



# Ants can exert a diverse effect on soil carbon and nitrogen pools in a Xishuangbanna tropical forest



Shaojun Wang<sup>a, b, \*</sup>, Hong Wang<sup>a</sup>, Jihang Li<sup>a</sup>, Zhe Zhang<sup>a</sup>

<sup>a</sup> Department of Environmental Science and Engineering, Southwest Forestry University, 300 Bailongsi, Kunming 650224, PR China

<sup>b</sup> Co-Innovation Center for Sustainable Forestry in Southern China, 159 Longpan Road, Nanjing 210037, PR China

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## ABSTRACT

Ants are known as important ecosystem engineers for their potentials in modifying the energy flows and nutrient cycles of soil. However, the direction and degree of these modifications vary with ant species and inhabiting environments. In this study, three underground-nesting ants with different feeding-behaviors (*Pheidole capellini* - predominantly honeydew harvester, *Pheidologeton affinis* - scavenger, and *Odontoponera transversa* - predominantly predator) were employed to explore their effects on soil carbon (C) and nitrogen (N) pools in a Xishuangbanna tropical forest in southwestern China. We observed a pronounced effect of ants on components of soil C and N pools, and the effect varied with ant species. Microbial biomass carbon (MBC), total organic C (TOC), total nitrogen (TN) and  $\text{NH}_4^+$  were higher in all ant nests than in the reference soils. However, readily oxidizable organic C (ROC) was only increased in *Ph. capellini* and *O. transversa* nests, dissolved organic nitrogen (DON) in *Ph. affinis* and *O. transversa* nests, and  $\text{NO}_3^-$  in *Ph. affinis* nests. Ants significantly increased spatial variability of C and N pools with the higher values in deeper soil layers compared with reference soil. *Pheidole capellini* nests had the greatest increases of MBC (196.85%), TOC (86.82%) and ROC (68.64%) in 10–15 cm soil depth, whereas there were the highest increase of TN in 10–15 cm soil layer of *O. transversa* nests, DON in 5–10 cm soil layer of *Ph. affinis* nests, and  $\text{NH}_4^+$  in 10–15 cm soil layer of *Ph. capellini* nests. The greatest increase of C pools ( $101.2 \text{ kg ha}^{-1}$  TOC,  $15.49 \text{ kg ha}^{-1}$  MBC, and  $4.89 \text{ kg ha}^{-1}$  ROC) was found in *Ph. capellini* nests, while that of N pools ( $6380 \text{ g ha}^{-1}$  TN,  $110.44 \text{ g ha}^{-1}$  DON,  $128.88 \text{ g ha}^{-1}$   $\text{NH}_4^+$  and  $10.17 \text{ g ha}^{-1}$   $\text{NO}_3^-$ ) was in *Ph. affinis* nests. We conclude that different feeding-behavior ants have a diverse contribution to soil carbon and nitrogen pools in the tropical forest.

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## 1. Introduction

Ants are important components in ecosystems primarily as engineers that affect the flows of energy and nutrients across nearly all types of terrestrial ecosystems (Whitford, 2002; Schumacher, 2011). Most ant species can build corridors and galleries in their nests above- or below-ground (Mikheyev and Tschinkel, 2004; Gosselin et al., 2016). The ability to build biogenic structures is a foundation of soil engineering concept (Bottinelli et al., 2015; Franco, 2015). The processes of nest-building involve the mixing and gathering of soils from different sources, horizons and soil vertical layers, and the transport of organic material from the surroundings into the nests as food or building material. These

activities of ants are crucial for the modification of soil properties and processes (Nkem et al., 2000; Frouz and Jilkova, 2008).

The activities of ant nesting can greatly affect soil physical, chemical, and biological properties, which is closely associated with the size and composition of soil C and N pools (Kilpelainen et al., 2007; Wu et al., 2013). The impacts on physical soil properties, including increases in soil porosity, soil water infiltration in moist or wet conditions, and usually soil temperature, are associated with the building of tunnels or chambers (Cammeraat and Risch, 2008; Chen et al., 2012). These can induce an indirect effect on the formation processes (e.g., decomposition, mineralization and denitrification) of soil C and N pools (Wu et al., 2013). Ants can directly mediate soil chemical changes, characterized mainly by an increase in soil C and N in ant-affected soil (Frouz and Jilkova, 2008). These effects are coincided with the accumulation of food in the nests and the effect on biological processes, such as acceleration of decomposition rate (Stadler et al., 2006). The effects on

\* Corresponding author. Department of Environmental Science and Engineering, Southwest Forestry University, Bailongshi 650224, Kunming, PR China.

E-mail address: [shaojunwang2009@163.com](mailto:shaojunwang2009@163.com) (S. Wang).

biological soil properties may be connected with increased or decreased microbial activity, which is affected by the accumulation of organic matter and internal changes in nest temperature and especially moisture (Holec and Frouz, 2006; Mueller et al., 2011). These effects on the properties and processes in soil vary with different ant species and habitat environments (Frouz and Jilkova, 2008; Wu et al., 2013).

A lot of studies have documented the effects of ants on soil, mainly in European temperate forests, grasslands, agricultural lands and wetlands (Risch et al., 2005; Lane and BassiriRad, 2005; Amador and Gorres, 2007; Wu et al., 2013). Most of these published studies have focused on a few species of harvesting ant, red wood ant, and leaf-cutting ant with above-ground nesting (Wagner et al., 2004; Domisch et al., 2008; Hudson et al., 2009). However, little is known about the effects of underground-nesting ants with different feeding-behavior on soil C and N pools in tropical forests, where high species diversity and abundance occurs.

Xishuangbanna is the main distribution area of tropical forests in China, where ants were found to have high diversity with diverse feeding-behaviors because of diverse food resources and micro-climate (Xu, 1999; Yang et al., 2001). However, there is no study about the effect of these ants on soil properties and processes. In this study, we explored the effects of three underground-nesting ant species with different feeding-behaviors (*Pheidole capellini* - predominantly honeydew harvester, *Pheidologeton affinis* - scavenger, and *Odontoponera transversa* - predominantly predator) on soil C and N pools in a tropical forest of Xishuangbanna (Sonthichai et al., 2006). The main queries of this study were: (1) Do soil C and N pools differ between ant nests and surrounding reference soil? (2) Do the vertical distributions of soil C and N pools vary among the three ant species with different feeding-behavior? (3) What is the effect of three ant species on the storage of soil C and N in the tropical forest?

## 2. Materials and methods

### 2.1. Site description and experimental design

This study was conducted in the Xishuangbanna Tropical Botanical Garden (21°55' N, 101°16' E), southern Yunnan, China. This region has a typical monsoon climate, thus has a distinct dry season from November to April. Mean annual total precipitation is about 1557 mm, and about 87% precipitation occurs in the wet season from May to October. Mean annual temperature is 21.5 °C, with monthly temperatures ranging from 15.1 to 21.7 °C. Although this region is at the northern edge of the tropics, the mountains and plateaus in the North and the West shelter it from cold air during the winter. In addition, heavy fog occurs from midnight to noon every day in the first 4 months of the dry season, maintaining soil moisture levels (through reduced evapotranspiration) over a large proportion of the dry season. As a result, monsoon forests and tropical rain forests flourish in this region, with a relatively high abundance of lianas.

The tropical rain forest of *Syzygium oblatum* community was selected to explore the relationships between ant nesting activity and soil C and N pools. The forest is composed of *S. oblatum*, *Milletia leptobotrya*, *Ficus semicordata*, *Castanopsis indica*, *Engelhardia spicata*, *Fissistigma polyanthum*, *Prismatomeris connata*, *Tectaria polymorpha*, *Alpinia galang*, and *Pandanus furcatus*, and covered an area of 8 ha. The tree canopy was approximately 46-yr old with mean diameters of 26 cm. The mean height of the tree canopy was approximately 27 m and its percentage of coverage reaches 95%. The soil taxonomy belongs to oxisols (laterite soil in China) originated from cretaceous sand stone. The soils are highly weathered and leached in the tropics.

Four plots (20 m × 20 m) at least 50 m apart were randomly replicated to assess ant nests distribution across the forest, and counts of ant nests and measurements of their diameters were conducted (Table 1). Nests were grouped by the ant species present. The ant species were discerned by bait method as the three types of nests were created almost below-ground. The baits of bread crumbs impregnating animal fat were sprinkled in 5 cm-wide strips (30 cm apart) and followed the trails of the ants back to their nests. The border of ant nests was identified by the piled materials (e.g., litter, petals, and nesting soil) around the nest entrances. Three kind nests of ants with different feeding-behaviors (i.e., *Ph. capellini* - predominantly honeydew harvester, *Ph. affinis* - scavenger, and *O. transversa* - predominantly predator) were selected for studying ant effects on the pools of soil C and N. Within each plot, five average-sized nests (5 m apart) of each ant species were sampled randomly to ensure independence. Simultaneously, five pair reference points were also selected randomly, within 5 m from each other or from ant nests.

### 2.2. Sampling and analysis

Soils were sampled in middle June when ants were active. We stratified our sampling for soil physical and chemical analyses of five average-sized ant nests, and five reference points at the depths of 0–5 cm, 5–10 cm, and 10–15 cm in each plot, respectively. Soil cores (5 cm diameter by 5 cm deep) were taken for each soil layer at six sampling locations at each nest, i.e., from the center part, and the east, south, west, and north of the edge of nest discs, and the reference. Soil samples placed separately into labeled ziploc bags, stored immediately in a cooler with ice and once back at the laboratory refrigerated at 4 °C until analysis. Sub-samples were dried to constant weight at 40 °C, milled, and passed through a 2-mm sieve for elemental analysis after ants, stones and other impurities were carefully removed.

Soil bulk density was measured by the core method (5 cm diameter by 5 cm deep). Soil pH was determined using a glass electrode in a 1:2.5 soil:water solution (w/v). Soil MBC was determined using the chloroform fumigation extraction method (Jenkinson and Powlson, 1976). TOC was analyzed by the dichromate oxidation with external heating procedure, and TN by the Kjeldahl digestion method. Soil ROC was measured by treating air-dried soil with 0.02 mmol L<sup>-1</sup> KMnO<sub>4</sub>. DON was determined using a TOC-VCN analyzer (Shimadzu Scientific Instruments, Columbia). Soil inorganic N contents (NO<sub>3</sub>-N and NH<sub>4</sub>-N) were extracted from approximately 30 g field-moist, 2-mm-seived soil sub-samples with 2 M KCl solution and determined with a UV-VIS spectrophotometer (UV mini 1240, Shimadzu, Japan).

### 2.3. Statistical analyses

Total C and N storage (kg m<sup>-2</sup>) in ant nests was calculated by multiplying the soil nutrient concentrations by the area-based ant nest masses (Wang et al., 2016a). Analysis of variance (ANOVA) was performed to examine the effects of habitat ant species, soil depth, and their interactions. Differences in soil C and N concentrations and stocks, and bulk densities among nests of the three ant species, and between ant nests and reference soils were tested using a one-way ANOVA. Data for concentration and storage of C and N pools were all ln (x + 1) transformed before analysis to improve normality and to reduce heterogeneity of variance. Least significant difference tests were used to compare treatment means. Differences were considered statistically significant if *p* < 0.05. The statistical analyses were conducted with the software SPSS 16.0 package.

**Table 1**  
Characteristics of three ant nests in tropical forest of Xishuangbanna.

Ant species	Nest density (No. ha <sup>-1</sup> )	Diameter of nest discs (cm)	Total area of nest discs (m <sup>2</sup> ha <sup>-1</sup> )
<i>Pheidole capellini</i>	1025 ± 104 <sup>a</sup>	6.8 ± 0.31 <sup>a</sup>	20.12 ± 3.44 <sup>a</sup>
<i>Pheidologeton affinis</i>	878 ± 72 <sup>b</sup>	2.4 ± 0.13 <sup>b</sup>	17.23 ± 2.25 <sup>b</sup>
<i>Odontoponera transversa</i>	466 ± 36 <sup>c</sup>	1.5 ± 0.05 <sup>c</sup>	9.15 ± 1.16 <sup>c</sup>

Note: Values of nest density, diameter of nest discs, and total area of nest discs are mean ± SE. Different letters indicate significant differences ( $p < 0.05$ ) among the ant species.

### 3. Results

#### 3.1. Soil carbon and nutrient concentrations in ant nests and the reference soil

The concentrations of MBC ( $F = 11.57$ ,  $p = 0.001$ ) and TOC ( $F = 9.42$ ,  $p = 0.020$ ) in nests of three ant species were significantly different from those in reference soils, while the significant differences of ROC contents were observed between *Ph. capellini* nests or *O. transversa* nests, and the reference soils (Fig. 1). Generally, the MBC, TOC, and ROC concentrations in the reference soil showed a decline vertical distribution with the maximum in the 0–5 cm layer. In contrast, the maximum values of MBC, TOC and ROC in *Ph. affinis* nests were in 5–10 cm soil layers, with 3.63, 1.46, and 1.54 times greater than those in reference soil, respectively; the peak values of MBC, TOC and ROC in nests of *Ph. capellini* (6.47, 2.67, and 1.99 times greater than reference soil, respectively) and *O. transversa* (2.19, 1.98, and 2.03 times greater than reference soil, respectively) were found at 10–15 cm soil depth.

TOC ( $F = 7.02$ ,  $p = 0.04$ ) and MBC ( $F = 9.74$ ,  $p = 0.03$ ) were significantly different among the ant species (Fig. 1 A and C). The concentrations of ROC ( $F = 38.24$ ,  $p < 0.02$ ) differed only between *Ph. capellini* nests and *O. transversa* or *Ph. affinis* nests, but these latter two species did not differ (Fig. 1 B). The increased concentration of MBC (196.85%), TOC (86.82%), and ROC (68.64%) in *Ph. capellini* nests was ranked the largest, in comparison to the reference soil.

There were significant differences in TN concentrations between ant nests and the reference soil (Fig. 2A;  $F = 9.34$ ,  $p < 0.01$ ), while a significant difference in DON was only found between *Ph. affinis* nests or *O. transversa* nests, and the reference soil (Fig. 2B). In the nests of *Ph. capellini* and *O. transversa*, the maximum values (2.15 and 2.67 times greater than reference soil) of TN concentration were in the 10–15 cm soil layer, but those of DON (1.05 and 1.28 times greater than reference soil) in the 0–5 cm soil layer. Whereas in *Ph. affinis* nests, the maximum values of TN and DON (2.02 and 1.99 times greater than reference soil) were in the 5–10 cm soil layers. The mean TN and DON concentrations for each soil layer of *Ph. affinis* nests were higher than those in *O. transversa* and *Ph. capellini* nests, but there were no differences of TN concentrations between *O. transversa* and *Ph. Capellini* nests.

Three types of nests and the reference soil differed considerably in the concentration of  $\text{NH}_4^+$  (Fig. 2C;  $F = 38.35$ ,  $p < 0.001$ ), but that difference in  $\text{NO}_3^-$  was only in *Ph. affinis* nests (Fig. 2D;  $F = 10.54$ ,  $p > 0.05$ ). The maximum values of average  $\text{NH}_4^+$  were found at 10–15 cm depth, with 151%, 83.6% and 121.4% higher in *Ph. capellini*, *Ph. affinis*, and *O. transversa* nests, respectively, than in surrounding reference soil. The differences in  $\text{NH}_4^+$  values across the soil layers were significant for three ant species, while those in  $\text{NO}_3^-$  were only found for *Ph. affinis* nests and the reference soil. The interactions between ant species and soil layer were all significant for TOC (Table 2;  $F = 11.24$ ,  $p < 0.01$ ), ROC ( $F = 27.46$ ,  $p < 0.01$ ), TN ( $F = 6.42$ ,  $p < 0.02$ ), DON ( $F = 51.21$ ,  $p < 0.01$ ),  $\text{NH}_4^+$  ( $F = 56.48$ ,  $p < 0.001$ ), but not for MBC ( $F = 4.52$ ,  $p > 0.05$ ) and  $\text{NO}_3^-$  ( $F = 4.24$ ,  $p > 0.05$ ).

#### 3.2. Soil carbon and nutrient storage in ant nests and the reference soil

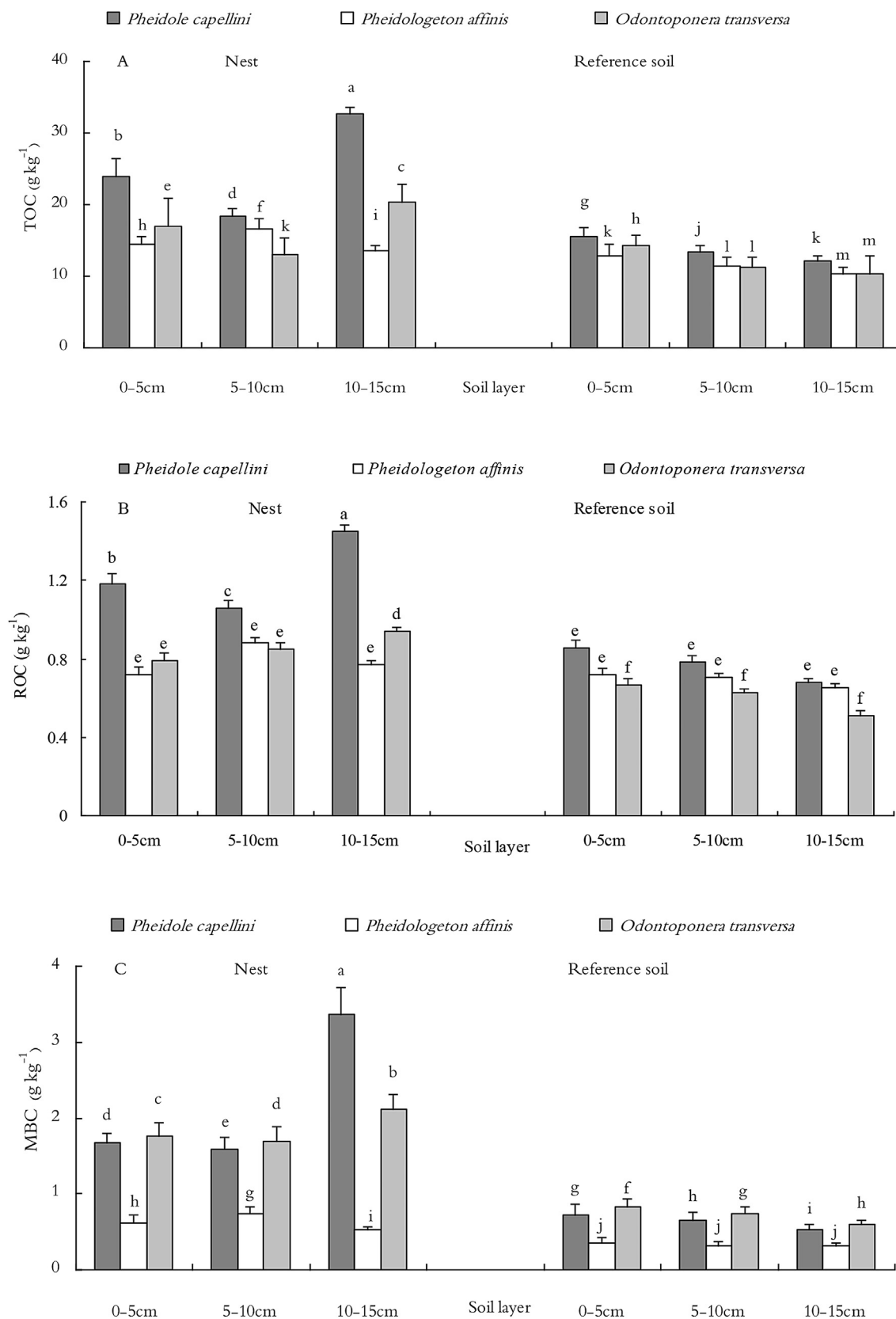
Soil bulk densities (1.03–1.26 g cm<sup>-3</sup>) were lower in three ant nests than in reference soils ( $F = 8.24$ ,  $p < 0.03$ ), except at 0–5 cm in *Ph. affinis* nests (Table 3). TOC and ROC pools (12.38 kg m<sup>-2</sup> and 6.03 kg m<sup>-2</sup>, respectively) in *Ph. capellini* nests were significantly higher than those in *O. transversa* and *Ph. affinis* nests and in the reference soil (Fig. 3). However, no significant differences were observed for the pools of TOC and ROC between *Ph. affinis* nests and the reference soil. MBC pools were significantly higher in *Ph. capellini* and *O. transversa* nests, but they had no significant difference between *Ph. affinis* nests and the reference soil. The *Ph. capellini* nests had the greatest increase of C pools (101.2 kg ha<sup>-1</sup> TOC, 15.49 kg ha<sup>-1</sup> MBC, and kg ha<sup>-1</sup> ROC) in the tropic forest (Table 4).

There were significantly higher TN and DON pools in the ant nests than in the reference soil, except for *Ph. capellini* nests (Fig. 4). The pools of TN (1.05 kg m<sup>-2</sup>) and DON (18.28 g m<sup>-2</sup>) values were considerably higher in *Ph. affinis* nests than in *Ph. capellini* and *O. transversa* nests, and in the reference soil ( $F = 11.58$ ,  $p < 0.05$ ; and  $F = 17.27$ ,  $p < 0.01$ , respectively). The  $\text{NH}_4^+$  pools were insignificantly higher in nests of three ant species than in the reference soil, but  $\text{NO}_3^-$  pools were significantly lower in *Ph. capellini* nests than in the reference soil.  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools in *Ph. affinis* nests were higher than those in *Ph. capellini* and *O. transversa* nests. The *Ph. affinis* nests had the greatest increase of N pools (6380 g ha<sup>-1</sup> TN, 110.44 g ha<sup>-1</sup> DON, 128.88 g ha<sup>-1</sup>  $\text{NH}_4^+$ , and 10.17 g ha<sup>-1</sup>  $\text{NO}_3^-$ ) in the tropic forest (Table 4).

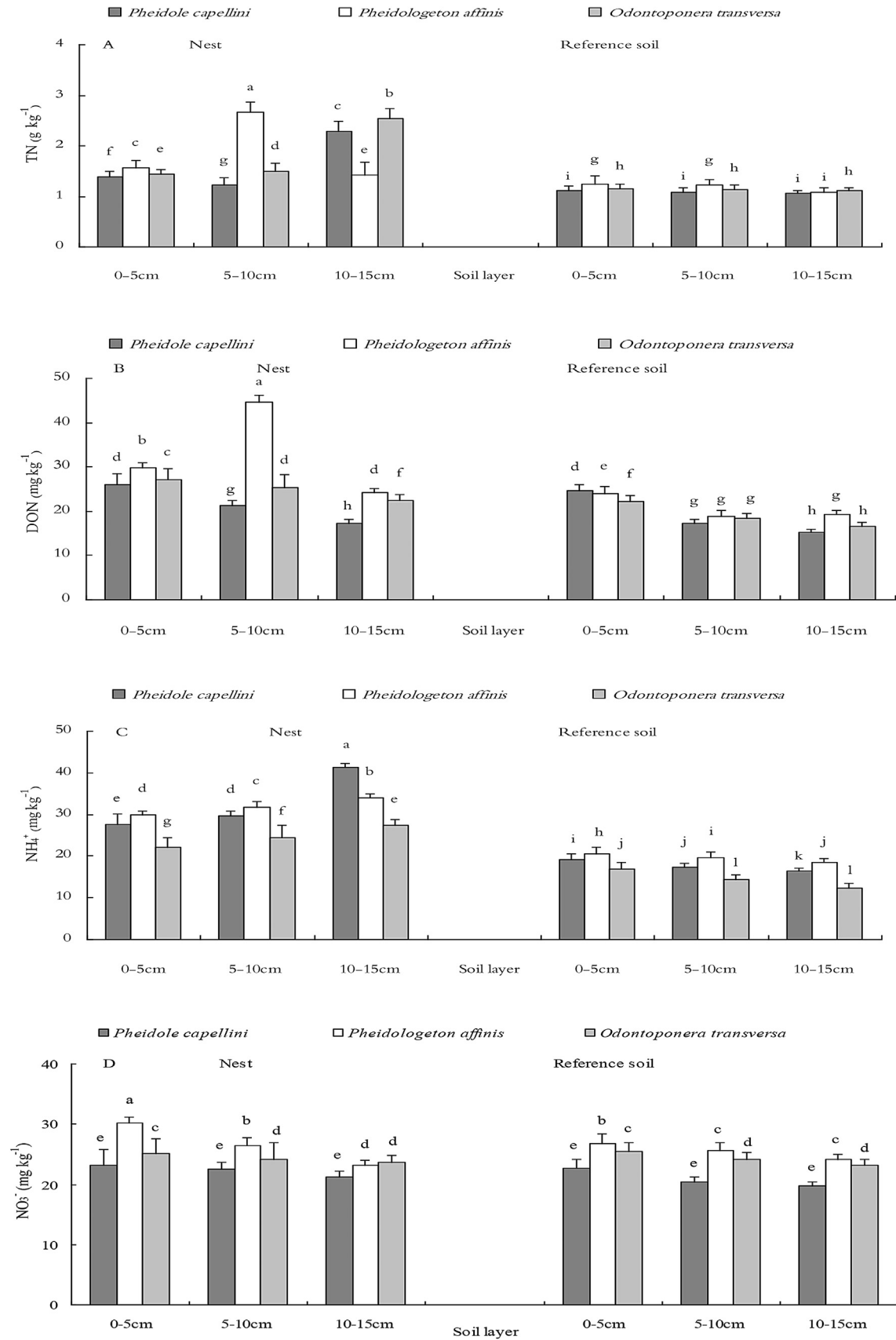
### 4. Discussion

Studies about ant effect on soils often focus on above-ground nesting species, seldom on under-ground nesting ants (Domisch et al., 2008; Hudson et al., 2009). In this study, we selected three ant species of different feeding-behaviors in tropic forest of southwestern China, which built their nest almost below-ground with high diversity of nests (Table 1), and observed a pronounced effect of ants on soil carbon and nitrogen pools. In this tropic environment with diversity of food resources and microclimates, ants can have quite variable effects on soil C and N pools and cycling (Cammeraat and Risch, 2008). Therefore, much attention should be paid to the effect of ants on soil processes in tropical ecosystems.

The ant effect on components of soil C and N pools varied with ant species. The fractions of C pool are commonly differentiated in TOC, ROC or MBC, and those of N pool in TN, DON,  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . In this study, MBC, TOC, TN and  $\text{NH}_4^+$  concentrations were higher in all ant nests than in the reference soils, however, the significant increases of ROC contents were observed in *Ph. capellini*, *O. transversa* nests, DON in *Ph. affinis* and *O. transversa* nests, and  $\text{NO}_3^-$  in *Ph. affinis* nests. Generally, the concentrations of these forms of nutrients can show higher or unchanged levels in ant nests compared to the reference soils. The reason is likely related to the changes in C and N turnover of organic material and microbial activity, which varies among different ant species (e.g., MacMahon et al., 2000;



**Fig. 1.** Concentrations of carbon pools (TOC, ROC and MBC) in three ant nests versus reference soil in tropical forest of Xishuangbanna (bars indicate  $\pm$ SE). TOC: total organic carbon; ROC: readily oxidizable organic carbon; MBC: microbial biomass carbon. The same letters have no significant difference ( $p > 0.05$ ) among ant species and the reference soil.



**Fig. 2.** Concentrations of nitrogen pools (TN, DON, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) in three ant nests versus reference soil in tropical forest of Xishuangbanna (bars indicate  $\pm$  SE). TN: total nitrogen; DON: dissolved organic nitrogen; NH<sub>4</sub><sup>+</sup>: NH<sub>4</sub>-N; NO<sub>3</sub><sup>-</sup>: NO<sub>3</sub>-N. The same letters have no significant difference ( $p > 0.05$ ) among ant species and the reference soil.

**Table 2**

Effects (F statistics) of three ant species (A) and soil layer (S) on soil carbon (C) and nitrogen (N) pools in tropical forest of Xishuangbanna.

Source	df	TOC	ROC	MBC	TN	DON	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>
A	2	7.02*	18.24*	9.74*	6.56*	46.45**	38.35**	10.54
S	2	6.65*	11.18*	5.42	6.14*	22.14	40.06*	9.67
A × S	4	11.24**	27.46**	4.52	6.42*	51.21**	56.48**	4.24

TOC: total organic carbon; ROC: readily oxidizable organic carbon; MBC: microbial biomass carbon; TN: total nitrogen; DON: dissolved organic nitrogen; NH<sub>4</sub><sup>+</sup>: NH<sub>4</sub>-N; NO<sub>3</sub><sup>-</sup>: NO<sub>3</sub>-N. Significant levels: \*\**P* < 0.01, \**P* < 0.05.

**Table 3**

Mean values of soil bulk density (g cm<sup>-3</sup>) in three ant nests and natural forest soil (0–15 cm) in tropical forest of Xishuangbanna.

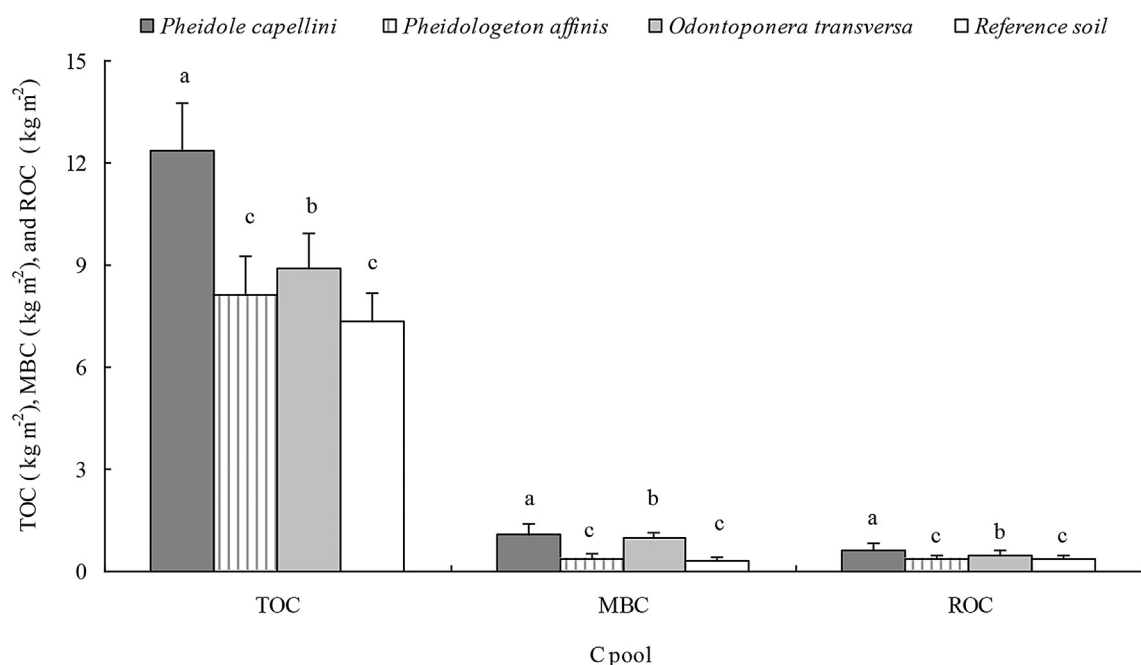
Ant species	Soil layer		
	0–5 cm	5–10 cm	10–15 cm
<i>Pheidole capellini</i>	1.03 ± 0.10 <sup>c</sup>	1.09 ± 0.12 <sup>d</sup>	1.18 ± 0.11 <sup>d</sup>
<i>Pheidologeton affinis</i>	1.22 ± 0.15 <sup>a</sup>	1.25 ± 0.17 <sup>b</sup>	1.27 ± 0.19 <sup>b</sup>
<i>Odontoponera transversa</i>	1.17 ± 0.16 <sup>b</sup>	1.12 ± 0.14 <sup>c</sup>	1.24 ± 0.21 <sup>c</sup>
Reference forest soil	1.24 ± 0.20 <sup>a</sup>	1.33 ± 0.25 <sup>a</sup>	1.42 ± 0.28 <sup>a</sup>

Values of soil bulk density are mean ± SE. Different letters indicate significant differences (*p* < 0.05) among ant species and the reference soil.

Jimenez et al., 2008; Jones and Wagner, 2006). The differences between ant nests and the surrounding soil observed in our study may mainly depend on feeding strategy of ants, in agreement with

Wagner et al. (2004) and Kilpelainen et al. (2007). There were relative higher C concentrations in *Ph. capellini* ants and higher N concentrations in *Ph. affinis* ants compared with the other ants and the reference soil. This was probably due to different feeding strategies. We observed that *Ph. capellini* mainly gathered a great deal of honeydew above-ground and from plant root aphids underground, while *Ph. affinis* ants are scavengers invertebrates feeding almost any detritus of plant and insects they can find in meadows. More organic matter as honeydew is concentrated in the top soil layers of *Ph. capellini* nests, which may lead to greater C concentration in *Ph. capellini* nests. In contrast, inputs of high-protein plant and animal tissues to ant nests as a direct result of ant foraging behavior often result in higher N concentrations in *Ph. affinis* nests (Wagner et al., 2004). In addition, the higher concentrations of MBC in three ant nests than in the reference soil were observed, which may induce higher microbial effect on the concentration increase of C and N pools in ant nests. Especially, the highest MBC in *Ph. capellini* nests may coincide well with the greatest C concentration in comparison with other ant nests and reference soils.

Our results also showed that ant nests considerably increased spatial variability of the concentrations of C pools (i.e., MBC, TOC) and N pools (i.e., TN, ROC, TN, DON, NH<sub>4</sub><sup>+</sup>), with the higher values in deeper soil layers (5–10 cm or 10–15 cm), in comparison with the reference soil. *Ph. capellini* nests had the greater increased concentration of MBC (106.85%), TOC (66.82%), and ROC (48.64%) in 10–15 cm, in comparison with reference soil, while there were



**Fig. 3.** Mean values of storages for carbon pools (TOC, ROC and MBC) in ant nests and reference soil to 15 cm depth in tropical forest of Xishuangbanna (bars indicate ± SE). TOC: total organic carbon; ROC: readily oxidizable organic carbon; MBC: microbial biomass. The same letters have no significant difference (*p* > 0.05) among ant species and the reference soil.

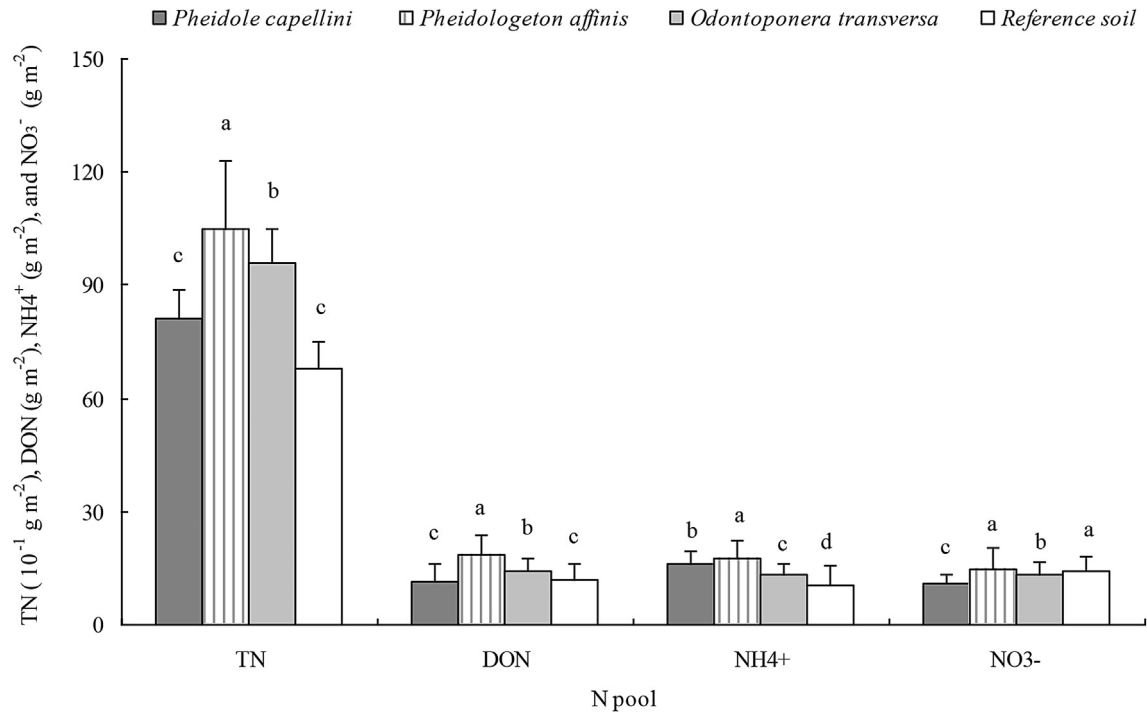
**Table 4**

Increased carbon (kg ha<sup>-1</sup>) and nitrogen (g ha<sup>-1</sup>) pools of soil in three ant species compared to the reference soils in tropical forest of Xishuangbanna.

Ant species	TOC	ROC	MBC	TN	DON	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>
<i>Pheidole capellini</i>	101.2	4.89	15.49	2620	-7.85	121.52	-60.56
<i>Pheidologeton affinis</i>	13.44	0.24	0.34	6380	110.44	128.88	10.17
<i>Odontoponera transversa</i>	14.27	1.04	5.95	2560	22.05	26.08	-7.41

TOC: total organic carbon; ROC: readily oxidizable organic carbon; MBC: microbial biomass carbon; TN: total nitrogen; DON: dissolved organic nitrogen; NH<sub>4</sub><sup>+</sup>: NH<sub>4</sub>-N; NO<sub>3</sub><sup>-</sup>: NO<sub>3</sub>-N.





**Fig. 4.** Mean values of storages for nitrogen pools (TN, DON,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in ant nests and reference soil to 15 cm depth in tropical forest of Xishuangbanna (bars indicate  $\pm$  SE). TOC: total organic carbon; ROC: Dissolved organic carbon; MBC: microbial biomass. The same letters have no significant difference ( $p > 0.05$ ) among ant species and the reference soil.

highest increases of TN concentrations in 10–15 cm nest of *O. transversa*, DON in 5–10 cm nest of *Ph. affinis*,  $\text{NH}_4^+$  in 10–15 cm nest of *Ph. capellini*. The vertical pattern of C and N pools in ant nests differed according to ant species, in agreement with previous studies (Jimenez et al., 2008; Ginzburg et al., 2008; Hudson et al., 2009). Ants differ in activities of feeding and mixing, and transport surface nutrient material to the subsurface, which would lead to the reduction of nutrient content in the surface layers and an increase in subsurface soil. This change may be weakened, when the modifying activities of ants are ceased after the nest is abandoned. In addition, the vertical properties of soil C and N pools may vary with distance to the nests where the turnover of organic material and ant activity are different. The lateral spatial heterogeneity can exert additional effects on the vertical variations of soil C and N pools.

The nest structures (e.g., chambers, soil aggregates, and maculae) and the activities of ant nesting affect soil physical and biological properties, which can exert a significant effect on soil C and N pools (Wu et al., 2013). In this study, we found that the three underground-nesting ants led to a tendency towards a decrease in soil bulk density and increase in soil moisture (Wang et al., 2016b). MBC content was found a greater increase in ant nests, which might lead to a change in soil C and N contents, as soil moisture can stir microbial activities in ant nests (Holec and Frouz, 2006; Ginzburg et al., 2008). The building nest also increased potential enzyme activities and altered the kinetic properties of the enzymes, and further significantly increased ROC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in litter solutions (Stadler et al., 2006). In addition, many specialized soil fauna inhabit in ant nests, which can impact the pools of soil C and N. Therefore, the physical changes brought about by constructing activities of ants can affect soil biology and microbial activities, which may have a crucial effect on the soil C and N pools.

Soil C and N concentrations were probably also influenced by changes in soil processes such as decomposition, mineralization

and denitrification. Ants could have an accelerating effect on litter decomposition through direct physical effect and indirect trophic effects via aphids and micro-organisms (Domisch et al., 2008). In this study, the higher concentrations of MBC indicate a fast decomposition and mineralization (Berg and McClaugherty, 2003). The higher  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents indicate that a high rate of N mineralization and nitrifying microorganisms seem to have been activated in *Ph. capellini* and *Ph. affinis* nests, because there were enough organic matter and suitable moisture conditions especially in the tropic environment. However, higher levels of inorganic N in *Ph. capellini* and *Ph. affinis* nests can result from enhanced resource availability and improved conditions for microorganism mineralization, as well as a low rate of N uptake by plants. Thus a detailed study need to further investigation on which microbial populations are enhanced or inhibited in these biogenic structures.

Our results observed a pronounced effect on soil C and N pools and the effects varied with ant species. In this study, the stock of C pools (i.e., TOC, ROC and MBC) in *Ph. capellini* nests was higher than that in *O. transversa* and *Ph. affinis* nests, and the reference soil. The increases in storage of soil C pools were associated with changes in both nutrient concentrations and bulk density in the nests (Wu et al., 2013). The greater C pools in *Ph. capellini* nests were due to the higher concentrations of TOC, ROC and MBC, though the bulk densities in the nests of *Ph. capellini* were the lowest. The lower stocks of C pools were observed in *Ph. affinis* nests may be due to the lower nutrient concentrations and not enough higher bulk density in the nests. The stocks of N pools (i.e., TN, DON,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in *Ph. affinis* nests were much higher than those in *Ph. capellini* and *O. transversa* nests, and the reference soil. These may be due to higher concentrations and not too lower bulk densities for the *Ph. affinis* nests. Higher N pools in ant nests agree with the findings of Wagner et al. (2004) and Kilpelainen et al. (2007). The lower storage of DON was found in *Ph. capellini* nests, and  $\text{NO}_3^-$  in nests of *Ph. capellini* and *O. transversa* than that in the reference soil,

which mainly dues to their lower bulk densities in nests than in the reference soil.

We can conclude that the activities of ant nesting significantly increased the content, spatial variability, and chemical form of soil carbon and nitrogen pools at the soil centimeter scale. The direction and degree of these soil modifications vary among ant species. The construction of ant nests can be an important process in structuring the soil resource environment and through this, in driving ecological processes in tropical ecosystems.

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