single-phase chloroform-methanol-citrate buffer (1:2:0.8) system with the amount of citrate buffer corrected to account for existing soil water content (Ferre et al. 2012; Zhang et al. 2013a). Phospholipids were extracted from neutral lipids and glycolipids on silica solid-phase extraction columns (Supelco, Inc., Bellefonte, USA) by elution with chloroform, acetone, and methanol, respectively. The methyl ester fatty acids from phospholipids were produced by a methylation procedure (Pietri and Brookes 2009). The fatty acid methyl esters were detected on a Hewlett-Packard (HP 6890) gas chromatograph. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as the internal standard before the methylation step. The identification of fatty acid methyl esters was based on comparison with chromatograms of fatty acid methyl ester standard compounds (Bacterial Acid Methyl Esters Mix from Supelco) and on structural analysis verified by gas chromatography mass spectrometry (Spitzer 1996). The abundance of individual fatty acids was determined as nanomole per gram of dry soil.

Soil microbial biomass was estimated by the total amount of extracted PLFAs. Total bacterial biomass was measured by summing the following PLFAs 14:0, 18:0, 19:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:0 N alcohol, 16:1ω7c, i17:1G, i17:1ω5c, 15:0 3OH, cy17:0, 16:1 2OH, 18:1ω9t, 18:1ω5c, 18:1ω11c, 19:1ω8 alcohol, cy19:0 c11-12, and cy19:0 c11-12 2OH. The fungal biomass was identified by the PLFAs

16:0, 16:1ω5c, 18:1ω9c, and 18:2ω6c (Vestal and White 1989; Frostegard and Bååth 1996). The Gram-positive bacteria were represented by the sum of branched lipids i15:0, a15:0, i16:0, i17:0, and a17:0 (Frostegard and Bååth 1996; Zelles 1999). The Gram-negative bacteria were calculated by the sum of monoenoic and cyclopropane unsaturated PLFAs 16:0 N alcohol, 16:1ω7c, i17:1G, i17:1ω5c, 15:0 3OH, cy17:0, 16:1 2OH, 18:1ω9t, 18:1ω5c, 18:1ω11c, 19:1ω8 alcohol, cy19:0 c11-12, and cy19:0 c11-12 2OH (Frostegard and Bååth 1996). The ratios of Gram-positive to Gram-negative (G^+/G^-) bacterial PLFAs and fungal to bacterial (F/B) PLFAs were calculated to estimate the composition of soil microbial community.

Statistical analysis

Repeated measures analysis of variance (ANOVA) with honestly significant difference (HSD) test was applied to examine the differences of soil CO_2 flux, soil microbial biomass, and community composition among the five treatments. Experimental treatment was set as between-subjects factor, and measurement date was selected as within-subjects variable. Standardized regression analysis was used to examine the relationships between the net change of soil CO_2 flux (Δsoil CO_2 flux) and the net change of microbial biomass and community composition (ΔPLFAs, ΔF/B ratio). All statistical analyses were performed using SPSS software package (version 16.0; SPSS, Inc.), and statistical significant difference was set with P value=0.05 unless otherwise stated.

Results

Soil CO_2 flux

Soil CO_2 flux across all five treatments showed an obvious seasonality with the maximum and minimum occurring in August and December, respectively (Fig. 1, Table 1, $P<0.001$). In the control plots, soil CO_2 flux averaged 67.99 and 21.29 mg C m^{-2} h^{-1} in the active growing and nonactive growing seasons, respectively (Fig. 1b). The average CO_2 flux from the subtropical plantation soil was 48.67 mg C m^{-2} h^{-1} during the whole study period (Fig. 1b). The added amount and form significantly changed soil CO_2 flux in the active growing season (Table 1, $P<0.001$ and $P=0.011$), while no significant difference in soil CO_2 flux was found in the nonactive growing season. When summed up across the annual timescale, the impacts were significant as well (Table 1, $P<0.001$ and $P=0.043$). Furthermore, a significant interaction between N level and N form was found, and low level of $NaNO_3$ addition significantly increased soil CO_2 flux in the active growing season (Table 1, $P=0.001$, Fig. 1b).

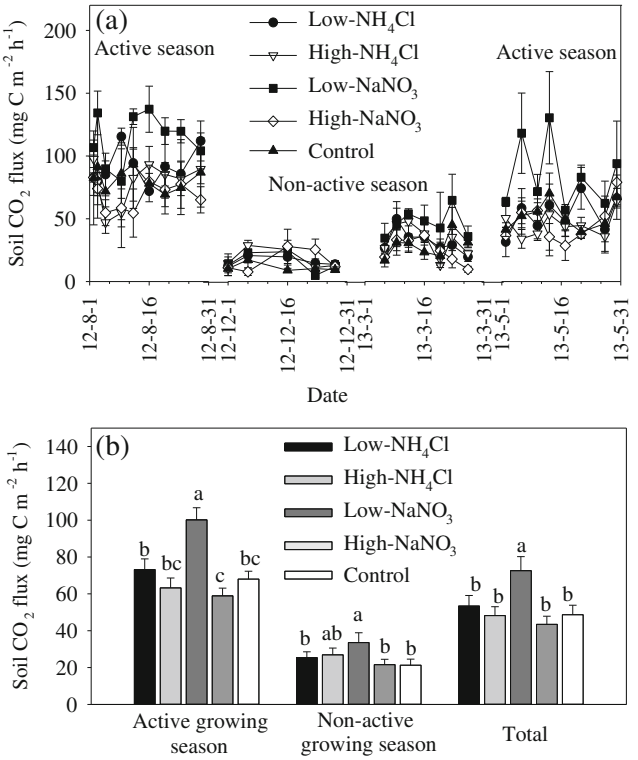

Fig. 1 Seasonal variation of soil CO_2 flux under the five experimental treatments. Data are shown as means with standard errors. *Different letters above the columns* mean significant differences among experimental treatments

Soil microbial groups

Except the fungal PLFA, total PLFA and other groups' PLFAs show a pattern of seasonal changes (Table 2). The maximum and minimum of total PLFA and bacterial PLFA occurred in May and August, respectively (Fig. 2a, b), and the similar seasonality was also found in the Gram-positive and Gram-negative bacterial PLFAs (Fig. 3a, b). In the control, there were no significant differences in both total PLFA and each group' PLFAs between the active growing season and nonactive growing seasons (Figs. 2 and 3). Low level of N addition tended to increase fungal PLFA, but high level of N addition tended to decrease it (Fig. 2f, Table 2, $P=0.023$). The difference in fungal PLFA between low-NH_4Cl and high-NH_4Cl treatments was significant in the nonactive growing season and the whole observation (Fig. 2f). However, N form had no significant effects on the abundance of each microbial group (Table 2).

For microbial community composition, significant seasonal variations in both F/B ratio and G^+/G^- ratio were found (Table 2, $P<0.001$). The maximum and minimum of soil F/B ratio occurred in August and May, respectively, but the seasonal variation of soil G^+/G^- ratio was opposite (Figs. 2g and 3e). N level rather than N form significantly changed soil

Table 1 Results of repeated measures ANOVA on the effects on N level, N form, date, and their interaction on soil CO_2 flux

Source of variation	Active growing season		Nonactive growing season		Whole observation	
	F	*P*	*F*	*P*	*F*	*P*
Between subjects						
N level	29.83	<0.001	1.14	0.30	26.37	<0.001
N form	6.93	0.011	0.003	0.96	4.27	0.043
N level×N form	10.69	0.002	0.56	0.46	11.03	0.001
Within subjects						
Date	17.44	<0.001	16.46	<0.001	0.014	<0.001
Date×N level	0.42	0.74	1.47	0.23	8.75	<0.001
Date×N form	1.02	0.39	1.61	0.19	2.57	0.061
Date×N level×N form	1.52	0.22	1.28	0.29	2.93	0.040

F/B ratio in the subtropical plantation (Table 2, P=0.028). Moreover, there was a significant interaction for soil F/B ratio between N level and N form (Table 2, P=0.037). Compared with control, high NH_4Cl significantly decreased soil F/B ratio by 12.79 and 17.73 % in the nonactive growing season and the whole observation, respectively (Fig. 2h).

Relationships between soil CO_2 fluxes and soil microbial community composition

The net change in soil CO_2 flux (ΔCO_2 flux) was positively correlated with the net changes of fungal PLFA (Δfungal PLFA) and microbial community composition (ΔF/B ratio) in the nonactive growing season, respectively (Fig. 4c, d). The Δfungal PLFA and ΔF/B ratio could explain 37 and 30 % of the ΔCO_2 flux, respectively (Fig. 4c, d). However, no significant relationships between them were observed in the active growing season (Fig. 4a, b).

Discussion

Effects of N addition on active soil microbial biomass and composition

Low level of N addition tended to increase soil total and fungal PLFAs and thus F/B ratio, whereas high level of N addition had an opposite effect, especially in the nonactive growing season (Fig. 2f, h). This confirms our first hypothesis that low N increased soil microbial biomass, whereas high N tended to reduce it. The F/B ratio increased in the low-N-fertilized plots, suggesting that fungi are relatively more limited by N than bacteria (Fanin et al. 2015). Based on our prior research, we found that soil acidification and NO_3^- accumulation could be responsible for the opposing effects between low N and high N treatments (Wang et al. 2014). Both Högberg et al. (2013) and Stark et al. (2014) documented negative relationships between fungal biomass and soil NO_3^- content, pH, and Al^{3+}, and soil nutrient availability and pH together controlled extracellular enzyme activities and soil

Table 2 Results of repeated measures ANOVA on the effects of N level, N form, month, and their interactions on soil PLFAs

Source of variation	Total PLFA		Bacterial PLFA		Fungal PLFA		F/B ratio		Gram-positive bacteria PLFA		Gram-negative bacteria PLFA		G^+/G^- ratio	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between subjects														
N level	3.57	0.088	2.56	0.14	7.21	0.023	6.55	0.028	2.61	0.14	1.53	0.25	0.88	0.37
N form	0.36	0.57	0.32	0.59	0.36	0.56	0.005	0.95	0.039	0.85	0.57	0.47	0.093	0.77
N level×N form	0.007	0.94	0.050	0.83	0.46	0.51	5.80	0.037	0.42	0.53	0.009	0.93	0.31	0.59
Within subjects														
Month	6.03	0.002	7.27	0.001	1.66	0.20	84.69	<0.001	9.01	<0.001	8.90	0.014	14.42	0.001
Month×N level	0.54	0.67	0.32	0.81	2.71	0.063	5.91	0.020	0.34	0.80	0.21	0.66	0.66	0.60
Month×N form	0.60	0.62	0.56	0.65	0.83	0.49	3.41	0.073	0.38	0.77	0.84	0.38	0.81	0.52
Month×N level×N form	1.67	0.19	1.38	0.27	1.71	0.19	3.10	0.089	1.77	0.18	1.06	0.33	1.13	0.39

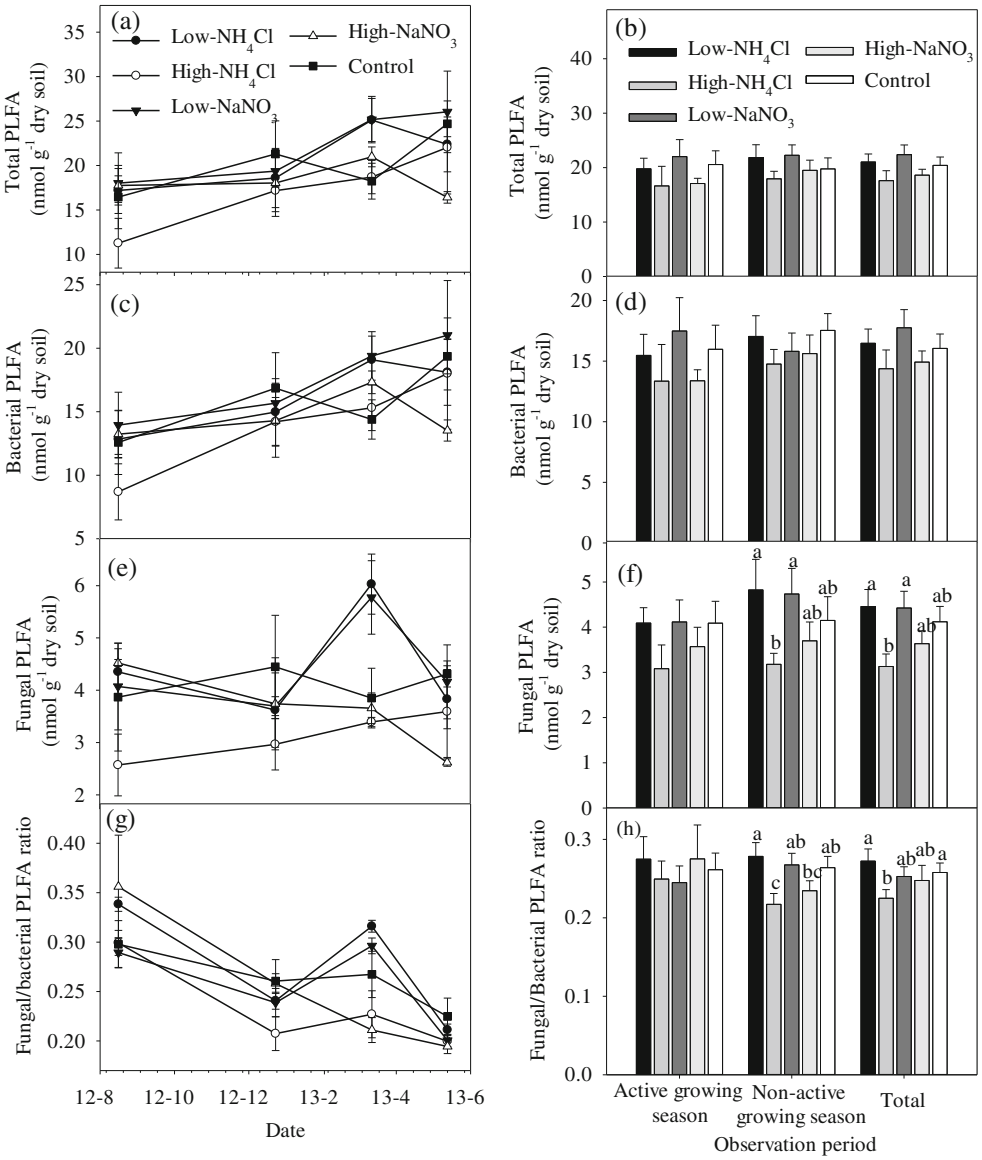

Fig. 2 Seasonal variation of soil total PLFA, bacterial and fungal PLFAs, and ratio of fungal to bacterial (F/B) PLFAs under the five experimental treatments. Data are shown as means with standard errors. *Different letters above the columns* mean significant differences among experimental treatments

organic matter (SOM) dynamics. In the early of N addition, N limitation is alleviated by fertilization, microbial biomass, and enzymatic capacity for cellulose decomposition increase, which facilitates greater decomposition of SOM and CO_2 emission (Gilliam et al. 2011). However, a high N supply increases bacterial abundance for some groups and suppresses decomposer fungal abundance, with detectable increases in complex C compounds in SOM (Fang et al. 2014b). Therefore, our results indicate that N addition leads to declines in labile C compounds and increases complex C compounds through increasing hydrolytic enzyme activities and decreasing oxidative enzyme activities in the N-rich subtropical plantation forest (Cusack et al. 2011; Nannipieri et al. 2012). Similarly, N fertilization in northern US hardwood and pine forests also suppresses fungal biomass, oxidative enzyme activity, and the processing of some C compounds (Frey et al. 2004).

Additionally, we found that high level of NH_4Cl addition had the strong inhibition to soil microbial activity and community composition (Fig. 2f, h), which was consistent with our second hypothesis. Inorganic N inputs decrease the decomposition of organic matter through changing its chemical properties (e.g., C/N ratio) as well as microbial metabolic activity. Nearby our study site, Chen et al. (2012) reported that NH_4NO_3 addition at rates of 7.5 and 15 g N m^{-2} $year^{-1}$ significantly decreased the C/N ratio of masson pine litter by 27.6 % in a 540-day incubation study. The molecular mechanism mediating a decline in microbial activity under high level of N addition is the transcriptional downregulation of fungal genes encoding phenol oxidase, Mn peroxidase, and lignin peroxidase (Hassett et al. 2009; Entwistle et al. 2013). Such

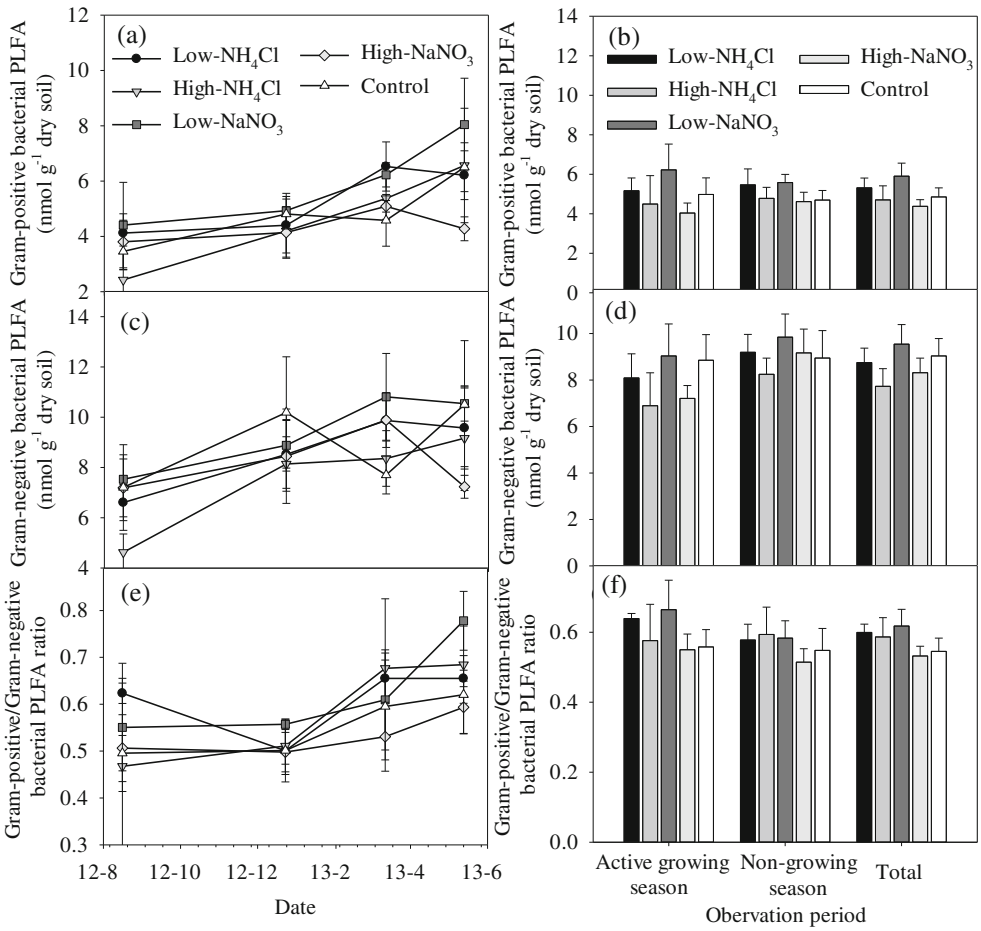

Fig. 3 Seasonal variation of soil Gram-positive and Gram-negative bacteria PLFAs and ratio of Gram-positive to Gram-negative bacteria (G^+/G^-) PLFAs under the five experimental treatments. Data are shown as means with standard errors

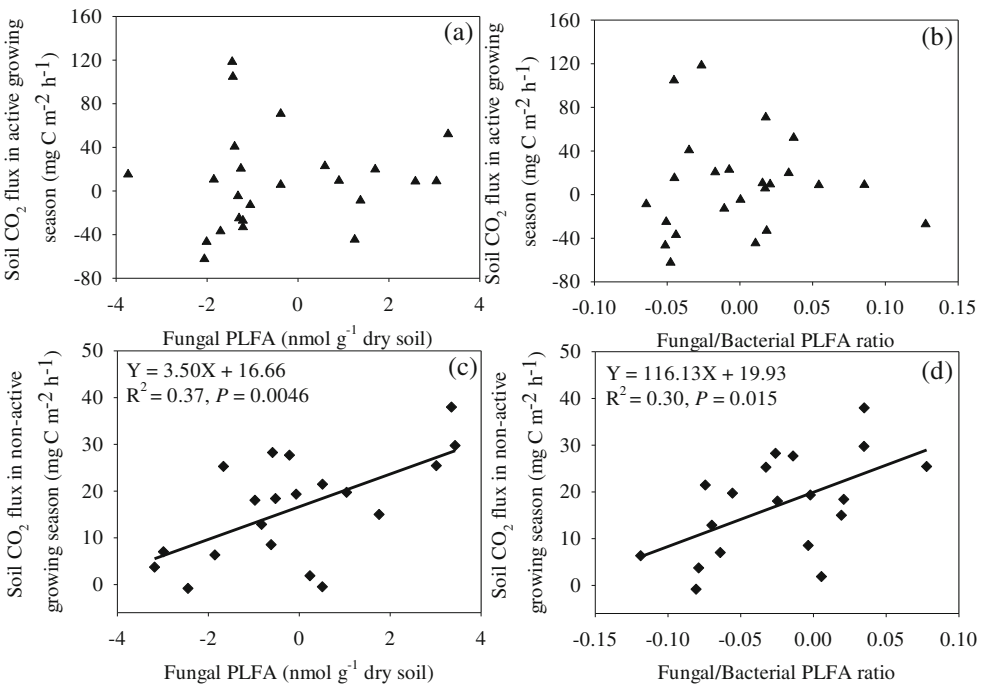

Fig. 4 Relationships between the change in soil CO_2 flux (Δsoil CO_2 flux) and the change in fungal PLFA (Δfungal PLFA), as well as between the change in soil CO_2 flux (Δsoil CO_2 flux) and the change in ratio of fungal to bacterial bacteria PLFAs (ΔF/B ratio) in the active and nonactive growing seasons (n=60)

a response could translate a decline in organic matter decomposition (Zak et al. 2008).

Furthermore, our results showed that the net change of soil CO_2 flux was positively related to the net changes of fungal PLFA content and F/B ratio in the nonactive growing season (Fig. 4c, d), which suggested that microbial respiration could dominate soil-atmospheric CO_2 exchange in the nonactive growing season. In the active growing season, however, soil CO_2 flux was primarily dominated by root autotrophic respiration although the microbial respiration plays an important role. Although we did not simultaneously measure root autotrophic respiration, the measured root biomass over the same period indirectly provided some explanation considering a positive relationship between root autotrophic respiration rate and fine root biomass (Mo et al. 2008). Based on our N addition experiment, Kou et al. (2015) found that N additions significantly increased the fine root biomass of slash pine, indicating an increase in root autotrophic respiration. The above result, to a certain extent, confirms our first work hypothesis, too.

Effects of N levels and forms on soil CO_2 flux

Soil CO_2 flux in the subtropical plantation forest exhibits a single-peak pattern with the maximum and minimum occurring in the active growing season and in the nonactive growing season, respectively, which indicates the temperature control on the seasonality of soil CO_2 flux (Tang et al. 2006; Mo et al. 2008). Under nature conditions, the average CO_2 flux from the slash pine plantation soils was 48.67 mg C m^{-2} h^{-1}, which is lower than those of masson pine forests nearby the study site (84.02 mg C m^{-2} h^{-1}) (Wang et al. 2011) and other subtropical plantation forests (56.2–66.0 mg C m^{-2} h^{-1}) (Mo et al. 2007; Wang et al. 2013). Probably, this depends on the fact that forest type, substrate availability, and microbial activity largely determine soil CO_2 flux at the local scale (Fang et al. 2014b).

For the N levels, low dose of N addition tended to increase soil CO_2 flux, but high N did not change soil CO_2 flux relative to the control. This is just partially consistent with our first hypothesis. We did not observe a significant decrease in soil CO_2 flux at the high N treatments, suggesting that the subtropical plantation is in the early or medium stages of N saturation (Aber et al. 1998). Generally, the promoting effect has a short duration (e.g., less than 1 year) and shifts to the neutral and inhibitory effects (Bowden et al. 2004; Fang et al. 2012). In our study, we found that each N treatment significantly increased soil CO_2 flux in the first 6 months of N addition (Wang et al. 2014), while the promotion was only observed at the low-$NaNO_3$ treatment after 1-year N addition (Fig. 1). Over the same period, N addition significantly increases plant root growth as well as root autotrophic respiration (Kou et al. 2015), which also indicates that soil N availability does not exceed plant demand for N. Furthermore, the proportion of microbial respiration to total respiration is more than 60 % in our study site (Wang et al. 2012), and microbial community dynamics could well explain the responses of soil CO_2 flux to N addition. Our findings differ from those of some grasslands and forests that the increase in N availability could decrease soil fauna abundance and microbial heterotrophic respiration (Fang et al. 2014b; Ochoa-Hueso et al. 2014). In our study site, most of the applied N is sequestered within the plants over the short term (1 year), and only a small amount of added N is used to increase microbial biomass (Sheng et al. 2014). No significant increase in total PLFA supported this speculation (Table 2). The potential inhibition of microbial respiration from high level of N addition could cancel out the N stimulation on root respiration, which could be an alternative explanation for neutral effects of high N level on soil CO_2 flux.

For the N forms, we found that low $NaNO_3$ had a significantly positive impact, whereas low NH_4Cl had a positive yet nonsignificant effect on soil CO_2 flux. This result confirms our second hypothesis that NO_3^--N fertilizer had a greater stimulation effect on soil CO_2 flux than NH_4^+-N fertilizer. It should be emphasized that the contrasting effects of deposited NO_3^- and NH_4^+ were only found at the low level of N addition. A positive correlation between soil CO_2 flux and soil NO_3^--N content is generally observed at large spatial scales (Fang et al. 2010). The higher promotion of CO_2 flux by NO_3^--N than by NH_4^+-N could be attributed to the following mechanism. First, deposited NH_4^+ and NO_3^- have distinct fate in the subtropical plantation forest: NH_4^+ is strongly absorbed onto cation exchange sites in SOM and clay minerals, while NO_3^- is very mobile and is absorbed by plants and soil microbes (Koba et al. 2003; Inselsbacher et al. 2010). Based on a ^{15}N tracer experiment in the subtropical forests of southern China, Sheng et al. (2014) also reported that more $^{15}NH_4^+$ was recovered from organic and mineral soils, while a large proportion of $^{15}NO_3^-$ was recovered from plant roots. In the early stage of N saturation, most of the deposited N is sequestered within the vegetation biomass (Bowden et al. 2004), and the increased C allocation to root systems could increase autotrophic respiration. Second, both plants and microbes are not able to directly use NO_3^- and have to reduce NO_3^- to NH_4^+ before assimilating it (Tischner 2000; Gavrichkova and Kuzyakov 2008). The high energy demands during NO_3^- reduction in roots and NO_3^- assimilation by rhizospheric microorganisms result in a greater increase in root-derived respiration compared to NH_4^+-N assimilation. NO_3^- can be immobilized by soil microflora if NH_4^+ concentration is below a certain level. Heterotrophic nitrification, the oxidation of organic amino N to NO_3^-, is prevalent in the acidic forest soils in subtropical region of China (Zhang et al. 2013b). This heterotrophic process will lead to extra CO_2 emission, too.

Effects of Na^+ and Cl^- on microbial community and soil C process

In the first year of N addition, we did not measure the concentrations of Na^+ and Cl^- in soils, leading to no direct knowledge on their effects on microbial activity and soil CO_2 flux. However, based on few related studies, we deduced that Na^+ and Cl^- inputs with N fertilizers could not significantly influence soil microbial community and C processes in the short term (e.g., 1 year). First, extensive microbial uptake and release of Cl^- and Na^+ occur over short timescales (from a week to a month) and are probably associated with changes in environmental conditions (Bastviken et al. 2007). High rainfall in our study site can remove lots of exogenous Na^+ and Cl^- via surface runoff. Second, Na^+ and Cl^- in soils are more likely to affect rhizosphere microbial community composition indirectly through root exudate quantity and/or quality than directly through microbial toxicity. Nelson and Mele (2007) documented that the addition of NaCl to soil changed rhizosphere microbial community structure indirectly through increased soil moisture and subtle changes in root exudate patterns and produced a more distinct change through increased osmotic pressure, leading to a greater increase in rhizodeposition of nutrients, especially carbohydrates. Similarly, Deforest et al. (2004) also reported that 10-year $NaNO_3$ addition at a rate of 30 kg N ha^{-1} $year^{-1}$ did not lead to a significant decrease in soil enzyme activities in the hardwood forests of North America.

Conclusions

This study emphasizes the contrasting effects of NO_3^- and NH_4^+ inputs on soil CO_2 flux, soil microbial biomass, and community composition in a subtropical coniferous plantation forest. Low level of NO_3^--N fertilizer addition slightly stimulated fungal activity, but high level of NH_4^+-N fertilizer addition significantly inhibited fungal activity and changed microbial community composition (F/B ratio). Although microbial respiration and root respiration dominate soil-atmospheric CO_2 exchange, together, the active microbial biomass and community composition can well explain the responses of soil CO_2 flux to N addition in the nonactive growing season. The contrasting impacts of NO_3^- and NH_4^+ should be incorporated into ecosystem model to better simulate the N impacts of ecosystem C cycle and feedback to the climate dynamics.

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