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# Cooperation of earthworm and arbuscular mycorrhizae enhanced plant N uptake by balancing absorption and supply of ammonia

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ABSTRACT

Earthworms and arbuscular mycorrhizal fungi (AMF) interact to regulate plant nitrogen (N) supply, but the mechanisms through which they affect plant N uptake are unclear. We hypothesized that earthworms, plants and the associated AMF exhibit different preferences for different forms of inorganic N ($NH_4^+$ and $NO_3^-$), which could regulate the effect of earthworms and AMF interaction on plant N acquisition. We outlined three independent but complementary experiments to test this hypothesis in the context of exotic earthworm, *Pontoscolex corethrurus*. The earthworm is dominating the plantation forests in subtropical and tropical regions of China, which have their understory dominated by the fern *Dicranopteris dichotoma*. By employing an excised root $^{15}N$ incubation experiment and a field *in situ* $^{15}N$ experiment, we found that the fern prefers to use $NH_4^+$ rather than $NO_3^-$. Then we did a 2 × 2 factorial microcosm experiment using AMF (*Rhizophagus intraradices*) and earthworms (*P. corethrurus*). The exotic earthworm increased soil $NH_4^+$ concentration but did not affect soil $NO_3^-$ concentration, while the AMF decreased soil $NH_4^+$ concentration but had no effect on soil $NO_3^-$ concentration. The increase in soil $NH_4^+$ induced by the earthworms was efficiently utilized by the AMF, and significantly increased the total N uptake by the fern. In contrast, the AMF alone increased the N concentration of leaves and coarse roots, but not the total plant N uptake, primarily due to the lower levels of available $NH_4^+$ compared with the earthworm treatments. The uninoculated fern did not benefit from the earthworm-induced increase in soil $NH_4^+$, suggesting that the root of the fern cannot access the '$NH_4^+$ hotspots' created by the earthworms. Our work suggests that successful cooperation of earthworms and AMF on plant N uptake depends on the correct match in N-form.

## 1. Introduction

Linkages between above- and below-ground biota are a cornerstone of modern ecology; they are critical in regulating ecosystem functioning (Wardle et al., 2004). Soil organisms affect nutrient supply for plants not only by decomposition but also through parasitism and mutualism. Thus, nutrient supply is best interpreted in the context of interactions between soil organisms (Lavelle et al., 2006; Wall and Bardgett, 2012).

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Earthworms and arbuscular mycorrhizal fungi (AMF) each trigger influences on plant nutrients, stimulating decomposition and root uptake, respectively (Hodge and Storer, 2015; Lavelle, 1988). How earthworms and AMF interact with each other, however, to affect plant nutrient uptake, has not been represented in perspectives on the linkages between above- and below-ground biota (Fitter and Garbaye, 1994; Paudel et al., 2016).

Soil nitrogen (N) is the main limiting nutrient for plant growth in most ecosystems (Chapin et al., 2011). Most soil N is present in organic forms; however, plants take up N mainly in inorganic forms ($NH_4^+$ and $NO_3^-$) (Vitousek and Howarth, 1991). Earthworms can enhance organic N mineralization by feeding, digestion and casting activities, which often lead to increased soil inorganic N (Araujo et al., 2004; Blair et al., 1997). This increase in N availability has been recognized as, perhaps, the most important effect of earthworms on plant growth (Brown et al., 2004; Barot et al., 2007), but it is still unclear whether plant N-uptake benefits from the increased N availability induced by earthworms (Blouin et al., 2006; Cao et al., 2016; Domínguez et al., 2004; Lubbers et al., 2011). Earthworms may have different effects on soil $NH_4^+$ and $NO_3^-$, and subsequently on N acquisition of plants (Domínguez et al., 2004; Hale et al., 2008; Lubbers et al., 2011). Such results may be because plant species prefer different inorganic N forms in different soil conditions. In general, plants that are adapted to low pH and reducing soil conditions tend to take up $NH_4^+$, whereas plants that adapted to higher pH and more aerobic soils prefer $NO_3^-$ (Maathuis, 2009; Masclaux-Daubresse et al., 2010). Therefore, a focus on soil inorganic N forms can improve predictions of the interactions between earthworm activities and plant N acquisition.

Another important determinant of plant N uptake is AMF, which form symbioses with more than two thirds of terrestrial plants (Hodge and Storer, 2015). However, it is unclear how their function responds to earthworms. AMF take up soil N and transport it to host plants (Jin et al., 2012; Thirkell et al., 2016). Most AMF species predominantly utilize $NH_4^+$, having low $NO_3^-$ assimilation rates (Cheng et al., 2012; Hodge and Storer, 2015; Perez-Tienda et al., 2012; Tanaka and Yano, 2005).

The cumulative influences of earthworms and AMF on plant N acquisition are complex. Earthworms may reduce AMF abundance by eating fungal hyphae or destroying them as they move through the soil. But earthworms may enhance AMF abundance by dispersing spores (Paudel et al., 2016). Earthworms may affect AMF functioning by enhancing N availability and altering the forms in which N occurs. Previous work has emphasized the amount, over the form, of N that may be present and available for AM fungal uptake (Li et al., 2013; Cao et al., 2016). Here, we propose that the effects of earthworms, AMF, and their interaction with AMF on plant N-uptake are strongly driven by inorganic N forms.

Introduced earthworms now occur in most terrestrial biogeographic regions (Hendrix et al., 2008). Previous studies suggests the effects of exotic earthworms on aboveground biota are mediated by AMF (Lawrence et al., 2003; Nuzzo et al., 2009), so AMF-mediated effects of exotic earthworms on ecosystems might be common (Paudel et al., 2016).

Earthworm invasion is common in disturbed plantations and secondary forests in southern China (Du et al., 2008; Gao et al., 2010; Zhang et al., 2010). These forests commonly have an understory that is dominated by a fern (*Dicranopteris dichotoma*) which grows rapidly when light intensity is high. *D. dichotoma* often forms a dense mat-like understory layer. This influences the soil microclimate (Zhao et al., 2013, 2012), nutrient cycling (Liu et al., 2012; Wu et al., 2011), biodiversity (Zhao et al., 2013, 2012), erosion and nutrient leaching (Zheng et al., 2008), and even overstory tree growth (Wan et al., 2014). In our study site, the pantropical peregrine earthworm *P. corethrurus* (González et al., 2006) accounts for 95% of the earthworm biomass, while *D. dichotoma* accounts for 40% of the understory plant biomass; they most likely co-exist (unpublished data).

In the present experiment, we focused on the interactive effects of the exotic earthworm *P. corethrurus* and the AMF *Rhizophagus intraradices* on *D. dichotoma* N uptake. We outlined three independent but complementary experiments. First, we asked which form of soil inorganic N ($NH_4^+$ or $NO_3^-$) the fern prefers; this involved two approaches; a lab experiment that measured uptake of $^{15}N$-substrates by excised root segments, and a field $^{15}N$ labeling experiment. Second, we asked which form of soil inorganic N the earthworms tend to stimulate and the AMF tend to absorb. We approached this by determining soil $NH_4^+$ and $NO_3^-$ in a $^{15}N$-microcosm experiment, in which earthworm and AMF abundance were manipulated as factors. Third, we asked whether N-uptake by the fern was influenced by the differential effects of earthworms and AMF on the balance of $NH_4^+$ vs. $NO_3^-$ in the microcosm experiment.

## 2. Materials and methods

### 2.1. Field site

The field site is located in a plantation mono-cultured with *Schima superba* Gardn. et Champ, at the Heshan National Field Research Station of Forest Ecosystem (112°54′E, 22°41′N), Chinese Academy of Science (CAS), Guangdong Province, China. The climate is subtropical monsoon with distinct wet (from April to September) and dry (from October to March) seasons. The mean annual precipitation is 1580.4 mm and the mean annual temperature is 21.9 °C from 1985 to 2014 (Gao et al., 2017). The soil is an Acrisol (FAO, 2006), the sand, silty and clay content are 48.5%, 13% and 38.5%, respectively. The soil C and, N contents are 56.1 and, 4.3 g $kg^{-1}$ dw soil, respectively. The soil pH was 3.7. The understory vegetation is dominated by *D. dichotoma*; other common understory plants include *Miscanthus sinensis* and *Rhodomyrtus tomentosa*.

### 2.2. Excised root segment experiment

An excised root technique was adopted to assay the physiological uptake capacity of *D. dichotoma* roots for $NH_4^+$ and $NO_3^-$ (Rothstein et al., 2000). In April 2017, three clusters of *D. dichotoma* were collected and brought to the laboratory within hours. Fine roots (< 1 mm) of each cluster were removed and washed under 0.5 mM CaCl to maintain membrane integrity. Fifteen 0.1 g fresh weight subsamples of fine roots from each cluster were used to assay for $^{15}N$ uptake rate. Three kinds of labeling markers were used, $^{15}NH_4NO_3$ (10 atom% $^{15}N$) with nitrification inhibitor dicyandiamide (DCD), $^{15}NH_4NO_3$ (10 atom% $^{15}N$) and $NH_4{}^{15}NO_3$ (10 atom% $^{15}N$). Each form of N label was tested at five concentrations (10, 50, 100, 250, and 500 μM). For all $^{15}NH_4NO_3$ + DCD solutions, the concentration of DCD was 120 μM. DCD was used to inhibit the conversion of $NH_4^+$ to $NO_3^-$ (McGeough et al., 2016); therefore, the uptake rate for $NH_4^+$ estimated by the treatment $^{15}NH_4NO_3$ + DCD may be more accurate than the results from the treatment without DCD. However, DCD may be toxic for some plants (Reeves and Touchton, 1986), so the treatment $^{15}NH_4NO_3$ without DCD was kept. All uptake solutions contained 0.5 mM CaCl and 1% sucrose. Each subsample was incubated in 100 ml labeling solution for 30 min. After incubation, roots were washed three times (with about 100 ml each time) with 5 mM KCl and 0.5 mM CaCl to remove any $^{15}N$ labeling marker absorbed by the root surface. The 45 labeled root samples and 3 unlabeled root samples (one per cluster) were oven dried at 60 °C for 3 days, then ball milled and sieved to 100 mesh before analyzing them for total N content and atom% $^{15}N$ of the root samples by an elemental analyzer (Vario EL Cube, Elementar Analysensysteme GmbH, Germany) interfaced to a IRMS (Iso-prime 100 IRMS, Isoprime Co., UK).

Atom% excess $^{15}N$ (APE) was calculated as the atom% $^{15}N$ difference between labeled samples and unlabeled samples. The $^{15}N$ uptake rate by roots was estimated by $^{15}N$ excess in unit mass of root per unit time (μg $^{15}N$ excess $g^{-1}$ dw root $h^{-1}$). This is calculated as:

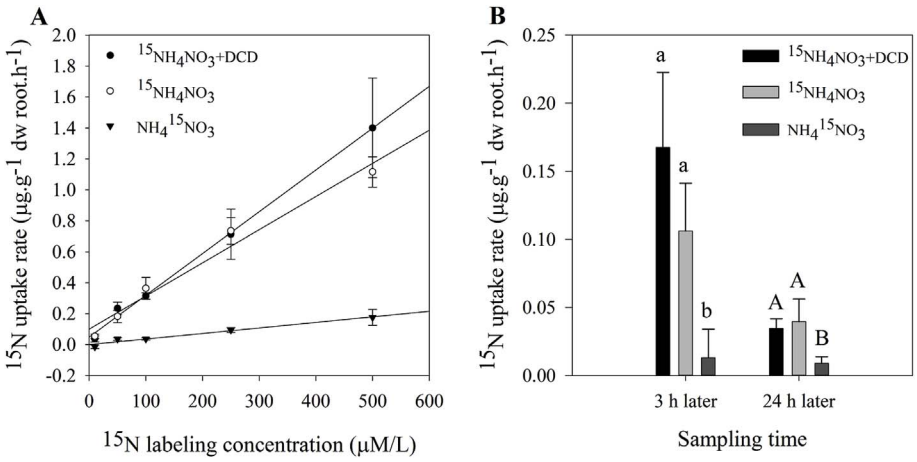


**Fig. 1.** Uptake pattern of the fern for $NH_4^+$ and $NO_3^-$. $^{15}N$ uptake rate by the fern estimated by excised root experiment (A) and field *in situ* estimation experiment (B). Different letters means that the difference of the means between treatments was significant (LSD test, $P < 0.05$).

APE / 100 × N% / 100 × Root Biomass (g) × $10^6$ (μg $g^{-1}$) / Root Biomass (g) / 0.5 (h).

### 2.3. Field in situ uptake estimation experiment

Although the excised root technique may give an accurate estimation of plant physiological preference for different forms of N, it cannot, by itself, indicate the real uptake pattern for $NH_4^+$ and $NO_3^-$ under field conditions. To address *in situ* uptake preferences, in April, 2017 we randomly selected twenty-eight clusters of *D. dichotoma* in the field site. The clusters were divided into three groups with each group including 8 individual plants. Each group of plants was labeled with one of the three labeling solutions, $^{15}NH_4NO_3$ (10 atom% $^{15}N$) with nitrification inhibitor dicyandiamide (DCD), $^{15}NH_4NO_3$ (10 atom% $^{15}N$) and $NH_4{}^{15}NO_3$ (10 atom% $^{15}N$). DCD was applied at a rate 10 μg $g^{-1}$ dry soil (McGeough et al., 2016). In detail, three 30 ml labeling solutions (6929 μM $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$) which account for 7.02 mg N were injected into 3 points of the surface soil, each point distributed at a distance of 5 cm from the plant, which was treated as the center of a triangle defined by the three points. The other four clusters of plants were treated as unlabeled controls; deionized water was injected into the soil in the same manner and volume as the isotope solutions had been. For each group, four clusters were harvested 3 h after the injection, while the remaining four clusters were sampled 24 h after injection. The harvested plants were broken down into: leaves, stems, coarse roots (2–3 mm) and fine roots (< 1 mm). Root samples were rinsed with 0.5 mM CaCl solution to remove any $^{15}N$ tracers adhering to the root surface before drying. Then all vegetation samples were prepared for isotopic analysis as described above (drying and grinding). $^{15}N$ uptake by plants was estimated by calculating $^{15}N$ excess in each plant part (APE/100 × biomass × N%/100) and then summing the total taken up into all components; this was then dived by root biomass to generate uptake per unit root mass. This was expressed as μg $^{15}N$ $g^{-1}$ dw root $h^{-1}$ (Xu et al., 2014).

### 2.4. Microcosm experiment

#### 2.4.1. Microcosm setup

The experiment was conducted in a greenhouse in the South China Botanical Garden, Guangzhou, China. The microcosm experiment was a 2 × 2 factorial experiment with a completely randomized design. There were four treatment combinations: soil with plant (P), soil with plant and inoculated with earthworms (P + E), soil with plant and inoculated with AMF (P + A), soil with plant and both inoculated with AMF and earthworms (P + A + E). Each treatment had 5 replicates. The earthworm *P. corethrurus* and the plant *D. dichotoma* were collected from the field site. The soil was collected from 0 to 20 cm soil layer of the field site, the rocks and earthworms in the soil were removed by sieving through 2 mm mesh and hand sorting. The AMF inocula was *Rhizophagus intraradices* BGC JX04 B (formerly known as *Glomus intraradices*).

Each microcosm was constructed by filling a plastic pot with 1.5 kg soil. The diameter of the opening and the bottom of the pot were 16 and 13 cm, respectively. The height of the pot was 17.5 cm. A hole of 2 cm diameter was drilled in the bottom of each to maintain drainage. The soil was packed in a nylon bag (20 cm wide × 40 cm long, mesh size = 0.25 mm) before placing it into the pot to prevent earthworms from escaping through the bottom (Huang et al., 2015). A 4 cm wide velcro tape was pasted onto the inner rim of the pot to prevent earthworms from escaping out the top of the pot (Lubbers et al., 2011). To simulate the litter fall layer in the forest, 10 g fresh and partially decomposed litter collected from the forest floor was add to the surface of each microcosm, which was equal to the litter standing crop of the field site (about 500 g $m^{-2}$). The %N of litter was 1.9%. *D. dichotoma* was transplanted into the microcosms (in June 2014) by placing a section of live coarse root with associated fine roots and new shoots (the root sections were 10 cm long and about 24 g in fresh weight). To simulate the soil condition during raining season, soil moisture was maintained at or above field capacity by watering with deionized water once a day until small amount of leachate came out the bottom. After 3 months, the shoots had grown to 5 cm height. At that time, for treatments with AMF inoculated plants, 50 g *R. intraradices* inocula consisting of sand mixed with *R. intraradices* hyphae and spores (BGC JX04 B) was mixed into the upper 5 cm of the soil. To maintain similar conditions between treatments with or without AMF inocula, 50 g sterilized *R. intraradices* inocula was added into each microcosm for treatments without AMF inoculation. Two months later, 8 adult *P. corethrurus* individuals (0.205 ± 0.006 g fresh weight per individual) were added per microcosm for treatments with earthworms (in December 26, 2014). The population density of 8 individuals per microcosm (ca. 400 individuals $m^{-2}$) was a little high compared to the average density of *P. corethrurus* in the plantations (ca. 100 individuals $m^{-2}$). However, in field, *P. corethrurus* distributed in an aggregated pattern where the population size can reach to 322 individuals $m^{-2}$ (Personal observation). Therefore, eight individuals per microcosm would be reasonable to reflect the real effects of *P. corethrurus* population. All microcosms were then moved to a chamber in the greenhouse; the temperature was maintained at 25 °C during the following experimental period. The day that earthworms were introduced was defined as day-0 of the experiment. At day-24, three 5.3 ml $^{15}NH_4{}^{15}NO_3$ (10 atom% $^{15}N$, 5.2 mM) solutions which was equal to 2.34 mg N were injected into 3 points of the surface soil, each point distributed at a distance of 3 cm from the plant as mentioned above. Each microcosm was then watered with 150 ml

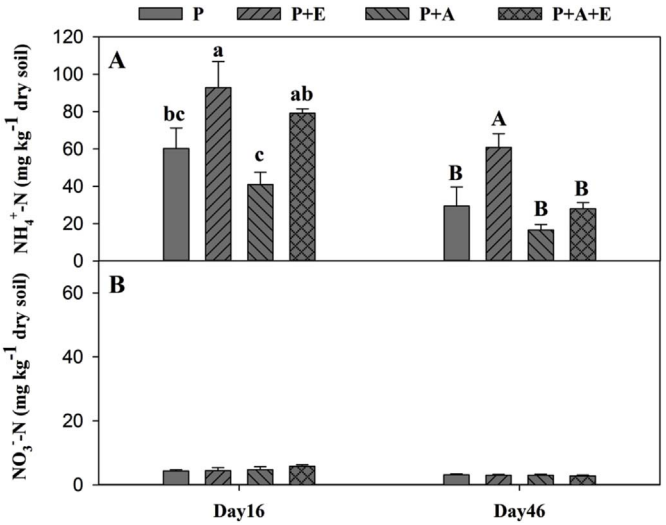


**Fig. 2.** The soil N-ammonia and N-nitrate amount. Means with standard error ($n$ = 5). Bars with different letters vary significantly (LSD test, $P$ < 0.05).

deionized water to distribute the tracer evenly in the soil.

#### 2.4.2. Sampling and analyses

On day-0, a small amount of soil and plant leaf were sampled from each microcosm to determine the $^{15}N$ atom% before labelling. On day-14, about 10 g soil was sampled per microcosm to measure soil $NH_4^+$-N and $NO_3^-$-N. The experiment lasted for 46 days, with a destructive sampling on 8 February, 2015. The nylon bag was drawn from the microcosm, then soil was separated from the plant by shaking gently. Soil that still adhered to the roots was rinsed off with tap water. Earthworms were collected, and the number of surviving earthworms in each microcosm was recorded. Earthworm biomass was determined by cleaning worms in water and drying them with a paper towel, before weighing them. A part of the soil was kept under 4 °C to measure soil $NH_4^+$-N, $NO_3$-N and soil microbial biomass carbon (C) and N (this was done within 3 days of sampling). Soil $NH_4^+$-N and $NO_3^-$-N were extracted by 2 M KCl (soil:solution ratio of 1:5) and determined with QuikChem® methods 10–107–04–1–A and 10–107–06–2–A on a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA). Microbial biomass C and N (MBC, MBN) were determined using the chloroform fumigation-direct extraction method (Brookes et al., 1985). The leaf, stem, coarse root and fine root of each plant were separately collected. The soil and plant materials were dried and ground for isotopic analysis as described above.

Atom% excess $^{15}N$ of each plant part was calculated as the atom% $^{15}N$ difference between samples before labeling and samples collected at the end of the experiment. Atom% excess $^{15}N$ ($APE_T$) of the $^{15}N$ tracer was calculated as the atom% $^{15}N$ of difference between the tracer and the background value in soil samples collected before labeling. $Biomass_S$ was the biomass of the plant samples. $Weight_T$ was the weight of the tracer applied in each pot. $\%N_S$ and $\%N_T$ was the N concentration of the sample and tracer, respectively. Recovery of the $^{15}NH_4{}^{15}NO_3$ in plant tissues can be used to estimate how much N plants took up during the labeling period. Recovery of the $^{15}N$ tracer in leaf, stem coarse root and fine root were estimated by calculating as:

$$\%Recovery_S = (Biomass_S \times \%N_S / 100 \times APE_S / 100) / (Weight_T \times \%N_T / 100 \times APE_T / 100) \times 100.$$

Recovery of the $^{15}N$ tracer in the total plant biomass was the sum of the $^{15}N$ tracer recovered in each plant part.

Mycorrhizal colonizations in the roots of *D. dichotoma* were measured by the method of McGonigle et al. (1990). The percentages of roots colonized by mycorrhizal hyphae, arbuscules and vesicles were determined using a microscope (AX70, Olympus) under 200 x magnification.

**Table 1**
Effects of earthworm (E), AMF (A) and their interaction on soil $NH_4^+$-N and $NO_3^-$-N amount on two sampling times (T), which were estimated by repeated-measures ANOVA.

| | $NH_4^+$-N | | $NO_3^-$-N | |
|---|---|---|---|---|
| | $F(1,16)$ | $P$ | $F(1,16)$ | $P$ |
| Between subjects | | | | |
| E | 18.33 | **0.001↑** | 0.297 | 0.594 |
| A | 8.75 | **0.009↓** | 0.94 | 0.347 |
| E*A | 0.30 | 0.591 | 0.30 | 0.592 |
| Within subjects | | | | |
| T | 49.75 | **< 0.001** | 21.04 | **< 0.001** |
| E*T | 2.03 | 0.174 | 0.868 | 0.365 |
| A*T | 0.415 | 0.529 | 1.828 | 0.195 |
| E*A*T | 1.712 | 0.209 | 0.468 | 0.504 |

'↑' indicates there was an increase when earthworms or AMF were inoculated.
'↓' indicates there was a reduction when earthworms or AMF were inoculated.
The $P$ values which are smaller than 0.1 have been shown in bold.

### 2.5. Statistical analyses

For the excised root experiment, because $^{15}N$ uptake kinetics appeared linear with tracer concentration, we calculated the first order rate constant $k$ (uptake rate = a + $k$ * [N-tracer type]) by performing linear regression for each tracer type in which **'$^{15}$N uptake rates'** and **'Tracer Concentration'** were chosen as dependent variable and independent variable, respectively. Uptake preference was assessed by comparing k values, with a higher value indicating a greater preference for that N-form. However, k values were compared indirectly by analysis of covariance (ANCOVA) in which **'Tracer Type'** and **'Tracer concentration'** were chosen as fix factor and covariate factor, respectively; 'Least Significant Difference (LSD)' test was used to compare the differences of estimated marginal means between tracer types.

For the field *in situ* experiment, the effect of **'Tracer Type'** on $^{15}N$ uptake rates of root were determined by a generalized linear model for each sampling time (3 or 24 h after labeling), in which "linear model" and "wald chi square test" were chosen, and post hoc contrasts were conducted with LSD test.

For the microcosm experiment, two-way analysis of variance (ANOVA) was performed to analyze the effects of **'Earthworm'**, **'AMF'** and their interaction on the following variables: root AMF colonization, plant biomass, the plant N amount, the $^{15}N$ tracer recovery in plants, MBC, and MBN. Residual normality and homoscedasticity were verified using Kolmogorov-Smirnov and Bartlett tests, respectively. Log10 transformation was used for variables to meet normality and homogeneity assumption if needed. Soil $NH_4^+$-N and $NO_3^-$-N were analyzed using repeated-measures ANOVA with 'Sampling Time (sampling at day 16 and 46)' as repeated factor and **'Earthworm'**, **'AMF'** as factors. The effects of **'Earthworm'**, **'AMF'** and their interactions on MBC/MBN ratio were determined by generalized linear model, in which "Gamma model" and "wald chi square test" were chosen. Multiple comparison of means were determined by LSD test. To test the effects of **'AMF'** on earthworm growth (earthworm number and biomass), two-tailed $t$-tests were made between treatments P + E and P + A + E.

Unless otherwise stated, results are shown as mean ± standard error. All statistics were performed in IBM SPSS Statistics 20 (IBM Corporation, New York, USA).

## 3. Results

### 3.1. The preference of the fern D. dichotoma for $NH_4^+$ vs. $NO_3^-$

In the excised root experiment, there was no significant difference

**Table 2**
Effects of earthworm (E), AMF (A) and their interaction on soil microbial biomass C (MBC), microbial biomass N (MBN) and the ratio of MBC to MBN, which were estimated by ANOVA or generalized linear model.

| | [a] MBC | | [a] MBN | | [b] MBC/MBN | |
|---|---|---|---|---|---|---|
| | $F$ (1, 16) | $P$ | $F$ (1, 16) | $P$ | *Wald* $\chi^2$ (1) | $P$ |
| E | 1.023 | 0.325 | 0.00004 | 0.985 | 0.837 | 0.36 |
| A | 3.632 | **0.075**↑ | 14.347 | **< 0.001**↑ | 18.219 | **< 0.0001**↓ |
| E*A | 0.907 | 0.355 | 0.008 | 0.928 | 3.028 | **0.082** |

'↑' indicates there was an increase when earthworms or AMF were inoculated.
'↓' indicates there was a reduction when earthworms or AMF were inoculated.
The $P$ values which are smaller than 0.1 have been shown in bold.
[a] indicates the variable was estimated by ANOVA.
[b] indicates the variable was estimated by generalized linear model.

between k value for $NH_4^+$ with or without DCD ($P$ = 0.091, Table S1, Fig. 1A), suggesting there was limited nitrification during the assay. However, the k value for $NH_4^+$ uptake was much higher than that for $NO_3^-$ ($P$ < 0.05, Table S1, Fig. 1A), indicating that the fern "prefers" $NH_4^+$ over $NO_3^+$.

The field experiment showed the same preference trend as did the excised root study: $^{15}N$ was taken up faster from $^{15}NH_4^+$ than from $^{15}NO_3^-$. This was true at each sampling time (3 h and 24 h; $P$ < 0.05, Fig. 1B). DCD did not change the $^{15}N$ uptake patterns significantly ($P$ = 0.38, $P$ = 0.79, after 3 h and 24 h, respectively).

### 3.2. Which form of inorganic N the earthworms tends to stimulate and the AMF tend to take up

For the microcosm experiment, the earthworm addition and AMF inocula application treatments were successful. An average of 4.6 ± 0.45 earthworms survived in each microcosm, and individual fresh body weight increased to 111 ± 4.7% of the initial fresh body weight. Neither the number nor the biomass of surviving earthworms was affected by AMF ($P$ > 0.1, Fig. S1). AMF inoculation increased the average percentage of mycorrhizal colonization of roots (increasing from 23% ± 0.6% without inoculation to 76% ± 0.9%). However, earthworms, and interaction of earthworms and AMF did not affect root AMF colonization ($P$ > 0.1, Fig. S2).

$NH_4^+$ was the predominant available N form on both sampling dates; $NO_3^-$ was generally less than one tenth of soil $NH_4^+$ concentrations (Fig. 2). Earthworms significantly increased the concentration of soil $NH_4^+$ ($F_{1,16}$ = 18.33, $P$ = 0.001, Table 1, Fig. 2A), but had no significant effect on soil $NO_3^-$ concentration ($F_{1,16}$ = 0.297, $P$ = 0.594, Table 1, Fig. 2B). AMF decreased soil $NH_4^+$ significantly when earthworms were present ($F_{1,16}$ = 8.76, $P$ = 0.009, Table 1, Fig. 2A), but did not change the soil $NO_3^-$ ($F_{1,16}$ = 0.94, $P$ = 0.347, Table 1, Fig. 2B).

### 3.3. Soil microbial C and N pool

AMF inoculation increased the amount of soil microbial biomass N (MBN) to 3.3 fold of that without AMF inoculation ($F_{1,16}$ = 14.347, $P$ < 0.001, Table 2, Fig. 3B), but the earthworms did not exert a significant effect ($F_{1,16}$ = 0.00004, $P$ = 0.985, Table 2, Fig. 3B). The soil microbial biomass C (MBC) pool also tend to increase when AMF inocula were applied, though the differences between treatments were not significant ($F_{1,16}$ = 3.632, $P$ = 0.075, Table 2, Fig. 3A). Furthermore, the ratio of MBC to MBN decreased when the AMF inocula were applied ($F_{1,16}$ = 18.219, $P$ < 0.001, Table 2, Fig. 3C).

### 3.4. Plant growth and nitrogen uptake

Plant shoot, root, total biomass and shoot/root biomass ratio were not significantly affected by earthworms, AMF, or their interactions (Table 3). Although not significant, AMF tended to increase coarse root biomass ($F_{1,16}$ = 3.26, $P$ = 0.09), interaction between earthworms and AMF tended to increase total shoot biomass ($F_{1,16}$ = 3.073, $P$ = 0.1, Table 3).

AMF increased the N concentration of leaves significantly ($F_{1,16}$ = 12.43, $P$ = 0.003) and coarse roots at marginally significant level ($F_{1,16}$ = 3.99, $P$ = 0.06, Table 4). Meanwhile, the C/N ratios of leaves and coarse roots were decreased by AMF ($F_{1,16}$ = 14.337, $P$ = 0.002 and $F_{1,16}$ = 5.936, $P$ = 0.027, respectively, Table 4). In addition, earthworm presence increased the N concentration of fine roots ($F_{1,16}$ = 5.07, $P$ = 0.04) and then decreased the C/N ratio significantly ($F_{1,16}$ = 5.224, $P$ = 0.039, Table 4). Although not significant, interaction between AMF and earthworms tended to increase fine roots N concentration ($F_{1,16}$ = 4.083, $P$ = 0.06) and decreased their C/N ratio ($F_{1,16}$ = 3.387, $P$ = 0.084, Table 4).

Two-way ANOVA results showed that AMF significantly affected the N amount in shoots ($F_{1,16}$ = 6.547, $P$ = 0.021) and in total plant biomass ($F_{1,16}$ = 7.022, $P$ = 0.017). Although not significant, interaction between AMF and earthworms tended to increase it in shoot ($F_{1,16}$ = 3.305, $P$ = 0.088) and in total plant biomass ($F_{1,16}$ = 3.087, $P$ = 0.098, Table 5, Fig. 4A). Further, one-way ANOVA results showed that the N amount in shoots and in total plant biomass increased only when both AMF and earthworms were inoculated (Fig. 4A). Similarly, the two-way ANOVA results showed that AMF significantly affected the $^{15}N$ recovery in shoots ($F_{1,16}$ = 4.78, $P$ = 0.044) and in total plant biomass ($F_{1,16}$ = 7.099, $P$ = 0.017), while interaction between AMF and earthworms affected it in shoot significantly ($F_{1,16}$ = 6.161, $P$ = 0.025) and in total plant biomass at marginally significant level ($F_{1,16}$ = 4.417, $P$ = 0.052, Table 5). One-way ANOVA results further suggested that the $^{15}N$ recovery in shoot and in total plant biomass

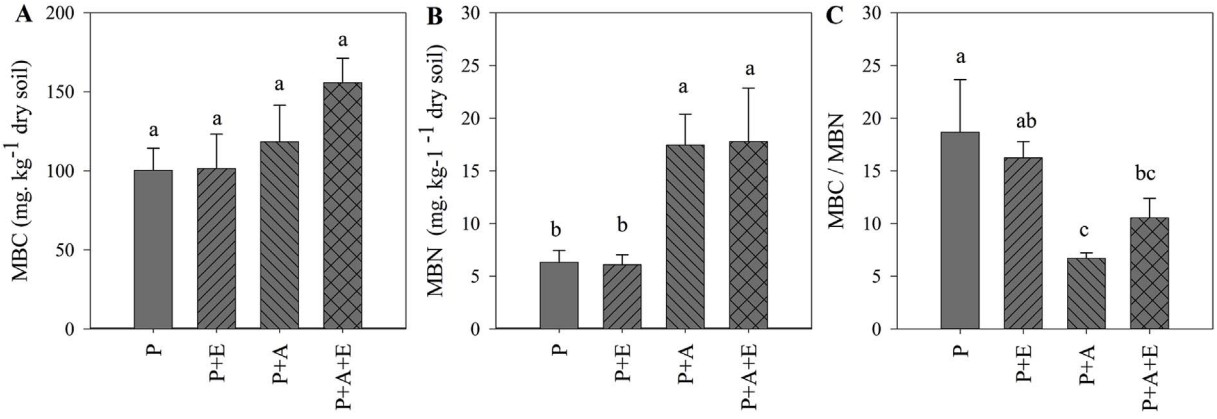


**Fig. 3.** The soil microbial biomass C, N (MBC, MBN) and MBC/MBN. Means with standard error ($n$ = 5). Bars with different letters vary significantly (LSD test, $P$ < 0.05).

**Table 3**
Biomass (g) of leaf, stem, shoot (leaf + stem), coarse root, fine root, root (coarse root + fine root), and total plant, and shoot biomass/root biomass ratio, means with standard error ($n$ = 5).

| | Shoot | | | Root | | | Total biomass | Shoot/root |
|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Total | Coarse root | Fine root | Total | | |
| P | 2.6(0.3) | 2.6(0.3) | 5.2(0.5) | 4.6(0.7) | 9.0(0.7) | 13.6(1.2) | 18.8(1.5) | 0.38(0.04) |
| P+E | 1.9(0.4) | 2.6(0.4) | 4.3(0.8) | 4.8(0.5) | 7.6(1.0) | 12.4(0.9) | 16.7(1.4) | 0.35(0.07) |
| P+A | 2.5(0.5) | 2.4(0.3) | 4.9(0.6) | 5.0(0.2) | 7.5(0.9) | 12.5(0.7) | 17.5(0.6) | 0.41(0.07) |
| P+A+E | 3.0(0.6) | 4.1(0.8) | 7.1(1.3) | 6.2(0.5) | 6.4(0.8) | 12.6(1.2) | 19.7(2.1) | 0.56(0.10) |
| ANOVA: full factorial, †, * | | | | | | | | |
| E | 0.83ns | 0.11ns | 0.78ns | 0.17ns | 0.14ns | 0.59ns | 0.96ns | 0.40ns |
| A | 0.23ns | 0.21ns | 0.18ns | **0.09†** | 0.12ns | 0.66ns | 0.59ns | 0.11ns |
| A × E | 0.19ns | 0.12ns | 0.100 | 0.34ns | 0.86ns | 0.55ns | 0.18ns | 0.19ns |

Levels of significance for ANOVA: '†' < 0.1; '∗' < 0.05; '∗∗' < 0.01; 'ns' not significant.
The *P* values which are smaller than 0.1 have been shown in bold.

increased only when both AMF inocula and earthworms were applied (Fig. 4B). Earthworms alone did not affect either the N amount or the $^{15}N$ recovery in plants (Table 5, Fig. 4.).

## 4. Discussion

In the present experiments, the effects of both the earthworms and AMF on soil inorganic N accumulation and/or uptake by the fern primarily relied on their influences on $NH_4^+$-N related processes. The exotic earthworm *P. corethrurus* increased soil $NH_4^+$ concentration but did not affect that of soil $NO_3^-$. The fern preferred to take up $NH_4^+$ over $NO_3^-$, but could not efficiently utilize the earthworm-induced soil $NH_4^+$ without the help of AMF. As a result of enhanced $NH_4^+$ production with earthworms and the $NH_4^+$-preference by the fern and the associated AMF, the combination of both organisms substantially increased N-acquisition of the fern.

The excised root experiment showed that the physiological uptake capacity of the fern *D. dichotoma* for $NH_4^+$ was larger than for $NO_3^-$; the field experiment reinforced the conclusion that *D. dichotoma* uses more $NH_4^+$ than $NO_3^-$ under *in situ* conditions. Our results thus agree with the general pattern that plants adapted to low pH and reducing soil conditions tend to take up $NH_4^+$ (Miller and Cramer, 2005).

Meanwhile, the earthworm *P. corethrurus* increased soil $NH_4^+$-N supply considerably. The fact that earthworms increased soil $NH_4^+$ and $NO_3^-$ to different extents has been reported previously (e.g. Blair et al., 1997; Domínguez et al., 2004; Lubbers et al., 2011). In this study, the dominance of $NH_4^+$ and the tendency of exotic earthworms to stimulate soil $NH_4^+$ may be explained by soil conditions and earthworm species characteristics. First, soil $NH_4^+$ is produced by mineralization of organic N (Booth et al., 2005), and earthworms can accelerate this process. We detected enhanced activity of the soil enzyme β-*N*-acetylglucosaminidase by the exotic earthworms (unpublished data), which can indicate that earthworms stimulated N mineralization (Dick, 2011). We found relatively little nitrification (Fig. 1); this is most likely explained by the soil's low pH (3.7), which is known to repress nitrification (Sahrawat, 1982; Ste-Marie and Pare, 1999). However, the soil clay content was also high (38.5%) and we maintained the microcosms at a relatively high moisture (at or above field capacity); this could create anaerobic conditions (Lavelle et al., 1987), which would suppress nitrification and stimulate denitrification, each of which would reduce soil $NO_3^-$ (Robertson, 1989). Furthermore, earthworms can either be regarded as compacting species or decompacting species, due to their different influences on soil porosity (Blanchart et al., 2004, 1999; Blouin et al., 2007); *P. corethrurus* is a compacting species, which could enhance anaerobiosis (Chauvel et al., 1999). So, the combination of low pH, high soil moisture, and the earthworm species we introduced may all have stimulated $NH_4^+$ accumulation. An alternative mechanism that earthworm may increase soil $NH_4^+$ accumulation was the input of N from dead earthworms. In the present experiment, on average 3.4 earthworm individuals died per microcosm, which was equal to 18.6 mg N input (Table S2). However, a previous study showed that only 4–7% of the N from earthworm tissues was transformed to mineral N (Whalen et al., 1999). Meanwhile, on average earthworm addition increased 49 and 47 mg $NH_4^+$-N per microcosm in Day16 and 46, respectively. Thus, the dead earthworms derived N may had minor effects (i.e., $< 18.6 \times 7\%/49 \approx 3\%$) on the increased $NH_4^+$ content, which also was supported by the insignificant correlations between the number of dead earthworms and soil $NH_4^+$-N concentration (Fig. S3).

Unexpectedly, the uninoculated fern could not benefit from the increased soil $NH_4^+$ induced by the exotic earthworms. This may result from the low accessibility of the earthworm-induced soil $NH_4^+$ -N. The $NH_4^+$ that accumulates from the activity of the exotic earthworms may not be evenly distributed in soil; rather it likely accumulates in '$NH_4^+$ hotspots,' such as earthworm casts, which are temporally and spatially

**Table 4**
N concentration and C/N ratio of leaf, stem, coarse root, fine root, means with standard error ($n$ = 5). Different letters means that the difference of the means between treatments was significant (LSD test, $P < 0.05$).

| Treatment | N concentration (%) | | | | C/N | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Coarse root | Fine root | Leaf | Stem | Coarse root | Fine root |
| P | 1.21(0.06)a | 0.26(0.05)a | 0.52(0.02)a | 0.38(0.02)a | 40(2.2)ab | 200(30.4)a | 105(6.9)a | 128(5.8)a |
| P+E | 1.14(0.02)a | 0.32(0.09)a | 0.56(0.05)a | 0.39(0.02)a | 42(0.8)a | 190(37.5)a | 94(9.1)b | 125(5.5)a |
| P+A | 1.40(0.06)b | 0.35(0.08)a | 0.64(0.04)a | 0.37(0.02)a | 34(1.4)c | 156(24.2)a | 82(5.5)b | 132(6.4)a |
| P+A+E | 1.33(0.05)b | 0.31(0.04)a | 0.61(0.03)a | 0.45(0.02)b | 36(1.5)b | 166(21.0)a | 85(4.3)b | 109(4.7)b |
| ANOVA: full factorial, †, *, ** | | | | | | | | |
| E | 0.22ns | 0.94ns | 0.9ns | **0.04*** | 0.17ns | 0.99ns | 0.53ns | **0.039*** |
| A | **0.003**** | 0.55ns | **0.06†** | 0.17ns | **0.002*** | 0.26ns | **0.027*** | 0.17ns |
| A × E | 0.99ns | 0.49ns | 0.45ns | **0.06†** | 0.91ns | 0.74ns | 0.36ns | **0.084†** |

Levels of significance for ANOVA: '†' < 0.1; '∗' < 0.05; '∗∗' < 0.01; 'ns' not significant.
The *P* values which are smaller than 0.1 have been shown in bold.

**Table 5**
Two-way ANOVA table showing *F*-values for the effects of earthworm (E), AMF (A) and their interaction on amount of N and the recovery of the $^{15}N$ tracer in shoot, root, and the total plant, and shoot/root ratio, respectively.

| Dependent variable | Independent variable | df | *F*-value | *P*-value |
|---|---|---|---|---|
| **Total N amount:** | | | | |
| Shoot | E | 1,16 | 0.256 | 0.62 |
| | **A** | **1,16** | **6.547** | **0.021** |
| | **A×E** | **1,16** | **3.305** | **0.088** |
| Root | E | 1,16 | 0.173 | 0.683 |
| | A | 1,16 | 1.316 | 0.268 |
| | A×E | 1,16 | 0.669 | 0.426 |
| total | E | 1,16 | 0.091 | 0.767 |
| | **A** | **1,16** | **7.022** | **0.017** |
| | **A×E** | **1,16** | **3.087** | **0.098** |
| shoot/root | E | 1,16 | 0.227 | 0.64 |
| | A | 1,16 | 2.093 | 0.167 |
| | A×E | 1,16 | 1.015 | 0.329 |
| **The $^{15}N$ tracer recovery:** | | | | |
| Shoot | E | 1,16 | 2.98 | 0.104 |
| | **A** | **1,16** | **4.78** | **0.044** |
| | **A×E** | **1,16** | **6.161** | **0.025** |
| Root | E | 1,16 | 0.348 | 0.564 |
| | A | 1,16 | 2.926 | 0.106 |
| | A×E | 1,16 | 1.604 | 0.223 |
| Total | E | 1,16 | 1.208 | 0.288 |
| | **A** | **1,16** | **7.099** | **0.017** |
| | **A×E** | **1,16** | **4.417** | **0.052** |
| shoot/root | E | 1,16 | 0.044 | 0.837 |
| | A | 1,16 | 0.136 | 0.717 |
| | A×E | 1,16 | 0.999 | 0.332 |

The *P* values which are smaller than 0.1 have been shown in bold.

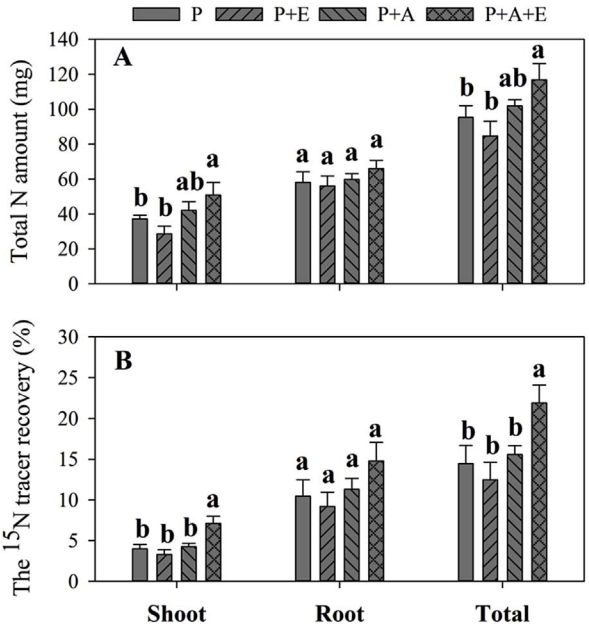


**Fig. 4.** The total amount of N and $^{15}N$ tracer recovery in the shoot, root, and the total biomass of the plant, and the shoot/root ratio. Means with standard error ($n = 5$). Bars with different letters vary significantly (LSD test, $P < 0.05$).

variable (Lavelle et al., 1992). Such heterogeneity may be driven particularly by endogeic earthworms such as *P. corethrurus* because they feed on soil organic matter and dig impermanent burrows (Lavelle, 1988). N mineralization in soil microsites can profoundly influence N cycling in the whole soil (Schimel and Bennett, 2004). The roots of the fern may fail to respond rapidly enough to forage N in hotspots, such as those created by burrowing earthworms. This might explain why the exotic earthworms did not increase plant N uptake in treatments without AMF inoculation.

However, the earthworm induced soil $NH_4^+$-N can be utilized efficiently by the fern with AMF inoculation. AMF was reported to prefer $NH_4^+$ over $NO_3^-$ (Cheng et al., 2012; Jin et al., 2005; Toussaint et al., 2004) and can transport N to host plants (Fellbaum et al., 2012). AMF hyphae are at least three orders of magnitude thinner than roots (Smith and Read, 2010), can extend more than 10 cm beyond the root surface (Cavagnaro et al., 2015, 2005), and have short lifespans (5–7 days; Treseder and Allen, 2000); this allows AMF to colonize hotspots quickly and extensively. In the present experiment, AMF inoculation decreased soil $NH_4^+$ (sampled at Day 46) by 34 mg N $kg^{-1}$ dw soil when earthworms were present. Moreover, AMF inoculation also increased microbial biomass N by 11 mg N $kg^{-1}$ dw soil (16.5 mg N per microcosm), and reduced the ratio of microbial biomass C/N. Although the initially introduced AMF inocula may theoretically contributes to the increased MBN, however, our present data partially indicated that the initial N input from AMF inocula was not the main source. Assuming that all the increased MBC induced by AMF inoculation (54 mg) was derived from the AMF inocula, then the associated input of MBN was 5.4 mg per microcosm (C:N of the AMF *R. intraradices* was 10:1 according to Larsen et al., 2008). Therefore, we considered that at least the extra 11 mg increased MBN per microcosm may primarily result from the increased uptake of $NH_4^+$-N rather than from the added biomass N of AMF spores and hyphae.

Note that AMF increased plant total N uptake only when soil $NH_4^+$ concentration was increased by the exotic earthworms. AMF alone increased N concentrations in plant leaves and coarse roots, but did not affect total plant N uptake. This implies that the amount of N transferred to the host plant was controlled by soil $NH_4^+$ supply. Furthermore, given that AMF may require a lot of N to support their growth and activity (Corrêa et al., 2015), thus, AMF may compete with their plant hosts for N (Eisenhauer et al., 2009; Wurst et al., 2004), especially when soil $NH_4^+$ supply is low. Disturbance or feeding on fungi mycelia and spores by earthworms did not affect root AMF colonization, thus this effect of earthworms may have had minimal influence on AMF mediated plant N-uptake. Although earthworms may selectively feed on fungal mycelia in some case (Bonkowski et al., 2000), the exotic earthworm *P. corethrurus* may not do this due to its geophagous feeding behavior.

Individually, the AMF and the exotic earthworms each had minor impacts on plant N uptake. Acting together, however, they stimulated plant N-uptake substantially. The mechanism that N forms mediate earthworm-AMF interaction on plant N uptake could be a general mechanism for explaining other soil fauna-AMF interactions on plant N uptake. In plantations that dominated by the fern *D. dichotoma* and the exotic earthworm *P. corethrurus*, the application of the AMF inocula may enhance plant N uptake, while reducing the risk of N loss caused by the invasion of the exotic earthworms.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.10.038.

## References


Araujo, Y., Luizão, F.J., Barros, E., 2004. Effect of earthworm addition on soil nitrogen availability, microbial biomass and litter decomposition in mesocosms. Biology and Fertility of Soils 39, 146–152.

Barot, S., Ugolini, A., Brikci, F.B., 2007. Nutrient cycling efficiency explains the long-term effect of ecosystem engineers on primary production. Functional Ecology 21, 1–10.

Blair, J.M., Parmelee, R.W., Allen, M.F., McCartney, D.A., Stinner, B.R., 1997. Changes in soil N pools in response to earthworm population manipulations in agroecosystems with different N sources. Soil Biology & Biochemistry 29, 361–367.

Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Gilot, C., Pashanasi, B., Lavelle, P., Brussaard, L., 1999. Effects of earthworms on soil structure and physical properties. Earthworm Management in Tropical Agroecosystems 5, 149–171.

Blanchart, E., Albrecht, A., Brown, G., Decaens, T., Duboisset, A., Lavelle, P., Mariani, L., Roose, E., 2004. Effects of tropical endogeic earthworms on soil erosion. Agriculture Ecosystems and Environment 104, 303–315.

Blouin, M., Barot, S., Lavelle, P., 2006. Earthworms (*Millsonia anomala*, Megascolecidae) do not increase rice growth through enhanced nitrogen mineralization. Soil Biology & Biochemistry 38, 2063–2068.

Blouin, M., Lavelle, P., Laffray, D., 2007. Drought stress in rice (*Oryza sativa* L.) is enhanced in the presence of the compacting earthworm *Millsonia anomala*. Environmental and Experimental Botany 60, 352–359.

Bonkowski, M., Griffiths, B.S., Ritz, K., 2000. Food preferences of earthworms for soil fungi. Pedobiologia 44, 666–676.

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs 75, 139–157.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837–842.

Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant growth: burrowing into the mechanisms. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, pp. 13–49.

Cao, J., Wang, C., Ji, D., 2016. Improvement of the soil nitrogen content and maize growth by earthworms and arbuscular mycorrhizal fungi in soils polluted by oxytetracycline. Science of the Total Environment 571, 926–934.

Cavagnaro, T.R., Bender, S.F., Asghari, H.R., van der Heijden, M.G.A., 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science 20, 283–290.

Cavagnaro, T.R., Smith, F.A., Smith, S.E., Jakobsen, I., 2005. Functional diversity in arbuscular mycorrhizas: exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell and Environment 28, 642–650.

Chapin III, F.S., Matson, P.A., Vitousek, P., 2011. Principles of Terrestrial Ecosystem Ecology. Springer, New York, USA 385–361.

Chauvel, A., Grimaldi, M., Barros, E., Blanchart, E., Desjardins, T., Sarrazin, M., Lavelle, P., 1999. Pasture damage by an Amazonian earthworm. Nature 398, 32–33.

Cheng, L., Booker, F.L., Tu, C., Burkey, K.O., Zhou, L., Shew, H.D., Rufty, T.W., Hu, S., 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated $CO_2$. Science 337, 1084–1087.

Corrêa, A., Cruz, C., Ferrol, N., 2015. Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza 25, 499–515.

Dick, R.P. (Ed.), 2011. Methods of Soil Enzymology. Soil Science Society of America, pp. 211–233 Madison, Wisconsin, USA.

Domínguez, J., Bohlen, P.J., Parmelee, R.W., 2004. Earthworms increase nitrogen leaching to greater soil depths in row crop agroecosystems. Ecosystems 7, 672–685.

Du, J., Yang, X., Zhang, H., Yu, G., 2008. Quantitative distribution of earthworms and its relationships with environmental factors in tropical secondary forest and rubber plantation in Xishuangbanna. Shengtaixue Zazhi 27, 1941–1947.

Eisenhauer, N., König, S., Sabais, A.C., Renker, C., Buscot, F., Scheu, S., 2009. Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. Soil Biology & Biochemistry 41, 561–567.

FAO, 2006. World Reference Base for Soil Resources 2006. World Soil Resources Report 103. FAO, Rome.

Fellbaum, C.R., Gachomo, E.W., Beesetty, Y., Choudhari, S., Strahan, G.D., Pfeffer, P.E., Kiers, E.T., Buecking, H., 2012. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences of the United States of America 109, 2666–2671.

Fitter, A.H., Garbaye, J., 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant and Soil 159, 123–132.

Gao, B., Zhang, W., Liu, S., Shao, Y., Xiong, Y., Zhou, C., Fu, S.-L., 2010. Short-term impacts of *Ocnerodrilus occidentalis* and *Evodia lepta* on soil $CO_2$ fluxes in an *Acacia auriculaeformis* plantation in Guangdong Province, China. Chinese Journal of Plant Ecology 34, 1243–1253.

Gao, J., Zhao, P., Shen, W., Rao, X., Hu, Y., 2017. Physiological homeostasis and morphological plasticity of two tree species subjected to precipitation seasonal distribution changes. Perspectives in Plant Ecology, Evolution and Systematics 25, 1–19.

González, G., Huang, C.Y., Zou, X., Rodriguez, C., 2006. Earthworm invasions in the tropics. Biological Invasions 8, 1247–1256.

Hale, C.M., Frelich, L.E., Reich, P.B., Pastor, J., 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155, 509–518.

Hendrix, P.F., Callaham Jr., M.A., Drake, J.M., Huang, C.-Y., James, S.W., Snyder, B.A., Zhang, W., 2008. Pandora's box contained bait: the global problem of introduced earthworms. Annual Review of Ecology Evolution and Systematics 39, 593–613.

Hodge, A., Storer, K., 2015. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant and Soil 386, 1–19.

Huang, J., Zhang, W., Liu, M., Briones, M.J.I., Eisenhauer, N., Shao, Y., Cai, X.a., Fu, S., Xia, H., 2015. Different impacts of native and exotic earthworms on rhizodeposit carbon sequestration in a subtropical soil. Soil Biology & Biochemistry 90, 152–160.

Jin, H., Liu, J., Liu, J., Huang, X., 2012. Forms of nitrogen uptake, translocation, and transfer via arbuscular mycorrhizal fungi: a review. Science China-Life Sciences 55, 474–482.

Jin, H., Pfeffer, P.E., Douds, D.D., Piotrowski, E., Lammers, P.J., Shachar-Hill, Y., 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytologist 168, 687–696.

Larsen, J., Johansen, A., Larsen, S.E., Heckmann, L.H., Jakobsen, I., Krogh, P.H., 2008. Population performance of collembolans feeding on soil fungi from different ecological niches. Soil Biology & Biochemistry 40, 360–369.

Lavelle, P., 1988. Earthworm activities and the soil system. Biology and Fertility of Soils 6, 237–251.

Lavelle, P., Barois, I., Cruz, I., Fragoso, C., Hernandez, A., Pineda, A., Rangel, P., 1987. Adaptive strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. Biology and Fertility of Soils 5, 188–194.

Lavelle, P., Melendez, G., Pashanasi, B., Schaefer, R., 1992. Nitrogen mineralization and reorganization in casts of the geophagous tropical earthworm *Pontoscolex corethrurus* (Glossoscolecidae). Biology and Fertility of Soils 14, 49–53.

Lavelle, P., Decaens, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology 42, S3–S15.

Lawrence, B., Fisk, M.C., Fahey, T.J., Suarez, E.R., 2003. Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytologist 157, 145–153.

Li, H., Wang, C., Li, X., Christie, P., Dou, Z., Zhang, J., Xiang, D., 2013. Impact of the earthworm *Aporrectodea trapezoides* and the arbuscular mycorrhizal fungus *Glomus intraradices* on N-15 uptake by maize from wheat straw. Biology and Fertility of Soils 49, 263–271.

Liu, Z., Wu, J., Zhou, L., Lin, Y., Fu, S., 2012. Effect of understory fern (*Dicranopteris dichotoma*) removal on substrate utilization patterns of culturable soil bacterial communities in subtropical *Eucalyptus* plantations. Pedobiologia 55, 7–13.

Lubbers, I.M., Brussaard, L., Otten, W., van Groenigen, J.W., 2011. Earthworm-induced N mineralization in fertilized grassland increases both $N_2O$ emission and crop-N uptake. European Journal of Soil Science 62, 152–161.

McGeough, K.L., Watson, C.J., Müeller, C., Laughlin, R.J., Chadwick, D.R., 2016. Evidence that the efficacy of the nitrification inhibitor dicyandiamide (DCD) is affected by soil properties in UK soils. Soil Biology & Biochemistry 94, 222–232.

McGonigle, T., Miller, M., Evans, D., Fairchild, G., Swan, J., 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytologist 115, 495–501.

Maathuis, F.J.M., 2009. Physiological functions of mineral macronutrients. Current Opinion in Plant Biology 12, 250–258.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany 105, 1141–1157.

Miller, A., Cramer, M., 2005. Root Nitrogen Acquisition and Assimilation, Root Physiology: from Gene to Function. Springer, New York, USA, pp. 1–36.

Nuzzo, V.A., Maerz, J.C., Blossey, B., 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conservation Biology 23, 966–974.

Paudel, S., Longcore, T., MacDonald, B., McCormick, M.K., Szlavecz, K., Wilson, G.W.T., Loss, S.R., 2016. Belowground interactions with aboveground consequences: invasive earthworms and arbuscular mycorrhizal fungi. Ecology 97, 605–614.

Perez-Tienda, J., Valderas, A., Camanes, G., Garcia-Agustin, P., Ferrol, N., 2012. Kinetics of $NH_4^+$ uptake by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Mycorrhiza 22, 485–491.

Reeves, D., Touchton, J., 1986. Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn, and grain sorghum. Soil Science Society of America Journal 50, 1353–1357.

Robertson, G., 1989. Nitrification and denitrification in humid tropical ecosystems: potential controls on nitrogen retention. In: Proctor, J. (Ed.), Mineral nutrients in Tropical Forest and Savanna Ecosystems. Blackwell Scientific, Cambridge, Massachusetts, USA, pp. 55–69.

Rothstein, D.E., Zak, D.R., Pregitzer, K.S., Curtis, P.S., 2000. Kinetics of nitrogen uptake by *Populus tremuloides* in relation to atmospheric $CO_2$ and soil nitrogen availability. Tree Physiology 20, 265–270.

Sahrawat, K., 1982. Nitrification in some tropical soils. Plant and Soil 65, 281–286.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.

Smith, S.E., Read, D.J., 2010. Mycorrhizal Symbiosis. Academic press, Cambridge, UK, pp. 66–99.

Ste-Marie, C., Paré, D., 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biology & Biochemistry 31, 1579–1589.

Tanaka, Y., Yano, K., 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell and Environment 28, 1247–1254.

Thirkell, T.J., Cameron, D.D., Hodge, A., 2016. Resolving the 'nitrogen paradox' of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. Plant Cell and Environment 39, 1683–1690.

Toussaint, J.P., St-Arnaud, M., Charest, C., 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an in vitro compartmented system. Canadian

Journal of Microbiology 50, 251–260.
Treseder, K.K., Allen, M.F., 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated $CO_2$ and nitrogen deposition. New Phytologist 147, 189–200.
Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13, 87–115.
Wall, D.H., Bardgett, R.D., 2012. Soil Ecology and Ecosystem Services. Oxford University Press, Oxford, UK.
Wan, S., Zhang, C., Chen, Y., Zhao, J., Wang, X., Wu, J., Zhou, L., Lin, Y., Liu, Z., Fu, S., 2014. The understory fern *Dicranopteris dichotoma* facilitates the overstory *Eucalyptus* trees in subtropical plantations. Ecosphere 5 (5), 1–12.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Whalen, J.K., Parmelee, R., McCartney, D.A., Vanarsdale, J.L., 1999. Movement of N from decomposing earthworm tissue to soil, microbial and plant N pools. Soil Biology & Biochemistry 31, 487–492.
Wu, J., Liu, Z., Wang, X., Sun, Y., Zhou, L., Lin, Y., Fu, S., 2011. Effects of understory removal and tree girdling on soil microbial community composition and litter decomposition in two *Eucalyptus* plantations in South China. Functional Ecology 25, 921–931.
Wurst, S., Dugassa-Gobena, D., Langel, R., Bonkowski, M., Scheu, S., 2004. Combined effects of earthworms and vesicular-arbuscular mycorrhizas on plant and aphid performance. New Phytologist 163, 169–176.
Xu, X., Li, Q., Wang, J., Zhang, L., Tian, S., Zhi, L., Li, Q., Sun, Y., 2014. Inorganic and organic nitrogen acquisition by a fern *Dicranopteris dichotoma* in a subtropical forest in South China. PLoS One 9 (5), e90075.
Zhang, M., Zou, X., Schaefer, D.A., 2010. Alteration of soil labile organic carbon by invasive earthworms (*Pontoscolex corethrurus*) in tropical rubber plantations. European Journal of Soil Biology 46, 74–79.
Zhao, J., Wan, S., Fu, S., Wang, X., Wang, M., Liang, C., Chen, Y., Zhu, X., 2013. Effects of understory removal and nitrogen fertilization on soil microbial communities in *Eucalyptus* plantations. Forest Ecology and Management 310, 80–86.
Zhao, J., Wan, S., Li, Z.a., Shao, Y., Xu, G., Liu, Z., Zhou, L., Fu, S., 2012. *Dicranopteris*-dominated understory as major driver of intensive forest ecosystem in humid subtropical and tropical region. Soil Biology & Biochemistry 49, 78–87.
Zheng, H., Chen, F., Ouyang, Z., Tu, N., Xu, W., Wang, X., Miao, H., Li, X., Tian, Y., 2008. Impacts of reforestation approaches on runoff control in the hilly red soil region of Southern China. Journal of Hydrology 356, 174–184.