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

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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 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 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 NH_4^+ in 10–15 cm soil layer of *Ph. capellini* nests. The greatest increase of C pools (101.2 kg ha^{-1} TOC, 15.49 kg ha^{-1} MBC, and 4.89 kg ha^{-1} ROC) was found in *Ph. capellini* nests, while that of N pools (6380 g ha^{-1} TN, 110.44 g ha^{-1} DON, 128.88 g ha^{-1} NH_4^+ and 10.17 g ha^{-1} 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.

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

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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 microclimate (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 evapotranspirtation) 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*, *Millettia 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^{-1} $KMnO_4$. DON was determined using a TOC-VCPN analyzer (Shimadzu Scientific Instruments, Columbia). Soil inorganic N contents (NO_3–N and NH_4–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^{-2}) 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.