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REGULAR ARTICLE

Effect of nitrogen additions on root morphology and chemistry in a subtropical bamboo forest

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Abstract
Aims Plant roots play a critical role in ecosystem underground processes, and are potentially influenced by elevated atmospheric nitrogen (N) deposition. However, the effects of N deposition or long-term N additions on plant root properties in forest ecosystems are not well understood. The aim of this study is to explore how N deposition influences root morphology, biomass, and chemistry in a subtropical bamboo forest.
Methods A field experiment was conducted with four N treatment levels (0, 50, 150, and 300 kg N ha^{-1} year^{-1}, applied monthly) in a *Pleioblastus amarus* forest beginning in November 2007. After more than 7 years of N additions, *P. amarus* root samples were collected, and analyzed by an order-based classification approach.
Results Nitrogen additions decreased specific root length (SRL) and specific root area (SRA) in orders (1 + 2), while the reverse was observed in order 3. The branch ratio (BR) of order (1 + 2): order 3 decreased by N additions. Root and rhizome biomass decreased in response to N additions. Nitrogen additions also decreased root tissue concentrations of N, P, K and Mn, and increased the concentration of Na and the ratios of C:N and N:P.
Conclusions Our study indicated that the growth and quality of lower-order roots were inhibited by N additions, potentially leading to a slower underground C cycle. Order-based classification approach revealed more accurate information about roots response to N additions than diameter-based classification method. Chronic N deposition may have pronounced effects on soil environments, plant root traits, and underground C and nutrient cycles in bamboo forest ecosystems.

Keywords Nitrogen deposition · Root morphology · Root chemistry · Root biomass

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Introduction

Rapid industrialization, fossil fuel use and intensification of agriculture since the beginning of the twentieth century have resulted in considerable production and release of anthropogenic reactive nitrogen (N). This N release has reached unprecedented levels that greatly exceed those typical in nature (Galloway and Cowling 2002; Galloway et al. 2004; Gruber and Galloway 2008). A large amount of active N in the atmosphere is subsequently returned to terrestrial and aquatic ecosystems worldwide, resulting in increased N deposition rates. It has been predicted that annual N deposition will increase considerably in the twenty-first century, given the growing N demands of global agriculture and industries, as well as the generally existing phenomenon of inefficient N use (Galloway et al. 2008; Liu et al. 2013).

Over the past few decades, numerous studies have focused on the effects of N deposition on terrestrial ecosystems. However, these effects are not well understood, particularly with reference to how ecosystems' underground processes respond to N deposition. Plant roots play a key role in underground carbon and nutrient cycles in terrestrial ecosystems. Fine roots (in general, defined as <2 mm in diameter) contribute more than 30 % of the net primary production in an ecosystem, despite contributing less than 2 % of the total ecosystem biomass (Vogt et al. 1996; Jackson et al. 1997). Respiration in live roots and decomposition of dead roots make a significant contribute to the flux of carbon (Gordon and Jackson 2000; Zhang and Wang 2015). Meanwhile, the morphological and chemical traits of plant roots are sensitive to environmental changes and exhibit a high degree of plasticity (Bardgett et al. 2014; Wurzburger and Wright 2015). Root morphological and chemical changes can effectively indicate changes in soil nutrient cycles. Thus, plant root behavior can be analyzed to determine how N deposition affects underground processes.

Plant root systems have evolved to maximize resource acquisition while minimizing the energy required for root tissue growth and maintenance (Eissenstat and Yanai 1997; Eissenstat et al. 2000; Wurzburger and Wright 2015). Therefore, root systems adapt to environmental changes by changing themselves. Many field studies have indicated that N deposition can change the local environment around plant roots through direct and indirect effects on the availability of soil N and other nutrients (Mitchell and Smethurst 2008; Lucas et al. 2011). In response, biomass (Liu et al. 2011), morphology (Noguchi et al. 2013), lifespan and turnover (Wang et al. 2012), respiration (Jia et al. 2010; Burton et al. 2012), carbon and nitrogen concentrations (Noguchi et al. 2013) and decomposition rates (Tu et al. 2015) of fine roots in forest ecosystems change accordingly. It must be noted that in the aforementioned studies, fine roots were defined broadly based on their maximum diameter (Pregitzer et al. 2002). However, there is significant evidence indicating that plant root systems exist in the form of branched structures, with roots on different branch orders being significantly different in lifespan, physiological processes, and element concentrations (McCormack et al. 2015). Most studies define fine roots as < 2 mm in diameter, a range that likely includes at least four branch orders (Sun et al. 2015). Thus, an order-based classification approach may be a more precise method of studying plant roots.

Additionally, most published studies on the responses of fine roots to N additions have been conducted in temperate zones. The limited studies that have been conducted in subtropical zones focused on only a few tree species (Li et al. 2015). Bamboo forests are among the most important types of forests, contributing about 10 % of the C stocks in living biomass of forests in China (Chen et al. 2009). Southern China is the distributional center of bamboo forests, which are currently experiencing the highest rate of N deposition in the world (Du et al. 2014; Zhan et al. 2015). Therefore, it is critical to address the effects of elevated atmospheric N depositions on key ecosystem processes in this region, particularly underground processes.

In order to reveal how root morphology and chemical traits respond to N deposition in bamboo forests, we conducted an experiment in a bamboo forest (*Pleioblastus amarus*) in southwestern China, where N was added monthly since November 2007. Our previous studies indicated that 6 years of high-rate N additions (300 kg N ha^{-1} $year^{-1}$) significantly increased the concentrations of exchangeable Al^{3+} by 70 % and NO^{3-}-N by 20 % and decreased soil pH in 0-20 cm soil (Chen et al. unpublished). Soil acidification and Al activation have the potential to disrupt root growth and morphology. Therefore, our hypotheses regarding the effects of more than 7 years of cumulative N additions were: (1) Alteration of root morphology such as specific root length (SRL) and specific root area (SRA); (2) a decrease in root biomass, and (3) elevation of root N concentrations.

Materials and methods

Site description

The study was conducted in a 10-ha *P. amarus* plantation in Liujiang (29°42'N, 103°14'E), Sichuan, China, near the western edge of the Sichuan Basin. The annual mean relative humidity is 86 %, and the monthly temperature ranges from 6.6 °C in January to 25.7 °C in July. The mean annual precipitation is 1490 mm. The annual frost-free period ranges from 352 to 360 days.

The study site was converted from cropland to a *P.amarus* plantation in 2000 as part of the National Project of Converting Farmland to Forests (NPCFF). The mean density of the study stand was 52,200 stems ha^{-1}, and the mean diameter at breast height was 2.3 cm in November 2007. The annual atmospheric wet N deposition was 95 kg N ha^{-1} year^{-1} in the study site (Tu et al. 2013). The soil at the site is classified as a Lithic Dystrudepts (according to USDA Soil Taxonomy), derived from purple sandstone and shale. The forest soil properties were shown in Table 1.

Experiment design

In October 2007, twelve plots (3 × 3 m) were established within the study site, at about 5 m intervals. These plots were randomly allocated to four treatments: control (CK, no N added), low-N (LN, 50 kg N ha^{-1} year^{-1}), medium-N (MN, 150 kg N ha^{-1} year^{-1}), and high-N (HN, 300 kg N ha^{-1} year^{-1}), with three replicates for each treatment. In order to simulate the nitrogen deposition increases 50 %, 150 %, 300 % scenarios in the future. Nitrogen additions were initiated in November 2007, through monthly applications of ammonium nitrate (NH_4NO_3). Before each application, the NH_4NO_3 was weighed, dissolved in 1 L of water, and applied to each plot using a portable sprayer. The control plot received 1 L water without NH_4NO_3.

Root collection, treatment, and morphology assessments

Root samples were collected in April 2015, after more than 7 years of N additions. Soil blocks were collected using an approach similar to that described by Guo et al. (2004). According to our previous study, more than 90 % of root/rhizome biomass of *P. amarus* occurs in the 0-20 cm soil layer. Then, the root collect soil block was 30 cm long, 30 cm wide and 20 cm deep. Specifically, in each plots, a bamboo plant was randomly chosen. Within a radius of 50 cm, a soil block was recovered using a custom-made sampler in the form of an iron box open at both ends. This enabled the recovery of rectangular soil blocks with sharp edges. The 12 soil blocks were placed in a foam box on ice and transported to the laboratory as quickly as possible. Live roots and bamboo rhizomes were extracted from the soil blocks, washed with tap water on a 2 mm screen cloth, and then rinsed with deionized water. They were subsequently stored at a maximum temperature of 4 °C until further treatment. In each plot, 20 living roots with large intact root branches were designated as for *subsample I*, while the rest of the roots were designated together as *subsample II*.

Roots from *subsample I* were kept moist with deionized water (4 °C) and dissected based on branch order into categories. The number of roots in each branch order was recorded, based on Pregitzer et al. (2002). Distal roots were classified as first-order, while second-order roots were defined as the joining of two first-order. Third, fourth and fifth order roots were defined in a similar manner. Based on actual observations, *P. amarus* roots were generally classifiable into only five orders, and the first-order roots of *P. amarus*is were shorter than 2 mm and less than 0.3 mm in diameter, making them difficult to dissect. Therefore, first- and second-order roots were studied together and are henceforth known as order (1 + 2). After dissection, all root sections were scanned at 300 dpi using an Epson digital scanner (Expression 10000XL, Epson Electronics Inc., San Jose, USA), separately for different plots and branch orders. The images were analyzed by WinRHIZO (2012b, Regent Instrument Inc, Canada) root analysis software to quantify the main root parameters, namely, total root length, average root diameter, total root volume, and total root superficial area. These data were used to calculating specific root length (SRL), specific root superficial area (SRA), root tissue density (RTD) and branch ratio (BR). Specific root length is the total root length divided by dry weight, while SRA is total root superficial area divided by dry weight. The root tissue density is the dry weight divided by total root volume, while BR = $\frac{N_i}{N_i+1}$ (N_i = the number of roots in order i,where i = 2, 3, 4).

Table 1 Soil properties (0–20 cm) of the *Pleioblastus amarus* plantation in October 2007 (Mean ± SE)

pH	Total organic carbon (g kg^{-1})	Total Nitrogen (g kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})
4.6 ± 0.1	8.9 ± 0.1	0.81 ± 0.01	7.6 ± 0.2	91 ± 0.6

Root biomass and chemical analysis

After *subsamples I* was scanned, all roots (*subsamples I, II*) and bamboo rhizomes were dried at 65 °C for 48 h before being weighed. The mass fraction and root biomass were calculated separately by root order. Subsample I was milled for determining root C, N, P, K, Ca, Mn, and Na concentrations. Root carbon concentrations were measured using the dry combustion method (Nelson and Sommers 1982). The concentrations of K, Ca, Mn and Na were analyzed using an atomic absorption spectrophotometer (TAS-986, PGENERAL, Beijing, China) after digesting the samples with perchloric acid-nitric acid ($HClO_4$-HNO_3). Nitrogen concentrations were determined by the Kjeldahl method (Grimshaw et al. 1989) using an automatic distillation unit (VELP, Milano, Italy). Finally, total P was determined with the ammonium molybdate-stannous chloride colorimetric method (Olsen and Sommers 1982).

Soil properties analyses methods are as follows. Soil pH was determined with a glass electrode in aqueous extracts. Soil total organic carbon (TOC) was measured by the dichromate digestion method (Kalembasa and Jenkinson 1973). Soil total nitrogen (TN) was determined through acid digestion, using the Kjeldahl method (Grimshaw et al. 1989). Soil available phosphorus was determined by the ammonium molybdate-stannous chloride colorimetric method after extract samples with hydrochloric acid-vitriol acid. Soil available potassium was analyzed using an atomic absorption spectrophotometer (TAS-986, PGENERAL, Beijing, China) after digesting the samples with ammonium acetate. NO_3^--N was extracted with a 2 M KCl solution, and their concentrations estimated using a colorimeter.

Data analysis

Plot mean values were used in all analyses. Two-way ANOVA was performed to analyze the effects of both N treatment and root order on diameter, SRL, SRA, RTD and the concentrations of the aforementioned chemical elements. One-way ANOVA with Tukey's Honestly Significant Difference test was carried out to analyze the effect of N treatments on all chemical, biomass and morphology indexes within root orders. All analyses were conducted using SPSS 20.0 for Windows (IBM SPSS Inc. Chicago, USA). Significant effects were determined by $\alpha = 0.05$ unless otherwise stated. Values are expressed as mean ± SE.

Results

Root characteristics of *P. amarus* by order

Roots of *P. amarus* usually categorized into five root orders. The mean root biomass was 651.7 ± 130.6 g m^{-2} in the 0–20 cm soil layer. The mass fractions of orders (1 + 2), 3, 4 and5 were 0.26, 0.22, 0.28, and 0.24, respectively (Table 2). Mean root diameters of orders (1 + 2), 3, 4 and 5 were 0.31 mm, 0.46 mm, 0.59 mm, and 0.82 mm, respectively. The mean diameter was positively correlated with root order and they had significant differences. Meanwhile, SRA and SRL decreased and RTD increased with increasing root order (Fig.1). Root element concentrations also displayed significant differences across root orders. A negative correlation was found between root order and the concentrations of C, N and Mn as well as the ratios of C:P and N:P. Conversely, the concentrations of K and P and the C:N ratio increased with increasing root order (Figs.2, and 3).

Root biomass

One-way ANOVA indicated that N additions did not significantly affect root biomass and rhizome biomass in *P. amarus*. However, evident differences on an arithmetic basis were observed; the root biomass in HN (432 g m^{-2}) was 34 % lower than that in CK (651 g m^{-2}), while the rhizome biomass in HN (967 g m^{-2}) and LN (967 g m^{-2}) were both 54 % lower than that in CK (2103 g m^{-2}, $P = 0.056$) (Fig. 4). Correlation analysis indicated that soil NO_3^--N concentration and pH significantly affected both root biomass and root rhizome biomass (Table 3). Nitrogen additions decreased the mass fraction in orders (1 + 2) and 3, but had the opposite effect in orders 4 and 5. Taking into consideration the change in root biomass, N additions were responsible for the decrease in biomass in all root orders, with the difference ranging from 18 % to 44 % in order (1 + 2) (Table 2).

Table. 2 Pleioblastus amarus root biomass by root order (g m^{-2}, Mean ± SE)

Treatment	Order 2	Order 3	Order 4	Order 5	Total
CK	174.5 ± 40.4a	142.3 ± 31.4a	191.7 ± 59.1a	143.3 ± 1.7a	651.7 ± 130.6a
LN	142.7 ± 24.8a	143.3 ± 32.2a	168.3 ± 14.6a	207.1 ± 115.0a	661.3 ± 183.9a
MN	134.3 ± 13.3a	125.6 ± 23.5a	183.7 ± 46.6a	228.4 ± 12.3a	672.0 ± 66.6a
HN	98.3 ± 34.6a	70.0 ± 22.5a	144.4 ± 47.2a	119.4 ± 25.9a	432.1 ± 120.4a

Different letters within the same row indicate significant differences among N treatments (One-way ANOVA with Tukey's Honestly Significant Difference test, $\alpha = 0.05$)

Root morphology

The results of two-way ANOVA indicated that N treatment significantly affected SRA and SRL, while root order significantly affected SRA, SRL, root average diameter and RTD (Fig.1). Effects of N additions on root morphology were primarily observed in order (1 + 2) and order 3. However, these orders exhibited the reverse pattern with respect to SRA, SRL and RTD. In order (1 + 2) (Fig.1), SRA (187.16 cm^2 g^{-1}) and SRL (20.04 m g^{-1}) in HN were significantly lower than in CK (330.73 cm^2 g^{-1}, 34.02 m g^{-1}, respectively); N treatments did not significantly affect RTD, while the RTD in HN (0.89 g cm^{-3}) is 128 % higher than that in CK (0.39 g cm^{-3}). In order 3 (Fig.1), SRA and SRL were higher in all three N treatments than CK and N additions decreased RTD Furthermore, N additions had no significantly effects on any of the morphological indexes in orders 4 and 5,while there still showed up some variation trends. In order 4 (Fig.1), N additions decreased the average diameter by 10 %–22 %, and increased RTD by 2 %–45 %. In order 5 (Fig.1), N

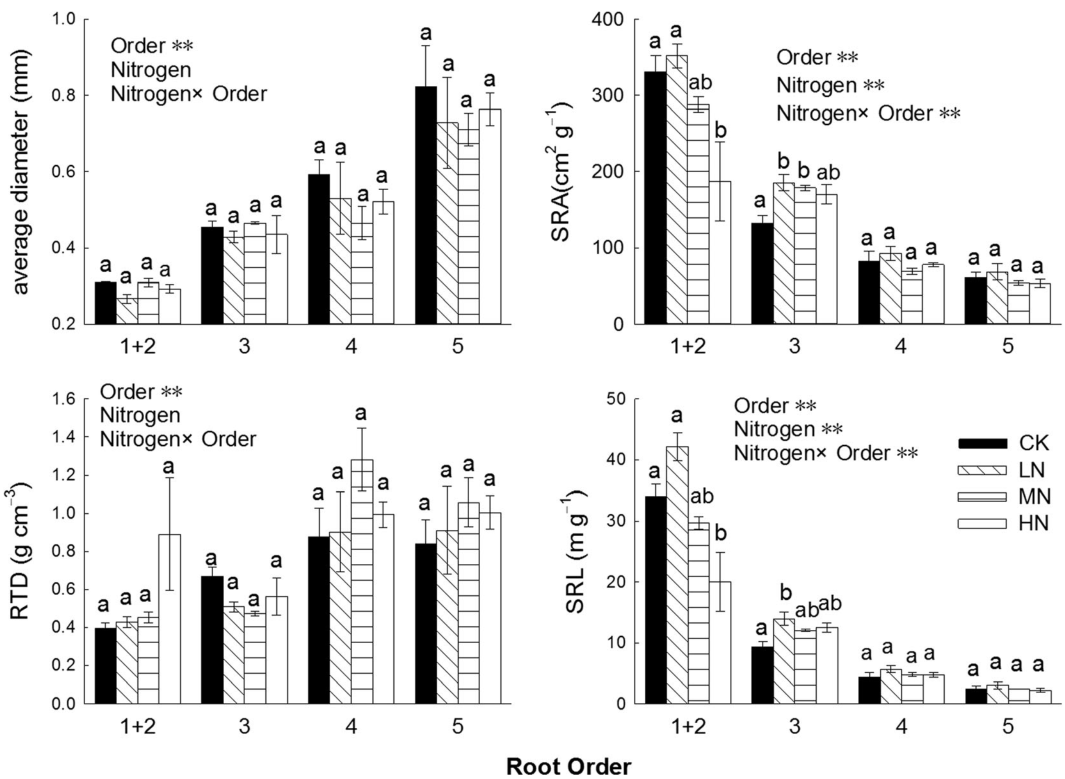

Fig. 1 Effect of N additions on *Pleioblastus amarus* mean root diameter, specific root superficial area (SRA), root tissue density (RTD), and specific root length (SRL) by root order (Mean ± SE). Results of two-way ANOVAs are shown in the text, * $P < 0.05$; ** $P < 0.01$. Different letters indicate significant differences among N additions ($P < 0.05$, Tukey's Honestly Significant Difference test)

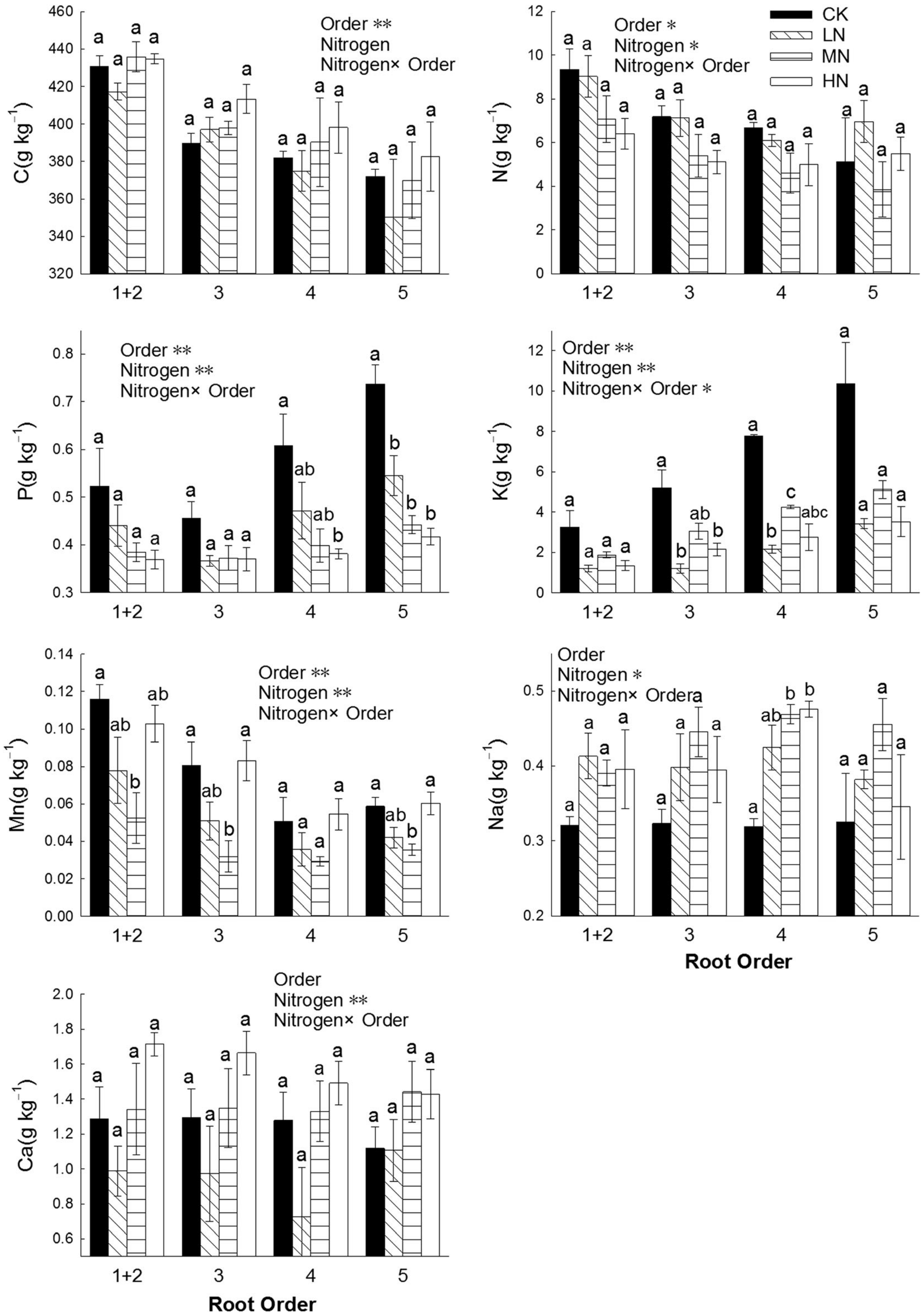

Fig. 2 Effect of N additions on *Pleioblastus amarus* root element concentrations by root order (Mean ± SE). Results of two-way ANOVAs are shown in the text, * $P < 0.05$; ** $P < 0.01$. Different letters indicate significant differences among N additions ($P < 0.05$, Tukey's Honestly Significant Difference test)

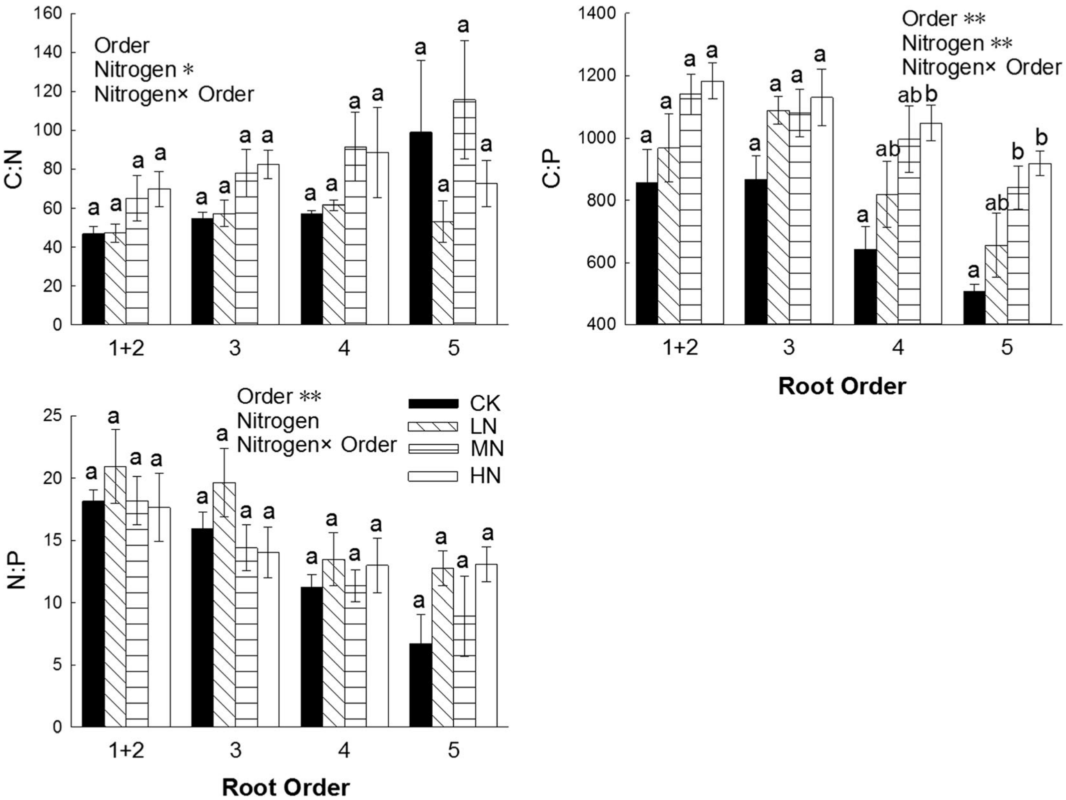

Fig. 3 Effect of N additions on *Pleioblastus amarus* root C:N, C:P, and N:P ratios by root order (Mean ± SE). Results of two-way ANOVAs are shown in the text, * $P < 0.05$; ** $P < 0.01$. Different letters indicate significant differences among N additions ($P < 0.05$, Tukey's Honestly Significant Difference test)

additions decreased average diameter by 7 %–13 %, and increased RTD by 8 % -26 %. The BR of order (1 + 2): order 3 decreased as N additions increased and the mean BR in HN (6.82) was 49 % lower than that in CK (13.42) (Fig.5).

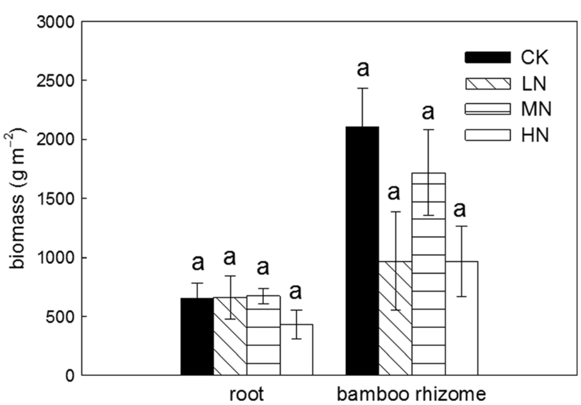

Fig. 4 Effect of N additions on *Pleioblastus amarus* root biomass and bamboo rhizome biomass (Mean ± SE). Different letters indicate significant differences among N additions ($P < 0.05$, Tukey's Honestly Significant Difference test)

Root chemistry

Nitrogen additions and root order significantly affected the concentrations of most of the elements studied. However, there was no interaction between N treatment and root order (Fig.2). Roots of order (1 + 2), 3, 4 and 5 had same response direction to N additions. Nitrogen additions increased Na concentrations and the ratio of C:N and C:P, while having the opposite effect on the concentrations of P, N, K and Mn. The results of one-way ANOVA indicated that N additions significantly

Table. 3 Correlation matrix of pH, soil NO_3^--N content, root biomass and root rhizome biomass

	pH	NO_3^--N	Root biomass
NO_3^--N	−0.780**		
Root biomass	0.529	−0.728**	
Rootrhizome biomass	0.609*	−0.599*	0.691*

* $P < 0.05$; **, $P < 0.01$

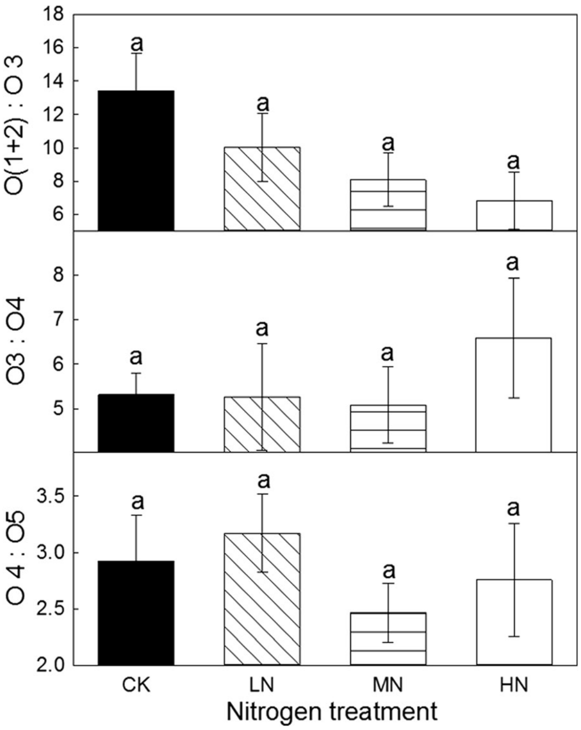

Fig. 5 Effect of N additions on *Pleioblastus amarus* root branch ratio (Mean ± SE). Different letters indicate significant differences among N additions ($P < 0.05$, Tukey's Honestly Significant Difference test). O (1 + 2):O3, O3:O4, O4:O5 present the root number ratio of the corresponding root level

decreased root P concentrations and increased the ratio of C:P in order 4 and5 in HN (Figs.2, and 3).

Discussion

Morphological and chemical variation among root orders of *P. amarus*

Based on a stream-order approach for the study of root branch orders, variation in root morphological and chemical characteristics among root orders have been studied in many different forest ecosystems (Pregitzer et al. 2002; Shi et al. 2008; Xiong et al. 2012). These studies revealed that most forest species exhibit similar trends in morphological and chemical variation as root order increases, although with different ranges in values. In other words, root diameter, C:N ratio and C concentration increase with increasing root order, while SRL and N concentration displays the opposite trend.

In this study, nearly all the morphological and chemical (except C concentration) properties of *P. amarus* roots exhibited trends similar to those reported in many other forest species (Pregitzer et al. 2002; Shi et al. 2008; Xiong et al. 2012). Interestingly, *P. amarus* roots were generally classifiable into only five orders, which are fewer than has been reported for many other plant species. This may be a unique characteristic of bamboo species.

Effects of N additions on root morphology

Confirming our first hypothesis, N additions significantly affected root morphology. Nitrogen additions decreased SRL and SRA in order (1 + 2) and had the opposite effect in order 3, and they decreased the branch ratio of order (1 + 2): order 3. In general, SRL is closely associated with root diameter and RTD. In this study, N additions had a minor effect on diameter, then, the SRL change may attribute to the change of RTD. These morphological changes may be attributed to two factors. First, root morphology is plastic and responsive to changes in the soil environment. When soil N availability increases because of N additions, plants prefer to invest more C in long-lived roots (i.e. higher-order roots), for increasing C use efficiency (Wang et al. 2012). Secondly, root functions degraded because of soil acidification and heightened Al^{3+} toxicity. After six years of N additions at the same site, N additions significantly decreased soil pH by 0.3 unit and increased the concentration of exchangeable Al^{3+} by 70 % in in HN treatment(Chen et al. unpublished). Roots avoid high Al^{3+} concentrations in the soil and aluminum inhibit cation uptake at the root epidermis (Zang et al. 2011; Hirano et al. 2003).

Specific root length is considered as an index of the cost-benefit relationship in root systems (Fitter et al. 1991). Many studies have found that SRL is positively correlated to root respiration, and negatively correlated to root lifespan (Eissenstat et al. 2000; Luke et al. 2012). The relatively low value of SRL in order (1 + 2) may imply that N additions reduce root metabolism, while increasing lifespan. Thus, the morphological responses in this study suggested that the growth and metabolism of lower-order roots were inhibited by N additions, potentially resulting in a slower underground C cycle.

Studying the responses of root morphology towards N additions using an order-based classification approach may be more scientific. This is because N additions change root morphology primarily in lower-order roots (Wang et al. 2013). In this study, N additions had a significant effect only on order (1 + 2) and order 3.

This maybe because lower-order roots have higher plasticity than higher-order roots (Guo et al. 2004), and changes higher-orders roots may more costly for plant (Wang et al. 2013). Moreover, roots of orders 4 and 5 are all less than 2 mm in diameter and therefore classified as fine roots by traditional definition. Thus, order-based classification approach can reveled more accurate information than diameter-based classification method.

Effect of N additions on root and rhizome biomass

Confirming our second hypothesis, N additions decreased root biomass in HN and decreased rhizome biomass in all three N addition treatments. This is similar to what has been observed in past studies (Mei et al. 2010; Jia et al. 2010; Wang et al. 2012), although some studies indicated that N additions increased or did not change fine root biomass (Noguchi et al. 2013; Kou et al. 2015). In this study, there was a decrease in the total root biomass following N additions; however, the mass fractions of order (1 + 2) and order 3 decreased, whereas they increased in orders 4 and 5. Those changes again suggest that N additions enhanced transport function at the expense of root absorption functions.

Over the long-term, N deposition or N additions can be expected to decrease fine root biomass. Based on cost-benefit analysis, an increase in soil nutrient availability increased induces plants to allocate a greater proportion of biomass in above-ground organs, resulting in a reduction in underground biomass (Eissenstat and Yanai 1997). This has been confirmed by several field studies (Mei et al. 2010; Jia et al. 2010; Wang et al. 2012). Another important reason maybe is Al toxicity. This is because the roots avoid high Al^{3+} concentrations in the soil (Zang et al. 2011; Chen et al. 2015). Our previous studies indicated that soil N availability, soil acidity, and Al^{3+} concentration increased after six years of N additions (Chen et al. unpublished). Finally, correlation analysis indicated that NO_3^--N and pH significantly affected both root biomass and root rhizome biomass, which may explain the observed trends.

Effect of N additions on root tissue chemistry

Nitrogen additions affected most element concentrations in all root orders. Nitrogen additions decreased the root tissue concentrations of N, P, K and Mn, and increased the concentration of Na and the ratios of C:N, C:P and N:P. Nitrogen additions had a minor effect on C concentration, but N additions decreased total root biomass, there was a subsequent decrease in underground root carbon storage.

Contrary to our hypothesis and many previous studies, root N concentrations decreased in all root orders following N additions. Several studies have indicated that simulated N deposition or N additions increases inorganic N availability for root uptake and thus increase N concentrations in root tissues (Li et al. 2015). Our study suggests that this is not necessarily true for all locations and/or forest species. Root N concentrations usually display a significant interaction with root metabolism (Jia et al. 2013). Since N additions can acidify soil and induce Al^{3+} activation, resulting in further degradation in root function and metabolism, this may result in a corresponding decrease in root N concentration. Therefore, root N concentrations decreased maybe is a signal of root metabolic activity decrease.

Many studies have indicated that N additions can induce nutrient element loss and cause nutrient imbalances in the soil solution (Lucas et al. 2011). Thus, N additions may affect other elements' concentrations (with the exception of C and N) in roots, which were indeed observed in our study. Nitrogen additions may result in Al^{3+} activation. Actually, we have observed that soil exchangeable Al^{3+} in HN increased by 70 % compared with CK after 6 years of N additions experiment in this *P. amarus* forest (Chen et al. unpublished). Aluminum ion is known to inhibit cation uptake at the root epidermis, because it can block Ca^{2+} channels and K^+ into the plasma membrane (Cassmann and Schroeder 1994; Hirano et al. 2003). This may be the mechanism by which N additions decreased root K and Ca concentrations. LN and MN treatments decreased root Mn concentration in all root orders. Some leaf litter decomposition studies have indicated that Mn and litter decomposition rates exhibit a positive relationship, because fungi require Mn to synthesize lignin-decomposing peroxidases (Hobbie 2015). Based on those results, any significant changes of root Mn concentrations will influence long-term root decomposition. Root P concentrations decreased indicated soil P availability decreased and it has an important effect on root morphology and root tissue quality (Zambrosi et al. 2015). Thus, the changes of soil P availability may be an important mechanism for root morphology and tissue elements concentrations change.

Nitrogen additions decreased Nitrogen, phosphorus, and potassium concentrations in all root orders, suggesting that root function degeneration occurred. Our previous study demonstrated that root respiration contributes about 49 % of total soil respiration and plays an important role in the underground carbon cycle in same study site (Tu et al. 2013). After N treatments more than 7 years, N additions decreased root biomass and root N concentrations, which suggest a corresponding decrease in the proportion of soil C cycling contributed by roots. Taking into consideration the changes in root morphology, chemistry, and biomass, N additions have the potential to be a significant threat to the health of bamboo forest ecosystems.

Overall, our study demonstrated that seven years of N additions decreased root and bamboo rhizome biomass, and significantly affected lower-order root morphology and root N, P, K and Mn concentrations in all root orders. Nitrogen additions led to root function degradation and decreased root C storage. This suggests that the growth and metabolism of lower-order roots were inhibited by N additions, potentially leading to a slower underground C cycle. Chronic N deposition may have considerable effects on soil environments, plant root traits, and underground C and nutrient cycles in bamboo forest ecosystems. However, the degrees of these effects remain uncertain. Further long-term studies regarding C cycling under continuous increases in annual N deposition are necessary to supplement our knowledge regarding how forest C processes respond to N deposition.

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