Pedobiologia 55 (2012) 167–174

Contents lists available at SciVerse ScienceDirect

Pedobiologia - International Journal of Soil Biology

journal homepage: www.elsevier.de/pedobi

Isotopic evaluation of the role of arbuscular mycorrhizae in the nitrogen preference in Chinese fir seedlings

He-Chun Piao, Cong-Qiang Liu*, Shi-Jie Wang*

The State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 55002, China

ARTICLE INFO

Article history:
Received 21 October 2011
Received in revised form 22 February 2012
Accepted 3 March 2012

Keywords:
Arbuscular mycorrhiza
Nitrate
Ammonium
^{15}N natural abundance
Chinese fir seedling
Soil environment

ABSTRACT

Chinese fir seedlings grow well in shrubland (including deciduous forest) soils without or less fertilizer application, but they sometimes harbor disease and show symptoms of nitrogen deficiency in ploughed (including several rotation of Chinese fir plantation) soils, where agricultural practice and clear-felling reduce the abundance and diversity of mycorrhizal fungi, and lead to destruction of mycorrhizae. Based on measurements of foliar $\delta^{15}N$ or foliar $\delta^{15}N_{fol\text{-}soil}$ in seedlings collected from 33 nurseries, we compared the effect of an AM-mediated process on nitrogen resource use between shrubland and ploughed soils. In mycorrhizal seedlings growing in shrubland soils, both foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) were significantly higher than those in ploughed soils, likely because of enhanced high $\delta^{15}N/NO_3^-$ absorption through AM-mediated pathways. Those results showed that foliar $\delta^{15}N$ typically reflected the isotopic signature of the source pools of N. We suggest that the dominant N form taken up by fir seedlings growing in ploughed soils was NH_4^+-N rather than NO_3^--N, where colonized root epidermis play an important role in exploiting soil N resource. However, the N form taken up by fir seedling growing in shrubland soils was primarily NO_3^--N compared to NH_4^+-N, which is attributed to the high efficiency in an AM-mediated process rather than the dominance of N species in the different habitats. It is conceivable that combined colonized root epidermis with AM-mediated process may be more important than root epidermis alone in exploiting different forms of N in nursery soils. Therefore, in low N and acidic ecosystems, species other than the dominant N-NH_4^+, should be considered to satisfy the N demand for Chinese fir survival and growth, while the efficiency of an AM-mediated process should be determined by soil abiotic conditions.

Introduction

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is able to form both arbuscular mycorrhiza (AM) and ectomycorrhiza (EM). One or two-year-seedlings are not colonized with EM, but only with AM fungi (Piao and Liu 2011). Arbuscular mycorrhizal fungi can be found in almost all habitats and climates (Barea et al. 1997), and at different depths of soil (Michelsen and Rosendahl 1989). Arbuscular mycorrhizal fungi are an important component of agricultual and natural ecosystems, as they form symbiotic associations with more than 80% of vascular plants (Smith and Read 1997; Bainard et al. 2011). The importance of AM fungi in enhancing host plant growth is well known (Smith and Read 1997; Gosling et al. 2006), and has been explained by the ability of fungal extraradical hyphae to spread in soil and take up nutrients, such as P, Zn and N, which are then translocated to the host plant roots (Smith and Read 1997; Brundrett 2002; Chen et al. 2007). Intraradical structures include vesicles, arbuscules, coils, and hyphae, and extraradical structures include extraradical hyphae and spores (Johnson et al. 2003). In the AM symbiosis, nutrient movement begins with its uptake from the soil matrix by the nutrient transporters located in the extraradical hyphae, followed by its translocation to intraradical fungal structures and subsequent transfer to the colonized root (Ferrol et al. 2002; Bainard et al. 2011).

In an AM plant, there are two nutrient absorption pathway: one is absorption directly at the soil-root interface through the root epidermis and root hairs, the other is an AM-mediated pathway via AM extraradical hyphae in soil (Smith et al. 2010). If extraradical structures are not connected with intraradical structures for nutrient transfer between the symbionts, the mycorrhizal uptake pathway does not function, and plant nutrients are absorbed directly through either the root epidermis and root hairs (Smith et al. 2010). Our previous study shows that even where an AM-mediated process should not establish, roots were colonized with AM fungi in seedlings grown on ploughed soils (Piao and Liu 2011) where agricultural practices and clear-felling had reduced the abundance and diversity of mycorrhizal fungi (Brundrett 2009). The method of Trypan blue

* Corresponding authors. Tel.: +86 0851 5891534.
E-mail addresses: liucongqiang@vip.skleg.cn (C.-Q. Liu), wangshijie@vip.gyig.ac.cn (S.-J. Wang).

0031-4056/$ – see front matter © 2012 Published by Elsevier GmbH.
doi:10.1016/j.pedobi.2012.03.003

staining of roots is a good indicator of fungal establishment into the root and of fungal colonization, but not of symbiotic efficiency (Smith and Gianinazzi-Pearson 1990; Azcón et al. 2008), which is defined as the ability of plants colonized with AM fungi to more efficiently deliver N from soil through an AM-mediated absorption pathway. McGonigle (1988) also found that total intraradical AM colonization is a poor predictor of mutualistic functioning. Therefore, more detailed measurements of intraradical and extraradical structures might be useful for linking mycorrhizal structure and function (Johnson et al. 2003). Extraradical hyphae that are developed in the soil are a major determinant of the efficiency of AM fungi to take up nutrients since the hyphae are linked to plant nutrition (Cornejo et al. 2007). However, the behaviour of extraradical hyphae can be adversely affected in some soil environments, such as compacted soils, leading to reduced nutrient uptake, fungal productivity and survival (Drew et al. 2003).

Although AM fungi have been shown to take up and transfer significant amounts of N to plants (Fitter et al. 2011), reports about fungal effects on plant N status are controversial (Blanke et al. 2011). For example, AM fungi have little capacity to enhance plant uptake of mobile ions such as NO_3^- because these ions move rapidly in soils (Tinker and Nye 2000; Tu et al. 2006). Theoretically, NO_3^- in soils could be extracted by a nonmycorrhizal root system assuming high fine-root density. Thus mycorrhizas would add little advantage to NO_3^- uptake (Tinker and Nye 2000; He et al. 2005). However, a number of studies show that AM fungi can take up a wide range of inorganic and organic sources. For example, AM fungi have the ability to acquire N from soil (Requena et al. 2003; Cavagnaro et al. 2012), under vitro conditions (Govindarajulu et al. 2005). Therefore, their importance in plant N nutrition has not been fully appreciated until recently (Read and Perez-Moreno 2003).

Several studies have shown that mycorrhizal fungi deliver isotopically depleted N to plants (Hobbie et al. 2000). However, AM fungi deliver less of this isotopically depleted N than EM fungi (Michelsen et al. 1998), AM fungal fractionation against ^{15}N during uptake and translocation is, therefore, probably small (Michelsen and Sprent 1994). Moreover, both NO_3^- and NH_4^+ can move to fungal extraradical hyphae, where it is converted into arginine, and then transferred to the intraradial structure, where it is broken down to release NH_4^+ for transfer to host plant roots (Tian et al. 2010) where NH_4^+ is immediately assimilated, so that there would be no difference in $\delta^{15}N$ between foliage and roots. Therefore, the forms of N (NO_3^-, NH_4^+ or organic N) taken up by plants will infuence the tissue N isotopic composition (Bustamante et al., 2004) in both a root epidermis absorption pathway and an AM-mediated absorption pathway. The products of both nitrification and denitrification processes become relatively lighter, or less enriched in ^{15}N, while the substrates from which they were formed become heavier or more enriched in ^{15}N because of fractionation against ^{15}N (Templer et al. 2007). Accordingly, in the reaction leading to nitrification the $\delta^{15}N$ values of soil NO_3^- are generally isotopically lighter than that of NH_4^+ (Miller and Bowman 2002; Dijkstra et al. 2006), while greater denitrification would lead to an enrichment of the ^{15}N isotope and higher $\delta^{15}N$ values for a substrate of NO_3^- (Kahmen et al. 2008; Stewart et al. 2011). Averill and Finzi (2011) reported that foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil)—the difference in $\delta^{15}N$ between foliage and soils—increase with increasing elevation, and that organic forms of N with higher $\delta^{15}N$ than that of inorganic N become the dominant source of N taken up by coniferous tree species with increasing elevation. Thus, they suggest that variations in foliar $\delta^{15}N$ between sites or among tree species reflect differences in organic vs. inorganic N uptake rather than fractionation by EM fungi, because foliar $\delta^{15}N$ typically reflects the isotopic signature of the source pools of N (Averill and Finzi 2011). In the denitrification process, light $^{14}NO_3^-$ does preferentially convert to N gas, which would be lost and thus, would lead to $^{15}NO_3^-$ enrichment in residual nitrate (Perakis et al. 2011). Both nitrification and denitrification rates should be very low in well-drained acid forest soils (Compton et al. 2007). Chinese fir normally grows on acid and moist soils under N-limited conditions; moreover, plant species with a preference for NH_4^+-N grow in acid soils (Tabuchi et al. 2007). But the forms of N preferred by fir seedlings remain unknown. The contributions of soil N forms to plant N can be studied by determining several parameters such as foliar $\delta^{15}N$ (fol-soil) (Amundson et al. 2003; Kahmen et al. 2008; Averill and Finzi 2011), or the enrichment ε factor ($\delta^{15}N$ in topsoil – $\delta^{15}N$ in leaves) (Pörtl et al. 2007), which indicates the deviation of plant from soil N sources (Kahmen et al. 2008). The foliar $\delta^{15}N$ (fol-soil) values are significantly positively correlated with NO_3^--N/NH_4^+-N uptake ratios for most plants (Kahmen et al. 2008), but for some plants the values are negatively correlated with these ratio (Miller and Bowman 2002; Falkengren-Grerup et al. 2004), the correlation depends on whether or not $\delta^{15}N/NO_3^-$-N is higher than $\delta^{15}N/NH_4^+$-N (Kahmen et al. 2008).

Like our previous study (Piao and Liu 2011), we divided the samples (collected at January 2008, 2009 and 2010) into two groups: the first included soils from land under deciduous forest, shrub/grassland (6 sites) and one rotation plantations (11 sites) prepared by slash burning (named shrubland). The second group represented soils from plantations with more than two rotations (8 sites) and ploughed soils, mainly paddy soils (8 sites) (named plough). Although the pathways for N transfer to the plant are subject to debate (Chalot et al. 2006), there is increasing evidence that AM fungi can preferentially assimilate immobile NH_4^+, but not the highly diffusible NO_3^- (Chalot et al. 2006; Veresoglou et al., 2012). Previously, a lower effectiveness of extraradical hyphae in NO_3^--N transfer was attributed to the high mobility of NO_3^-. The aim of this study was to provide some information to show that fir seedlings colonized with AM fungi can take up NO_3^-, and transfer it to the host plant roots through an AM-mediated pathway. In this paper we assumed that foliar $\delta^{15}N$ typically reflected the isotopic signature of the source pools of N (Averill and Finzi 2011), and AM fungi did not appear to deliver isotopically depleted N to plants (Michelsen et al. 1998). Accordingly, foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) values could provide useful benchmarks for comparisons of different forms of N delivery in fir seedlings through two absorption pathways. We hypotheses that if an AM-mediated process does not exist, nutrients would be directly absorbed at the soil-root interface through the root epidermis and root hairs, which should occur in ploughed soils. If an AM-mediated process does exist, nutrients should be absorbed through an AM-mediated pathway via AM extraradical hyphae in soil, which is likely to occur in shrubland soils. We also expect differences in the species of N absorbed by seedlings in shrubland and ploughed soils.

Materials and methods

Research site

The study area was located in a transitional zone between the lowland and hills in the mountainous terrain of Guizhou Province of southwest China. Elevations ranged from 280 m to 1510 m above sea level. Regional mean annual air temperatures decreased with altitude, and ranged from 18.4–14.3 °C. Most parts of Guizhou Province have total annual precipitation of >1100 mm (Piao and Liu 2011). Sampling sites were located in sandstone areas except for one site, and the latitude and longitude ranged from 25°59′E to 26°69′E and from 105°25′N to 109°14′N, respectively. The distribution of soil types varies with elevations with yellowish-red soils (Ultisols) found at altitudes below 600 m above sea level,

and yellow soils (Ultisols) at altitudes between 600 and 1500 m (Piao et al. 2001; Piao and Liu 2011). Site selection aimed to capture a significant range of the variability in soil associated with land use histories. Accordingly, 33 nurseries distributed widely in Guizhou Province were chosen as the sampling sites. Soil samples were taken from 0 to 25 cm depth to provide an indication of available nutrients with more than ten replicated plots for each soil sample. Seedlings were collected in early January of 2008 (12 samples), 2009 (12 samples) and 2010 (9 samples). The total biomass of each seedling was measured at ground height, and was subsequently separated into leaves, stems and roots. Nurseries growing fir seedlings varied in size from about 100 m^2 to around 1000 m^2. Chemical fertilizers (15% N, 8% K_2O and 15% P_2O_5) were added to the soil surface in all 33 nurseries before sowing seeds. The soil was ploughed to a depth of 25 cm in early spring. Thus, variation in soil $\delta^{15}N$ values with depth should not affect $\delta^{15}N$ values in the seedlings. Generally, the farmers sow seeds in February, and remove weeds at regular intervals. The nurseries on the shrub/grassland sites and some of the one-rotation plantation sites did not receive fertilizer additions of urea, but those on ploughed soils and those with >2-rotations had urea added once or twice during one growing period.

Laboratory analysis

Plant samples for laboratory analysis were dried for 48 h at 60 °C and ground with a mortar and pestle. Total C and N were determined with a CHNS autoanalyzer (PE 2400-II). Plant P was digested using nitric-perchloric acid digestion and analyzed by the vanadomolybdate colorimetric method. Olsen extractable phosphorus (Olsen P) was measured with 0.5 M $NaHCO_3$ (adjusted to pH 8.5 with NaOH) (Olsen et al., 1954). Total soil P was determined after combustion of 1 g soil for 2 h at 550 °C followed by digestion with 6 M HCl (Graetz et al. 1999). NH_4^+-N was measured by the phenate method after extraction with 2 M KCl (Ghosh and Kashyap 2003). NO_3^--N was measured with 0.5 M K_2SO_4 extractant (Kooijman and Hedenäs 2009) followed by the phenol disulfonic acid method. Nitrification potentials were determined using the method of Fortuna et al. (2003). Briefly, 15 g soil was placed in a 250 ml flask that contained 100 ml of a mixture of 1.5 mM NH_4^+, and 1 mM PO_4^{3-}, then incubated on an orbital shaker at 180 rpm for 24 h at 25 °C. The generated nitrate was measured using the phenol disulfonic acid method. The samples of Chinese fir seedlings were sent to the laboratory of China Agricultural University and examined for mycorrhizal colonization following the method of Gai et al. (2006). Briefly, after clearing the roots with 10% KOH, they were acidified in lactic acid, and stained with Trypan blue, to be subsequently examined for their level of colonization under a compound microscope. For soluble sugar determination, 0.25 g of air-dried material was extracted four times with distilled water at 75 °C, modified from the method of Chinnasamy and Bal (2003) whereby water was used instead of 80% of ethanol and the water temperate was 75 °C rather than boiling. After each extraction, samples were filtered (Whatman No 42 filter paper), and the filtrates were used to determine soluble sugar colorimetrically through anthrone reaction (Piao and Liu 2011). N isotope was measured using a Euro EA 3000 elemental analyzer interfaced with an IsoPrime isotope ratio mass spectrometer (Elementar Analysensysteme GmbH GER). The N_2 generated from the combustion was purified in a gas chromatographic column (Erovector S.p.A. Milano ITA.) and passed directly to the inlet of a gas isotope ratio mass spectrometer (IRMS, Isoprime Ltd. UK). C isotopes were measured using a mass spectrometer. CO_2 generated in the combustion tubes was separated by cryogenic distillation, collected in break seal test tubes and analyzed on a mass spectrometer (MAT 252). The ratio of heavy to light isotopes in the sample material (R_{sample}) was measured using mass spectrometry as the deviation from the isotopic ratio of a standards ($R_{sandard}$); where R denotes the ratio of stable C ($^{13}C/^{12}C$) or N ($^{15}N/^{14}N$) isotopes, expressed in δ notation; for example for C: $\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$; for N: $\delta^{15}N\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$; where, R_{sample} and $R_{standard}$ are the $^{13}C:^{12}C$ ratios for C, or $^{15}N:^{14}N$ ratios for N of the sample and the standard, respectively. The V-PDB ($\delta^{13}C = 0‰$) and atmospheric N ($\delta^{15}N = 0‰$) serve as international standards for stable C and N, respectively. The $\delta^{15}N$ values of extractable NH_4^+-N and NO_3^--N in 8 of the 33 soil samples were determined in duplicate after pretreatment with 2 M KCl using the method of Stephan and Kavanagh (2009). Briefly, the soil solution extracted with 2 M KCl (containing ca. 50 μg N) was poured into a 250 ml polypropylene specimen cup with a tight-fitting polypropylene snap lid. First, MgO was added to dissociate NH_4^+, which diffused into an acid trap on a quartz fiber filter disk. Next, Devarda's alloy and MgO were added to diffuse NO_3^- as NH_3 gas, which dissolved into the acid trap. After freeze-drying the disk, the $\delta^{15}N$ of NH_4^+-N or NO_3^--N was analysed using isotope ratio mass spectrometer (IRMS). The precision of the analyses was ±0.2‰ for $\delta^{15}N$, and ±0.1‰ for $\delta^{13}C$, respectively.

Statistical analyses

Student's *t*-tests were used to determine statistical differences between mean values of nutrient concentrations in plants growing in ploughed and shrubland soils. Pearson correlation coefficients were performed to assess relationships between each plant tissue and soil parameters, and linear regression to assess relationships between leaf N or N:P ratio and $\delta^{15}N$. For all statistical tests, differences were considered signification at the $P < 0.05$ level.

Results

Colonization with mycorrhizae

All of the collected Chinese fir seedlings were colonized by AM fungi, but not by EM fungi. Colonization levels with AM fungi varied significantly from <10% to 80%, with no differences between shrubland and ploughed soils. According to field investigations, all of the Chinese fir seedlings grown on shrubland soils harboured neither root rot nor any above ground diseases, however, some of the seedlings grown on ploughed soils did. However, the cause of the disease and the N deficiency symptoms remains unclear.

Soil and Chinese fir tissue parameters

Variations in soil pH were very narrow, ranging from 4.08 to 4.96 in shrubland soils, and from 4.18 to 5.44 in ploughed soils with one exception of 6.89. The mean soil pH in shrubland soils, however, were significantly lower that in ploughed soils (Table 1). On average, the concentrations of exchangeable Ca and Mg (0.38 ± 0.31 mg g^{-1} and 0.043 ± 0.021 mg g^{-1}) in shrubland soils were lower, but not significantly, than that (0.71 ± 0.66 mg g^{-1} and 0.11 ± 0.16 mg g^{-1}) in ploughed soils, respectively. The average concentration of soil organic C (33.8 ± 1.1 mg g^{-1}) in shrubland soils was relatively, but not significantly, higher than that (28.1 ± 0.9 mg g^{-1}) in ploughed soils. The average ratio of soil C:N (13.3 ± 2.6) in shrubland soils was similar to that (12.1 ± 2.6) in ploughed soils. The nitrification potential (4.0 ± 5.4 μg g^{-1} day^{-1}) ranged from 0.1 μg g^{-1} day^{-1} to 20.9 μg g^{-1} day^{-1} in shrubland soils, and that (3.2 ± 2.3 μg g^{-1} day^{-1}) observed in ploughed soils ranged from 0.5 μg g^{-1} day^{-1} to 5.2 μg g^{-1} day^{-1}. Extractable NH_4^+-N varied between 7.9 μg g^{-1} and 40.8 μg g^{-1} in shrubland soils except for one outlying value of 118.6 μg g^{-1} and between 2.4 μg g^{-1} and 96.2 μg g^{-1} in ploughed soils. Extractable NO_3^--N

Table 1
The mean ± SD (standard deviation in parentheses) values for soil pH, total N, P and Olsen P, C:N ratio, $\delta^{15}N$, and extractable NH_4^+-N and NO_3^--N concentrations. Significant differences between shrubland and ploughed soils were determined using student's *t*-test. Soils were collected from 33 fir nurseries distributed across the Guizhou Province of Southwest China. The shrub groups in the table include shrub/grassland, deciduous forest and one-rotation plantation soils; while the plough group included ploughed and >2 rotation plantation soils.

	pH (H_2O)	$\delta^{15}N$ (‰)	Total N (mg g^{-1})	Total P (mg g^{-1})	Olsen P (mg g^{-1})	C:N ratio (mass)	NH_4^+-N (μg g^{-1})	NO_3^--N (μg g^{-1})
Shrub $N = 17$	4.44 (0.25)	4.8 (1.1)	2.4 (0.7)	0.52 (0.30)	0.025 (0.01)	13.3 (2.6)	28.2 (26.0)	9.3 (8.0)
Plough $N = 16$	4.91 (0.64)	4.3 (1.1)	2.4 (0.8)	0.71 (0.29)	0.038 (0.018)	12.1 (2.6)	35.3 (26.4)	5.6 (1.3)
t-test	$P < 0.01$	NS	NS	NS	$P < 0.05$	NS	NS	NS

NS: no significance.

Table 2
The mean ± SD (standard deviation in parentheses) values of foliar and root N and P concentration, N:P ratio, $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) or root $\delta^{15}N$ (root-soil). Significant differences between two groups of shrubland and ploughed soils were calculated using student's *t*-test. The soils were collected from 33 fir nurseries distributed across the Guizhou Province of Southwest China. Shrub groups in the table include shrub/grassland, deciduous forest and one-rotation plantation sites; plough group included ploughed and >2 rotation plantation sites. The significant differences for the mean values of foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) between two groups of shrubland and ploughed sites were tested using an analysis of variance (ANOVA). The results were: $P = 0.001$ for foliar $\delta^{15}N$ and $P = 0.009$ for foliar $\delta^{15}N$ (fol-soil).

Tissue	Foliage					Root				
	N (mg g^{-1})	P (mg g^{-1})	N:P (mass)	$\delta^{15}N$ (‰)	Fol-soil $\delta^{15}N$ (‰)	N (mg g^{-1})	P (mg g^{-1})	N:P (mass)	$\delta^{15}N$ (‰)	Root-soil $\delta^{15}N$ (‰)
Shrub $N = 17$	20.4 (3.8)	1.4 (0.3)	14.3 (2.0)	4.7 (1.7)	−0.07 (2.0)	8.5 (2.1)	0.8 (0.2)	10.6 (3.0)	5.1 (1.4)	0.3 (1.7)
Plough $N = 16$	18.5 (4.9)	1.8 (0.5)	10.6 (2.2)	2.3 (1.7)	−1.9 (1.7)	8.7 (3.5)	1.2 (0.4)	7.8 (4.9)	2.8 (1.5)	−1.4 (1.5)
t-test	NS	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS	$P < 0.01$	NS	$P < 0.001$	$P < 0.01$

NS: no significance.

ranged from 4.5 μg g^{-1} to 37.4 μg g^{-1} in shrubland soils, and from 3.9 μg g^{-1} to 7.6 μg g^{-1} in ploughed soils.

No significant differences in biomass (from ground height) between seedlings grown on shrubland soils (37.4 ± 9.2 cm, $n = 17$) and ploughed soils (37.7 ± 10.3 cm, $n = 16$) were found. The average concentration of foliar N, which ranged from 10.1 mg g^{-1} to 27.8 mg g^{-1} in shrubland soils, was higher, although not significantly so, compared to concentrations found in ploughed soils, ranging from 8.8 mg g^{-1} to 25.0 mg g^{-1} (Table 2). However, the average concentration of leaf P ranging from 0.9 mg g^{-1} to 2.1 mg g^{-1} in shrubland soils was significantly lower than in ploughed soils where the values ranged from 0.8 mg g^{-1} to 2.9 mg g^{-1} (Table 2). Foliar N:P, which varied between 11.6 to 19.3 (mass) in shrubland soils, was significantly higher than the foliar N:P values found for ploughed soils, where N:P ranged from 5.9 to 13.8 (mass; Table 2). Foliar N increased with increasing soil N ($r^2 = 0.18$, $P < 0.05$, $n = 33$). Foliar P was significantly correlated with total soil P ($r^2 = 0.176$, $P < 0.05$, $n = 33$) but not with Olsen P. Foliar sugar (82 ± 38 mg g^{-1}) in shrubland soils was significantly lower (116 ± 34 mg g^{-1}) in ploughed soils ($P < 0.05$, $n = 33$), and root sugar (54 ± 23 mg g^{-1}) in shrubland soils was also significantly lower (68 ± 26 mg g^{-1}) than root sugar in ploughed soils ($P < 0.05$, $n = 33$).

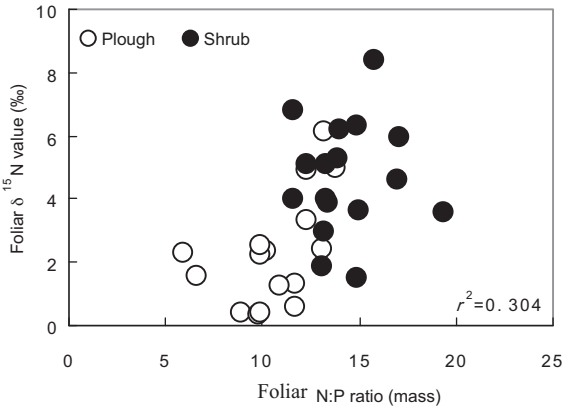

Fig. 1. Foliar $\delta^{15}N$ as affected by foliar N:P ratio (mass). The samples were collected from 33 fir nurseries distributed across the Guizhou Province of Southwest China. Shrub groups in the legend include shrub/grassland, deciduous forest and one-rotation plantation sites; plough group included ploughe and >2 rotation plantation sites.

$\delta^{15}N$ values in the plant tissue and soil

Extractable NH_4^+-N was negatively and significantly correlated with foliar N ($r^2 = 0.215$, $P < 0.01$, $n = 33$). Foliar N:P was positively and significantly correlated with foliar $\delta^{15}N$ ($r^2 = 0.304$, $P < 0.001$, $n = 33$; Fig. 1). Foliar $\delta^{15}N$ in seedlings from shrubland soils (1.5‰ ~ 8.4‰) was significantly higher than in seedlings from ploughed soils (0.4–6.2‰) ($P < 0.001$, $n = 33$). Root $\delta^{15}N$ in seedlings from shrubland soils (3.3–9.2‰) was also higher than what was observed for ploughed soils (0.5–6.1‰) ($P < 0.001$, $n = 33$; Table 2). Root $\delta^{15}N$ and foliar $\delta^{15}N$ values were both positively, but not significantly, correlated with soil $\delta^{15}N$. Extractable NH_4^+-N/NO_3^--N was negatively and significantly correlated with foliar N:P ($r^2 = 0.227$, $P < 0.01$, $n = 33$).

Foliar $\delta^{15}N$ (fol-soil) and root $\delta^{15}N$ (root-soil) were not always negative (Fig. 2a and b). Foliar $\delta^{15}N$ was mostly greater than the same values in the corresponding soils except for 8 out of the 17 samples in shrubland soils, while bulk soil $\delta^{15}N$ values were mostly greater than those in corresponding plant foliage except for 3 out of the 16 samples in ploughed soils. Root $\delta^{15}N$ (root-soil) was positively and significantly correlated with foliar N:P (Fig. 2a), and foliar $\delta^{15}N$ (fol-soil) was also positively and significantly correlated with foliar N:P (Fig. 2b).

Extractable NO_3^--N was positively correlated with foliar N and $\delta^{15}N$, and significantly with root $\delta^{15}N$ ($r^2 = 0.180$, $P < 0.05$, $n = 33$). Extractable NO_3^--N/NH_4^+-N was positively correlated with foliar $\delta^{15}N$ ($r^2 = 0.167$, $P < 0.05$, $n = 33$, Fig. 3a), and foliar $\delta^{15}N$ (fol-soil) ($r^2 = 0.303$, $P < 0.001$, $n = 33$, Fig. 3b). The average $\delta^{15}N$ of NH_4^+-N in extraction solutions minus the average soil $\delta^{15}N$ was −4.9 ± 3.6‰, while the average $\delta^{15}N$ of NO_3^--N in extraction solutions minus the average soil $\delta^{15}N$ was +6.5 ± 6.3‰ in the 8 soil samples. The value of $\delta^{15}N$/NO_3^--N was lower than that of $\delta^{15}N$/NH_4^+-N in one of the ploughed soils, which was located at high elevation (1477 m).

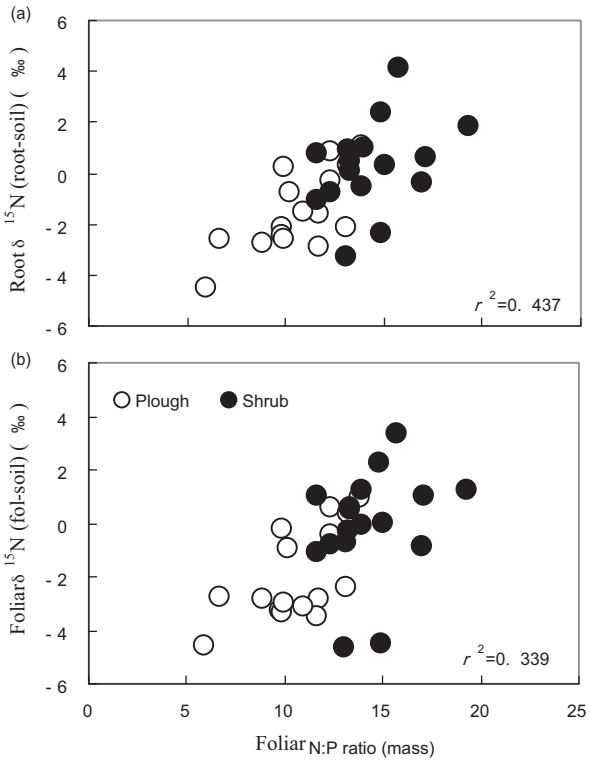

Fig. 2. (a) Relationships of foliar N:P ratio (mass) with root $\delta^{15}N$ (root-soil) and (b) with foliar $\delta^{15}N$ (fol-soil). The samples were collected from 33 fir nurseries distributed across the Guizhou Province of Southwest China. Shrubs group in the legend include shrub/grassland, deciduous forest and one-rotation plantation sites; plough group included ploughed and >2 rotation plantation sites.

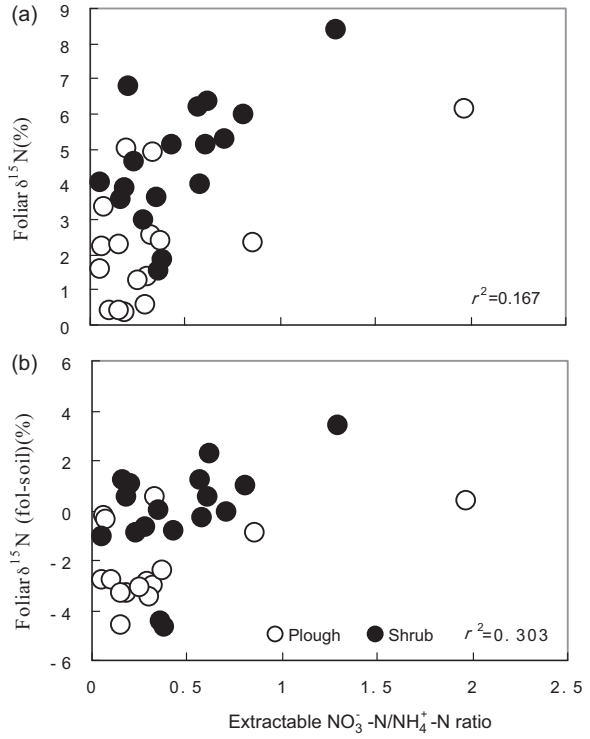

Fig. 3. (a) Relationships between foliar $\delta^{15}N$ and extractable NO_3^--N/NH_4^+-N ratio and (b) between foliar $\delta^{15}N$ (fol-soil) and NO_3^--N/NH_4^+-N ratio. The samples were collected from 33 fir nurseries distributed across the Guizhou Province of Southwest China. Shrub groups in the legend include shrub/grassland, deciduous forest and one-rotation plantation sites; plough group included ploughed and >2 rotation plantation sites.

Discussion

Effect of abiotic condition on AM-mediated process

There was no significant relationship between AM colonization levels and nutrient acquisitions in fir seedlings grown on either shrubland or ploughed soils. However, there was less sugar in tissues in shrubland soils compared to tissue sugar values in ploughed soils, which was similar to our previous results obtained from 24 samples collected January 2008 and 2009 (Piao and Liu 2011). In that study, the average concentration of sugar in foliage ($82 \pm 38\,mg\,g^{-1}$) in shrubland soils was significantly lower than that in ploughed soils ($116 \pm 34\,mg\,g^{-1}$). A key feature of AM-mediated process is the transfer of photosynthate from host plant to the AM fungal hyphae (Zhu and Miller 2003; Richardson et al. 2009; Piao and Liu 2011). For example, there is significantly less sucrose in both shoots and roots of summer wheat with AM fungus compared with summer wheat without AM fungus (Hawkins and George 2001). This study confirms that aboveground-belowground feedback loops play a fundamental role in controlling the interaction between Chinese fir seedlings and AM fungi, and that the lower concentrations of foliar and root sugar in shrubland soils compared to ploughed soils imply that more sugar are transferred to the AM fungi-mediated processes in the former than in the latter soils (Piao and Liu 2011). The actual sugar status of the root is generally low in mycorrhizal plant root, therefore does not necessarily reflect the degree to which sugar is available to the fungus (Hawkins and George 2001). Thus, it is clear that the capacity for the metabolic activity of a bi-directional nutrient transfer in an AM-mediated process should depend on soil conditions (Piao and Liu 2011), and should be a key fact in controlling survival and growth of Chinese fir. However, the interactions between abiotic and biotic are complex and must be site dependent (Karst et al. 2011).

The slight differences in soil organic C concentrations between shrubland ($33.8 \pm 1.1\,mg\,g^{-1}$, $n = 16$) and ploughed soils ($28.1 \pm 0.9\,mg\,g^{-1}$, $n = 17$), between soil organic matter $\delta^{13}C$ values between in shrubland ($-24.4 \pm 1.5‰$, $n = 16$) and ploughed soils ($-24.2 \pm 1.3‰$, $n = 17$), and exchangeable Ca concentrations in shrubland ($0.38 \pm 0.31\,mg\,g^{-1}$, $n = 16$) and ploughed soils ($0.71 \pm 0.66\,mg\,g^{-1}$, $n = 17$) seem to indicate that a more recalcitrant organic C and N accumulated in ploughed soils. The $\delta^{13}C$ values of bulk soil organic C seem to be determined by the ^{13}C content of microbial biomass in the long-term (Piao et al. 2006). Because some enzymes, particularly the phosphatases, may originate from roots or associated mycorrhizae (Nannipieri et al. 2002), AM fungi have a role in maintaining plant diversity in natural communities, contribute to organic matter cycling (Theuerl and Buscot 2010), and have a strong capacity to mobilize both N and P absorbed by host plants. However, AM fungi are obligate symbionts, and or their growth and activity, they depend on the supply of C compounds by the photosynthates of host plants (Ferrol et al. 2002; Smith et al. 2010). In addition, AM fungi are always searching for a new, uninfected root tip at the appropriate stage of development. The infection process lasts for a relatively short period of time, and AM infections are apparently transient. For example, AM-colonized roots of *Populus* had shorter life spans than uncolonized roots (Pregitzer et al., 2002). Therefore, it is apparent that destroyed hyphal network and reduced AM fungal populations through soil disturbance and high levels of compaction (Drew et al. 2006) are the main factors controlling establishment of an AM-mediated process in plant–soil systems.

Nitrogen preferences in Chinese fir seedlings

It appears that plant preferences for NO_3^- or NH_4^+ uptake are associated with the prevailing inorganic N form in the natural

habitat (Brix et al. 2002). The dominant inorganic soil N form here was NH_4^+, except for 3 out of 33 sites. Elevated NH_4^+ levels do occur predominantly in agricultural regions (Yoshida and Allen 2001), which are confirmed by our results, especially in the 8 paddy soils. It was therefore expected that the seedling preferences would be for NH_4^+ in both shrubland and ploughed soils. However, the decrease in NH_4^+ concentrations or NH_4^+/NO_3^- ratios in the relatively low-pH shrubland soils compared to ploughed soils, was reflected in a small though not significant increase in nitrification. This seems contrary to the common belief that nitrification decreases with decreasing soil pH (Falkengren-Grerup et al. 1998). In addition, the concentrations of both NH_4^+ and NO_3^- were only measured once during the study period. Different species of AM fungi can show preferential uptake of NO_3^--N or NH_4^+-N (Johansen et al. 1993), but the actual contribution of this fungal-mediated NO_3^- uptake is unknown (Hawkins and George 2001). It is difficult to distinguish the N form absorbed by fir seedlings in shrubland soils with high efficiency via the AM-mediated process, from the one taken up directionally by fir root epidermis under low efficiency via the AM-mediated process.

Responses of foliar $\delta^{15}N$ to N preferences

Generally, the highest values of soil $\delta^{15}N$ are found under agriculture and the lowest values under forestry, but there is a continuum and there is some overlap among all types of landuse (Norra et al. 2005). In this study, there were no differences in soil $\delta^{15}N$ between shrubland and ploughed soils despite the greater addition of fertilizer to the ploughed soils. Lobe et al. (2005) noted that, even after 98 years of arable cropping, bulk soil $\delta^{15}N$ values do not change. Any decreases in $\delta^{15}N$ values caused by mineral fertilizer N inputs and $\delta^{15}N$ changes in the whole soil pool are solely driven by microbial processes (Bol et al. 2008). The $\delta^{15}N$ values of soil NO_3^- were generally isotopically lighter than $\delta^{15}N$ values of NH_4^+ because of fractionation against ^{15}N taking place during nitrification (Miller and Bowman 2002; Dijkstra et al. 2006; Kahmen et al. 2008). Choi et al. (2005) reported that increasing foliar $\delta^{15}N$ values with increasing foliar N concentrations supports the hypothesis that increasing the contribution of ^{15}N-enriched N, particularly in the NH_4^+ pool, compared to foliar N concentrations leads to ^{15}N-enrichment of foliar N, and concluded that NH_4^+-N is preferentially assimilated by conifers.

In this study, foliar $\delta^{15}N$ values in shrubland soils were significantly higher than those in ploughed soils, and increases in foliar $\delta^{15}N$ did significantly respond to increasing foliar N:P ($r^2 = 0.304$, $P < 0.001$, $n = 33$). Both foliar N concentrations and foliar $\delta^{15}N$ values significantly decreased by increasing extractable NH_4^+ concentrations, but the changes in soil NO_3^- were minimal, ranging from 4.1 $\mu g\,g^{-1}$ to 8.2 $\mu g\,g^{-1}$ except for two samples. Hence, the occurrence of a positive correlation between leaf $\delta^{15}N$ and NO_3^--N/NH_4^+-N, and a negative one between leaf $\delta^{15}N$ and NH_4^+-N/NO_3^--N, was mainly determined by changes in extractable NH_4^+-N concentrations. Several studies have shown that NO_3^- can become enriched in ^{15}N, in contrast to NH_4^+ in ecosystems during denitrification (Falkengren-Grerup et al. 2004; Houlton et al. 2007; Pörtl et al. 2007; Kahmen et al. 2008). There is some evidence for the effects of denitrification on the changes of $\delta^{15}N/NO_3^-$ in soils: soil water $\delta^{15}N/NO_3^-$ is highly enriched relative to mineral soil $\delta^{15}N$ at low NO_3^- concentrations and becomes progressively depleted at higher NO_3^- concentrations, to a maximum of 4.2‰ relative to mineral soil N (Perakis et al. 2011). In our case, the $\delta^{15}N$ of NO_3^--N were higher than the $\delta^{15}N$ of NH_4^+-N and the bulk soil $\delta^{15}N$ in 7 of the 8 sampled soils, which was consistent with the results of Perakis et al. (2011). Therefore, we concluded that the lower foliar $\delta^{15}N$ values should respond to soil NH_4^+ with lower $\delta^{15}N$ for the ploughed soils, while the higher foliar $\delta^{15}N$ should respond to soil NO_3^- with higher $\delta^{15}N$ for the shrubland soils. Hence, it is reasonable to assume that the switch from NH_4^+-N in ploughed soils to NO_3^--N in shrubland soils as the N form taken up by fir seedlings was associated with a change from a low to a high efficiency AM-mediated process.

Responses of foliar $\delta^{15}N$ (fol-soil) to N preferences

In general, plant $\delta^{15}N$ are lower than those of soils (Amundson et al. 2003; Norra et al. 2005), but foliar $\delta^{15}N$ (fol-soil) range from positive to negative (Bol et al. 2002). Within a site, foliar $\delta^{15}N$ (fol-soil) increase with increasing NO_3^-/NH_4^+ uptake ratios (Kahmen et al. 2008), although Miller and Bowman (2002) and Falkengren-Grerup et al. (2004) reported that plant $\delta^{15}N$ decrease with increasing NO_3^-/NH_4^+ uptake ratios. Within an ecosystem, when NO_3^- is ^{15}N depleted compared with NH_4^+, plants should become depleted with increasing NO_3^-/NH_4^+ uptake, but when NO_3^- is enriched in ^{15}N compared to NH_4^+, plants should become enriched with increasing NO_3^-/NH_4^+ uptake (Kahmen et al. 2008). These contrasting results suggest that the results of ^{15}N enrichment with NH_4^+ or NO_3^- can vary significantly between ecosystems, depending on the nature of the N cycle. In this study, foliar $\delta^{15}N$ (fol-soil) increased by increasing extractable NO_3^--N/NH_4^+-N. This aligns with the findings of Kahmen et al. (2008), where all foliar $\delta^{15}N$ (fol-soil) were negative under a significant nutrient gradient. However, as discussed above, the significant correlation between foliar $\delta^{15}N$ (fol-soil) and NO_3^--N/NH_4^+-N was due to extractable NH_4^+-N concentrations rather than to higher soil NO_3^- concentrations (as shrubland soils did not differ significantly from ploughed soils). It is apparent that higher foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) in shrubland soils were determined by the amount of NO_3^- taken up by seedlings, which suggested that a high efficiency AM-mediated process should play a more important role in exploiting NO_3^-. By contrast, lower foliar $\delta^{15}N$ and foliar $\delta^{15}N$ (fol-soil) in ploughed soils was determined mainly by the level of NH_4^+ taken up by seedlings, where colonized root epidermis should play a more important role in exploiting NH_4^+ in soils with a low efficiency AM-mediated process. In addition, AM fungi can also take up organic N source from the soils (Tian et al. 2010). The $\delta^{15}N$ values of most individual amino acids are depleted compared to the bulk soil (Bol et al. 2002). Moreover, amino acids and other soil organic N compounds presumably are ^{15}N-depleted, similar to plant litter, and hence, EM plants with high uptake of these N forms have low foliar $\delta^{15}N$ (Michelsen et al. 1998). It is apparent that organic N with low $\delta^{15}N$ should not respond to high foliar $\delta^{15}N$ in fir seedlings grown on shrubland soils. However, Averill and Finzi (2011) reported that organic forms of N taken up by coniferous tree species, which are EM species, have higher $\delta^{15}N$ values than those of inorganic N. But this was not true in the soils in our investigation since they had higher $\delta^{15}N/NO_3^-$ values compared to the bulk soil due to denitrification. Chinese fir can also be colonized by EM fungi after transplanting seedlings. At present, there is a dearth of information regarding the variability of mycorrhizal colonization in Chinese fir grown in nurseries and plantations, therefore, more intensive sampling is required.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (grant no. 41021062; 40772207), and by the Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDA05070400).

References

Amundson, R., Austin, A.T., Schuur, E.A.G., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., Brenner, D., Baisden, W.T., 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles 17, 31-1–31-10.

Averill, C., Finzi, A., 2011. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem $\delta^{15}N$. Ecology 92, 883–891.

Azcón, R., Rodriguez, R., Amora-Lazcano, E., Ambrosano, E., 2008. Uptake and metabolism of nitrate in mycorrhizal plants as affected by water availability and N concentration in soil. European Journal of Soil Science 59, 131–138.

Bainard, L.D., Klironomos, J.N., Gordon, A.M., 2011. Arbuscular mycorrhizal fungi in tree-based intercropping systems: a review of their abundance and diversity. Pedobiologia 54, 57–61.

Barea, J.M., Azcón-Aguilar, C., Azcón, R., 1997. Interactions between mycorrhizal fungi and rhizosphere micro-organisms within the context of sustainable soil–plant systems. In: Gange, A.C., Brown, V.K. (Eds.), Multitrophic interactions in terrestrial systems. Backwell, Cambridge, pp. 65–77.

Blanke, V., Wagner, M., Renker, C., Lippert, H., Michulitz, M., Kuhn, A.J., Buscot, F., 2011. Arbuscular mycorrhizas in phosphate-polluted soil: interrelations between root colonization and nitrogen. Plant Soil 343, 379–392.

Bol, R., Ostle, N.J., Petzke, K.J., 2002. Compound specific plant amino acid $\delta^{15}N$ values differ with functional plant straties in temperate grassland. Journal of Plant Nutrition and Soil Science 165, 661–667.

Bol, R., Ostle, N.J., Petzke, K.J., Chenu, C., Balesdent, J., 2008. Amino acid $\delta^{15}N$ in long-term bare fallow soils: influence of annual N fertilizer and manure applications. European Journal of Soil Science 59, 617–629.

Brix, H., Dyhr-Jensen, K., Lorenzen, B., 2002. Root-zone acidity and nitrogen source affects *Typha latifolia* L. growth and uptake kinetics of ammonium and nitrate. Journal of Experimental Botany 53, 2441–2450.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist 154, 275–304.

Brundrett, M.C., 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320, 37–77.

Bustamante, M.M.C., Martinelli, L.A., Silva, D.A., Camargo, P.B., Klink, C.A., Domingues, T.F., Santos, R.V., 2004. ^{15}N natural abundance in woody plants and soils of central Brazilian savannas (Cerrado). Ecological Application 14 (Suppl. 4), S200–S213.

Cavagnaro, T.R., Barrios-Masias, F.H., Jackson, L.E., 2012. Arbuscular mycorrhizas and their role in plant growth, nitrogen interception and soil gas efflux in an organic production system. Plant Soil 353, 181–194.

Chalot, M., Blaudez, D., Brun, A., 2006. Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface. Trends in Plant Science 11, 263–266.

Chen, C.Y., Gao, M.Q., Liu, J.Y., Zhu, H.Y., 2007. Fungal symbiosis in rice requires and ortholog of a legume commen symbiosis gene encoding a $Ca_2{}^+$/calmodulin-dependent protein kinase. Plant Physiology 145, 1619–1628.

Chinnasamy, G., Bal, A.K., 2003. Seasonal changes in carbohydrates of perennial root nodules of beach pea. Journal of Plant Physiology 160, 1185–1192.

Choi, W.-J., Chang, S.X., Lee Allen, H., Kelting, D.L., Ro, H.-M., 2005. Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. Forest Ecology and Management 213, 90–101.

Compton, J.E., Hooker, T.D., Perakis, S.S., 2007. Ecosystem N distribution and $\delta^{15}N$ during a century of forest regrowth after agricultural abandonment. Ecosystems 10, 1197–1208.

Cornejo, P., Borie, F., Rubio, R., Azcón, R., 2007. Influence of nitrogen source on the viability, functionality and persistence of *Glomus etunicatum* fungal propagules in an Andisol. Applied Soil Ecology 35, 423–431.

Dijkstra, P., Menyailo, O.V., Doucett, R.R., Hart, S.C., Schwartz, E., Hungate, B.A., 2006. C and N availability affects the ^{15}N natural abundance of the soil microbial biomass across a cattle manure gradient. European Journal of Soil Science 57, 468–475.

Drew, E.A., Murray, R.S., Smith, S.E., 2006. Functional diversity of external hyphae of AM fungi: ability to colonise new hosts is influenced by fungal species, distance and soil conditions. Applied Soil Ecology 32, 350–365.

Drew, E.A., Murray, R.S., Smith, S.E., Jakobsen, I., 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant Soil 251, 105–114.

Falkengren-Grerup, U., Brunet, J., Dickmann, M., 1998. Nitrogen mineralisation in deciduous forest soils in south Sweden in gradients of soil acidity and deposition. Environmental Pollution 102 (Suppl. 1), 415–420.

Falkengren-Grerup, U., Michelsen, A., Olsson, M.O., Quarmby, C., Sleep, D., 2004. Plant nitrate use in deciduous woodland: the relationship between leaf N, ^{15}N natural abundance of forbs and soil N mineralisation. Soil Biology and Biochemistry 36, 1885–1891.

Ferrol, N., Barea, J.M., Azcón-Aguilar, C., 2002. Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. Plant Soil 244, 231–237.

Fitter, A.H., Helgason, T., Hodge, A., 2011. Nutritional exchange in the arbuscular mycorrhizal symbiosis: implications for sustainable agriculture. Fungal Biology Reviews 25, 68–72.

Fortuna, A., Harwood, R.R., Robertson, G.P., Fisk, J.W., Paul, E.A., 2003. Seasonal changes in nitrification potential associated with application of N fertilizer and compost in maize systems of southwest Michigan. Agriculture Ecosystems and Environment 97, 285–293.

Gai, J.P., Feng, G., Cai, X.B., Christie, P., Li, X.L., 2006. A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet. Mycorrhiza 16, 191–196.

Ghosh, P., Kashyap, A.K., 2003. Effect of rice cultivars on rate of N-mineralization, nitrification and nitrifier population size in an irrigated rice ecosystem. Applied Soil Ecology 24, 27–41.

Gosling, P., Hodge, A., Goodlass, G., Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. Agriculture Ecosystems and Environment 113, 17–35.

Govindarajulu, M., Pfeffer, P.E., Jin, H., Abubaker, J., Douds, D.D., Allen, J.W., Bücking, H., Lammers, P.J., Shachar-Hill, Y., 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435, 819–823.

Graetz, D.A., Nair, V.D., Portier, K.M., Voss, R.L., 1999. Phosphorus accumulation in manure-impacted Spodosols of Florida. Agriculture Ecosystems Environment 75, 31–40.

Hawkins, H.-J., George, E., 2001. Reduced ^{15}N-nitrogen transport through arbuscular mycorrhizal hyphae to *Triticum aestivum* L. supplied with ammonium vs. nitrate nutrition. Annals of Botany 87, 303–311.

He, X.H., Critchley, C., Ng, H., Bledsoe, C., 2005. Nodulated N_2-fixing *Casuarina cunninghamiana* is the sink for net N transfer from non-N_2-fixing *Eucalyptus maculata* via an ectomycorrhizal funus *Pisolithus* sp. Using $^{15}NH_4{}^+$ or $^{15}NO_3{}^-$ suppled as ammonium nitrate. New Phytologist 167, 897–912.

Hobbie, E.A., Macko, S.A., Williams, M., 2000. Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. Oecologia 122, 273–283.

Houlton, B.Z., Sigman, D.M., Schuur, A.G., Hedin, L.O., 2007. A climate-driven switch in plant nitrogen acquisition within tropical forest communities. PNAS 104, 8902–8906.

Johansen, A., Jakobsen, I., Jensen, E.S., 1993. Hyphal transport by vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. Biology and Fertility of Soils 16, 66–77.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M., Allen, E.B., 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84, 1895–1908.

Kahmen, A., Wanek, W., Buchmann, N., 2008. Foliar $\delta^{15}N$ values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. Oecologia 156, 861–870.

Karst, J., Hoeksema, J.D., Jones, M.D., Turkington, R., 2011. Parsing the roles of abiotic and biotic factors in Douglas-fir seedlings growth. Pedobiologia 54, 273–280.

Kooijman, A., Hedenäs, L., 2009. Changes in nutrient availability from calcareous to acid wetland habitats with closely related brown moss species: increase instead of decrease in N and P. Plant Soil 324, 267–278.

Lobe, I., Bol, R., Ludwig, B., Du Preez, C.C., Amelung, W., 2005. Savanna-derived organic matter remaining in arable soils of the South African Highveld long-term mixed cropping: Evidence from ^{13}C and ^{15}N natural abundance. Soil Biology and Biochemistry 37, 1898–1909.

McGonigle, T.P., 1988. A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. Functional Ecology 2, 473–478.

Michelsen, A., Quarmby, C., Sleep, D., Jonasson, S., 1998. Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115, 406–418.

Michelsen, A., Rosendahl, S., 1989. Propagule density of VA mycorrhizal fungi in semi-arid bushland in Somalia. Agriculture Ecosystems and Environment 29, 295–301.

Michelsen, A., Sprent, J.I., 1994. The influence of vesicular-arbuscular mycorrhizal fungi on the nitrogen fixation of nursery-grown Ethiopian acacias estimated by the ^{15}N natural abundance method. Plant Soil 160, 249–257.

Miller, A.E., Bowman, W.D., 2002. Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? Oecologia 130, 609–616.

Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Bums, R.G., Dick, W.A. (Eds.), Enzymes in the environment. Marrcel Dekker, New York, pp. 1–33.

Norra, S., Handley, L.L., Berner, Z., Stüben, D., 2005. ^{13}C and ^{15}N natural abundances of urban soils and herbaceous vegetation in Karlsruhe, Germany. European Journal of Soil Science 56, 607–620.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939. Washington, DC.

Perakis, S.S., Sinkhorn, E.R., Compton, J.E., 2011. $\delta^{15}N$ constraints on long-term nitrogen balances in temperate forests. Oecologia 167, 793–807.

Piao, H.C., Liu, C.-Q., 2011. Variations in nitrogen, zinc and sugar concentrations in Chinese fir seedlings grown on shrubland and ploughed soils in response to arbuscular mycorrhizae-mediated process. Biology and Fertility of Soils 47, 721–727.

Piao, H.C., Liu, G.S., Wu, Y.Y., Xu, W.B., 2001. Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest China. Biology and Fertility of Soils 33, 347–350.

Piao, H.C., Zhu, J.M., Liu, G.S., Liu, C.-Q., Tao, F.X., 2006. Changes of natural ^{13}C abundance in microbial biomass during litter decomposition. Applied Soil Ecology 33, 3–9.

Pörtl, K., Zechmeister-Boltenstern, S., Wanek, W., Ambus, P., Berger, T.W., 2007. Natural ^{15}N abundance of soil N pools and N_2O reflect the nitrogen dynamics of forest soils. Plant Soil 295, 79–94.

Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L., 2002. Fine root architecture of nine North American trees. Ecological Monographs 72, 293–309.

Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytologist 157, 475–492.

Requena, N., Breuninger, M., Franken, P., Ocón, A., 2003. Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiology 132, 1540–1549.

Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339.

Smith, S.E., Facelli, E., Pope, S., Smith, F.A., 2010. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326, 3–20.

Smith, S.E., Gianinazzi-Pearson, V., 1990. Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L. Effects of photon irradiance and phosphate nutrition. Australian Journal of Plant Physiology 17, 177–188.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, 2nd ed. Academic Press, San Diego, CA.

Stephan, K., Kavanagh, K.L., 2009. Suitability of the diffusion method for natural abundance nitrogen-15 analysis. Soil Science Society of American Journal 73, 293–302.

Stewart, K.J., Coxson, D., Siciliano, S.D., 2011. Small-scale spatial patterns in N_2-fixation and nutrient availability in an arctic hummock-hollow ecosystem. Soil Biology and Biochemistry 43, 133–140.

Tabuchi, M., Abiko, T., Yamaya, T., 2007. Assimilation of ammonium ions reutilization of nitrogen in rice (*Oryza sativa* L.). Journal of Experimental Botany 58, 2319–2327.

Templer, P.H., Arthur, M.A., Lovett, G.M., Weathers, K.C., 2007. Plant and soil natural abundance $\delta^{15}N$: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. Oecologia 153, 399–406.

Theuerl, S., Buscot, F., 2010. Laccases: toward disentangling their diversity and functions in relation to soil organic mater cycling. Biology and Fertility of Soils 46, 215–225.

Tian, C.J., Kasiborski, B., Koul, R., Lammers, P.J., Bücking, H., Shachar-Hill, Y., 2010. Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiology 153, 1175–1187.

Tinker, P.B., Nye, P.H., 2000. Solute Movement in the Rhizosphere. Oxford University Press, New York, NY, USA.

Tu, C., Booker, F.L., Watson, D.M., Chen, X., Rufty, T.W., Shi, W., Hu, S.J., 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: impact of mineral N inputs. Global Change Biology 12, 793–803.

Veresoglou, S.D., Chen, B.D., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology and Biochemistry 46, 53–62.

Yoshida, L.C., Allen, E.B., 2001. Response to ammonium and nitrate by a mycorrhizal annual invasive grass and native shrub in southern California. American Journal of Botany 88, 1430–1436.

Zhu, Y.-G., Miller, R.M., 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil–plant systems. Trends in Plant Science 8, 407–409.