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The contributions of underground-nesting ants to CO_2 emission from tropical forest soils vary with species

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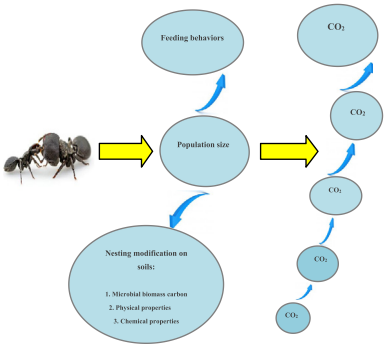
HIGHLIGHTS

- Underground ant nests were sources for contribute to CO_2 emission in tropical forests.
- Ants increased the heterogeneity of C flux in the wet tropical environments.
- Soil CO_2 emission varied with population size and feeding-habits of ant species.
- Ants affect C emission through modifying microbial and chemical properties of soil.

GRAPHICAL ABSTRACT

The aboveground ant nests are admitted as hot spots for CO_2 emissions which increase the heterogeneity of soil carbon (C) flux in forest ecosystems. However, little is known about the effects of underground-nesting ant species on C emissions in tropical forests, where the ants have high diversity and abundance.
In this study, we chose three underground-nesting ant species with different feeding-behaviors (*Pheidole capellini* - predominantly honeydew harvester, *Odontoponera transversa* - predominantly predator, and *Pheidologeton affinis* - scavenger) to explore their impacts on soil CO_2 emission in Xishuangbanna tropical forest, China. We observed a pronounced effect of ants on soil CO_2 emission, and the effect varied with ant species. The mean CO_2 emissions were 2.4 times higher in *P. capellini* nests than in the reference soils, while those in nests of *O. transversa* and *P. affinis* were 2.0 and 1.6 folds, respectively. The contribution of total CO_2 flux from the nests of three ant species comprised 0.01–0.54% of the total annual CO_2 efflux from the forest floor. The CO_2 emission in ant nests and reference soils increased exponentially with soil temperature and water. Soil water was considerably increased by ant nesting, which explained nearly 93–97% of CO_2 emissions. However, soil temperature was not significantly different between nests and reference soils, and it only explained about 54–70% of CO_2 emission. Ant species differed in increase of soil microbial biomass carbon, total organic carbon, readily oxidizable organic carbon, soil bulk density and pH, which also contributed to a diverse effect on soil CO_2 emission.
We suggest that the different effects of ant species on C emission may be closely associated with diversity of ant population size, feeding-behaviors and nesting modification on soil microbial biomass carbon, and physicochemical properties (i.e., temperature, moisture, pH, Bulk density, total and readily oxidizable organic carbon) in the tropical forest.



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1. Introduction

Forest soil CO_2 efflux accounting for 40–80% of the total forest ecosystem respiration, is one of the major processes involved in the forest C cycling (Domisch et al., 2006; IPCC, 2006). Small changes in CO_2 emission from forest soil could have great impact on the global C cycle (IPCC, 2007). Soil CO_2 emissions originate from a combination of various biological and physical processes, thus they have a great spatiotemporal heterogeneity (Ohashi et al., 2005). Unfortunately, our understanding of the origin and magnitude of this heterogeneity is considerably incomplete.

Ants as ecosystem engineers can contribute to the modification of carbon dynamics through CO_2 emissions from their nest soil, causing heterogeneity in soil CO_2 efflux within forest ecosystem (Ohashi et al., 2005; Frouz and Jilkova, 2008). A number of studies have indicated that ant nests are "hot spots" for CO_2 emission, and can produce more CO_2 (maximum up to 12 times) than the surrounding soils (Domisch et al., 2006; Ohashi et al., 2012). The contribution of ant colonies to CO_2 fluxes (<3%) is small relative to the total soil C budget (Ohashi et al., 2012). However, it is important to the understanding of ecosystem C balance, since even a small alteration of soil C flow can significantly impact the total C balance of forest ecosystem (Yuste et al., 2005; IPCC, 2007).

The soil CO_2 emissions from ant nests are mainly composed by several sources (i.e., the respiration of ants and other invertebrates living in and under the nests, microbial decomposition of nest organic matter, and respiration of plant roots that grow beneath the nests) (Risch et al., 2005). A great number of ants and invertebrate fauna within a nest can directly increase the efflux of CO_2 by their respirations (Jilkova and Frouz, 2014). Ants can also indirectly affect the CO_2 efflux by stirring the activity of soil microorganisms. Furthermore, the CO_2 efflux can be affected by modifying of ant nesting on soil properties (Frouz, 2000; Lenoir et al., 2001; Frouz and Jilkova, 2008). Therefore, changes in the micro-environment and/or the nesting activity of the ants can induce considerable variation in CO_2 efflux from the nests (Risch et al., 2005; Ohashi et al., 2007).

A number of studies have documented ant effects on CO_2 efflux in forest soils, mainly in the European temperate forests (Domisch et al., 2008; Ohashi et al., 2012; Jilkova and Frouz, 2014; Jilkova et al., 2015a, 2015b). All of these published studies focused on wood ant species mainly nesting aboveground. The underground-nesting ant species often differ in their feeding activities in tropic soils to input different organic materials into the nests, which can contribute to diverse effect on soil CO_2 emission (Ohashi et al., 2017). However, little is known about the impacts of underground-nesting ant species with different feeding-behavior on soil CO_2 emissions in tropical forests, where ants have high diversity and abundance.

Xishuangbanna distributes the largest area of tropical forests in China, where ants often occur in high diversity with diverse feeding-behaviors because of diverse food resources and micro-climate (Xu, 1999; Yang et al., 2001, Wang et al., 2016a). However, there is no study about the nesting influence of these ants on soil CO_2 emissions. In this study, we explored the impacts of three underground-nesting ant species with different feeding-behaviors (P. capellini - predominantly honeydew harvester, O. *transversa* - predominantly predator, and P. affinis-scavenger) (Sonthichai et al., 2006) on soil CO_2 emissions in tropical forest of Xishuangbanna. The main queries of this study were: (1) Do soil CO_2 emissions differ between ant nests and the reference soil? (2) Do soil CO_2 emissions varied among the nest types of three feeding-habit ant species? (3) What is the association of soil properties modified by three ant species to soil CO_2 emissions in the tropical forest?

2. Materials and methods

2.1. Site description and experimental design

We conducted this study in the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (21°55′ N, 101°16′ E), southern Yunnan, China. The study region has a typical monsoon climate with a distinct dry season from November to April. Mean annual total precipitation is about 1,557 mm with about 87% precipitation in the wet season from May to October. Mean annual temperature is 21.5 °C and monthly temperature from 15.1 to 21.7 °C. In addition, heavy fog occurs from midnight to noon every day in the first 4 months of the dry season, maintaining soil moisture levels over a large proportion of the dry season. Thus, monsoon forests and tropical rain forests flourish in this region, with a high abundance of lianas.

Three sites of Syzygium oblatum (Myrtaceae) community with similar soil and vegetation conditions were chosen to explore the impact of ant nesting activity on soil CO_2 emissions in the type of tropical forest. The experimental forest sites were composed of *S. oblatum*, *Millettia leptobotrya*, *Ficus semicordata*, *Castanopsis indica*, *Engelhardia spicata*, *Fissistigma polyanthum*, *Prismatomeris connata*, *Tectaria polymorpha*, *Alpinia galang*, and *Pandanus furcatus*. The tree canopy of coverage was about 95%. The mean height was approximately 17 m with mean diameters of 22 cm. The taxonomy of soil belongs to oxisols (laterite soil in China) originated from cretaceous sand stone.

Three ant species with different feeding-behaviors were chosen for assessing ant effects on soil CO_2 emissions. In each site, three plots (15 m × 10 m) at least 20 m apart were randomly replicated to measure the average values of ant population (i.e., abundance and body size), and nest density and diameters for each ant species (Table 1). The nest characteristics (i.e., diameter, depth and architecture) were

Table 1
The nest distribution and architecture characteristics of three ant species in the tropical forest in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Ant species	Abundance (ind. nest^{-1})	Body size (mm)	Nest numbers per plot	Nest density (nests ha^{-1})	Nest diameter (cm)	Nest depth (cm)	Nest architecture Above- and under-ground
Pheidole capellini	380 ± 44^{a}	2.5 ± 0.2^{b}	142 ± 26^{a}	1286 ± 115^{a}	11.4 ± 2.64^{a}	9.5 ± 1.8^{a}	5 cm piled petals for nesting ; 3 chambers in first layer (3.5 cm depth), 2 chambers in second layer
Odontoponera transversa	56 ± 15^{c}	8.1 ± 0.4^{a}	54 ± 9^{c}	488 ± 47^{c}	9.8 ± 0.29^{c}	10.5 ± 2.4^{a}	Few scattered soil particles; 2 chambers in first layer (4 cm depth), 1 chamber in second layer
Pheidologeton affinis	250 ± 23^{b}	1.2 ± 0.1^{c}	100 ± 17^{b}	904 ± 72^{b}	10.2 ± 1.72^{b}	7.3 ± 1.2^{a}	Few scattered soil particles; 2 chambers in first layer (3 cm depth), 1 chamber in second layer

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

assessed by digging after CO_2 efflux measurement. Within each plot, five average-diameter nests (Table 1) (about 5 m apart) of each ant species were sampled randomly to ensure independence. Simultaneously, five pair reference points were also selected randomly, about 5 m from each other or from ant nests. Nests were grouped by the ant species present. We used bait method (Wang et al., 2017) to identify ant species as the three types of nests were created almost underground. The bread crumbs baits impregnating animal fat were sprinkled in 5cm-wide strips (30cm apart) and followed the trails back to ant nests.

2.2. Soil CO_2 emissions measurement and soil analysis

Soil CO_2 emission from ant nests and the reference soils were measured twice (approx. day 20) each month in March, June, September, and December in 2015. Within each replicated plot, we conducted CO_2 emissions measurement on five nests and five pair reference points (about 5 m apart) for each species, using the Li 6400-09 soil respiration chamber (Li Inc, Lincoln, NE, USA) connected to a portable infrared gas analyzer (Li Inc.). All soil CO_2 efflux measurements were performed in about an hour to ensure the simultaneity (Jilkova et al., 2015a), since the measurements at each location (two times consecutively) can be finished within 10 min. Soil CO_2 emission was measured in a polyvinyl chloride (PVC) collar (10 cm in diameter and 5 cm in height) installed about 3 cm into the soil three days in advance. Because the ants of three species had the preferences of illuminating intensity, they usually performed their colony tasks outside the nests from 9 a.m to 7 p.m. at daytime. Therefore, CO_2 measurements were taken at 8–9 a.m. in the period of very little external activity of the colony in this study. On each measurement of CO_2 emissions from ant nests, PVC was covered across the nest because the diameter per nest was about 10 cm. We tried the best to choose the reference points with no surface vegetation. If this didn't work, we removed all ground vegetation at whole in the collars three days in advance to avoid interference of respiration from above plants. Soil temperature was monitored simultaneously using a copper/constantan thermocouple penetration probe (Li6400-09 TC, Li Inc.) inserted in the soil to a depth of 5 cm in the vicinity of the soil respiration chamber. Soil water in nests and reference soils was measured by portable soil temperature-moisture measuring instrument (SIN–TN8, China). The C flux (kg C a-1) per ha nest were calculated by multiplying the soil CO2 emission (kg C m^{-2} a-1) from the nests by the total nest areas (m^2) for each ant species (Wu et al., 2013). Whereas C flux (kg C ha^{-1} a-1) per ha forest floor was estimated by multiplying the soil CO_2 emission from the reference soil (kg C m^{-2} a-1) by 10000 m^2 forest floors.

Soil cores (10 cm diam.) were sampled from the soil surface down to 5 cm (nearly the depth of respiration measurement and most nest chambers distribution of ants) in the locations of PVC collars where soil respiration chambers were placed following respiration measurements in each plot. Ninety soil cores (10 ind. per plot × 3 plots × 3 sites) were sampled for each species in each sampling period. 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 (Wang et al., 2016a). Soil pH was determined using a glass electrode in a 1:2.5 soil:water solution (w/v). Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation extraction method (Vance et al., 1987). Total organic carbon (TOC) was analyzed by the dichromate oxidation with external heating procedure, and total nitrogen (TN) by the Kjeldahl digestion method. Soil readily oxidizable organic carbon (ROC) was measured by treating air-dried soil with 0.02 mmol L−1 KMnO4. Dissolved organic nitrogen (DON) was determined using a TOC-VCPN analyzer (Shimadzu Scientific Instruments, Columbia). Soil inorganic nitrogen contents (NO3–N and NH4–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

The regression models used in Wang et al. (2016b) were employed to fit the relationship between soil CO_2 emission (*SE*), and soil temperature (*ST*) or soil water (*SW*) with the data from 3 plots and 4 sampling time. However, we only listed the relationship of *SE* and *ST* with exponential regression function, and that of *SE* and *SW* with quadratic regression one, because the two equations fit the best. The differences of soil CO_2 emissions, soil temperature and soil water between each type of ant nests and paired reference soils were compared with a paired *t*-test. The effects of species and sampling period on soil CO_2 emissions, soil temperature and soil water were determined using a two-way ANOVA. Pearson's correlation coefficients were used to express the association of moisture/temperature and other selected soil properties to soil CO_2 emissions and C flux. Data for concentration of CO_2 emissions 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 19.0 package.

3. Results

3.1. Soil CO_2 emission from ant nests

In this study, CO_2 emissions were significantly higher in nests of three ant species [1.14 ± 0.43 (SE) g C m^{-2} h^{-1}] than in the surrounding reference soils [0.55 ± 0.18 (SE) g C m^{-2} h^{-1}] (*t*-test, $t = 6.4$, $p < 0.001$) (Fig. 1A). The CO_2 emissions were 1.6–2.9 times higher from *P. capellini* nests than from the reference soils, while those in nests of *O. transversa* and *P. affinis* were 1.4–2.2 and 1.1–1.9 folds, respectively. There was significant difference in soil CO_2 emission ($F = 12.35$, $df = 2$, $p = 0.001$) among the nests of three ant species (Table 2). The mean soil CO_2 emission in *P. capellini* nests (1.35 ± 0.51 g C m^{-2} h^{-1})

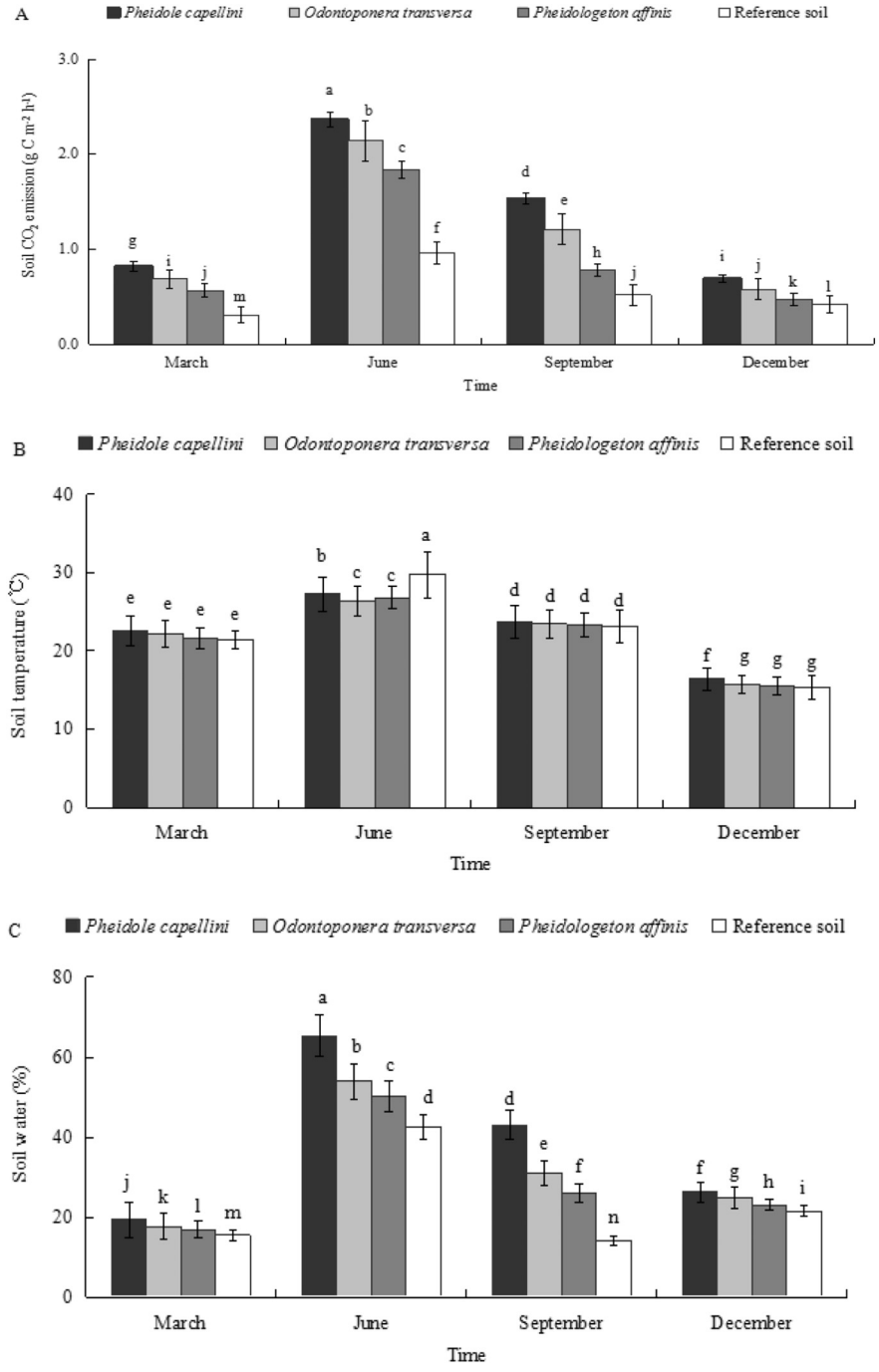

Fig. 1. Temporal variations of soil CO_2 emission (*SE*): A; soil temperature (*ST*): B and soil water (*SW*): C of ant nests and the reference soils in the tropical forest of Xishuangbanna. Bars are mean ± SE. Treatments with the same letter were not significantly different (ANOVA with Tukey-HSD, $p < 0.05$).

was higher than that in nests of *O. transversa* (1.14 ± 0.31 g C m^{-2} h^{-1}) and *P. affinis* (0.91 ± 0.25 g C m^{-2} h^{-1}), respectively (Fig. 1A).

Soil CO_2 emissions differed among sampling months ($F = 21.38$, df = 3, $p < 0.001$) and showed a difference of temporal pattern in ant nests vs. the reference soils (Table 2). The highest CO_2 production in nests of three ant species and the reference soils was all in June (Fig. 1A), which was closely associated with the highest soil temperature (27.5 ± 4.7 °C) and soil water (53.0 ± 9.3 %) in the months (Fig. 1B and C). Whereas, the lowest CO_2 production in nests of three ant species was in December when the soil temperature (15.9 ± 1.8 °C) had minimum values (Fig. 1A and B); in reference soils, the minimum CO_2 emission was in March in coincide with the lowest soil water (14.4 ± 1.4 %) in the months (Fig. 1A and C).

The CO_2 emission in nests of three ant species and reference soils increased exponentially with soil temperature and soil water (Figs. 2 and 3). Although there was no significant difference of temperature between ant nests (22.1 ± 3.7 °C) and the reference soils (22.4 ± 4.8 °C) (*t*-test, $t = 9.6$, $p > 0.05$) (Fig. 1B), soil temperature can explain 53.5–70.1 % of the CO_2 emission from ant nests. In contrast, soil water which was significantly higher in ant nests (33.1 ± 14.7%) than in reference soils (23.5 ± 10.5 %) (*t*-test, $t = 27.5$, $p < 0.001$) (Fig. 1C), can explain 93.4–96.9% of soil CO_2 emission in ant nests. Soil CO_2 emission in ant nests was 1.76–2.65 times higher in wet seasons (June and September) than in dry seasons (April and December).

Although the quantity of CO_2 emission from the ant nests of three species, expressed as g C m^{-2} h^{-1}, was much higher (1.4–2.9 times)

Table 2
Two-way ANOVA showing the F values of species (S) and periods (P) effects on soil CO_2 emissions, soil temperature and soil water in the tropical forest in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Source	df	Soil CO_2 emissions	Soil temperature	Soil water
S	2	12.35**	8.12	11.55*
P	3	21.38**	10.66*	29.04**
S × P	6	9.16*	8.98	13.73*

Significant levels: $^{**}p < 0.01$, $^{*}p < 0.05$.

than the efflux from the surrounding reference soils, the estimated total flux from the nests of three ant species comprised only 0.01–0.54 % of the total annual efflux from the forest floor (Table 3). The contribution of *P. capellini* to soil CO_2 emission was the higher than that of *O. transversa* and *P. affinis* (Table 3).

3.2. Soil properties modified by ant nesting

The soil properties were significantly affected by ant nesting, but these effects varied among three ant species (Table 4). Ant nesting had a significant increase in SW, while there was no difference in ST between each type of nests and its paired reference soil. MBC, TOC, ROC, TN, DON, NH_4^+ and NO_3^- were elevated by ant nesting, while soil bulk density was reduced by ant nesting. *P. capellini* had the greatest SW, pH, MBC, ROC and TOC, and the lowest soil bulk density. Whereas, there were the highest contents in TN, DON, NH_4^+ and NO_3^- in nests of *P. affinis*.

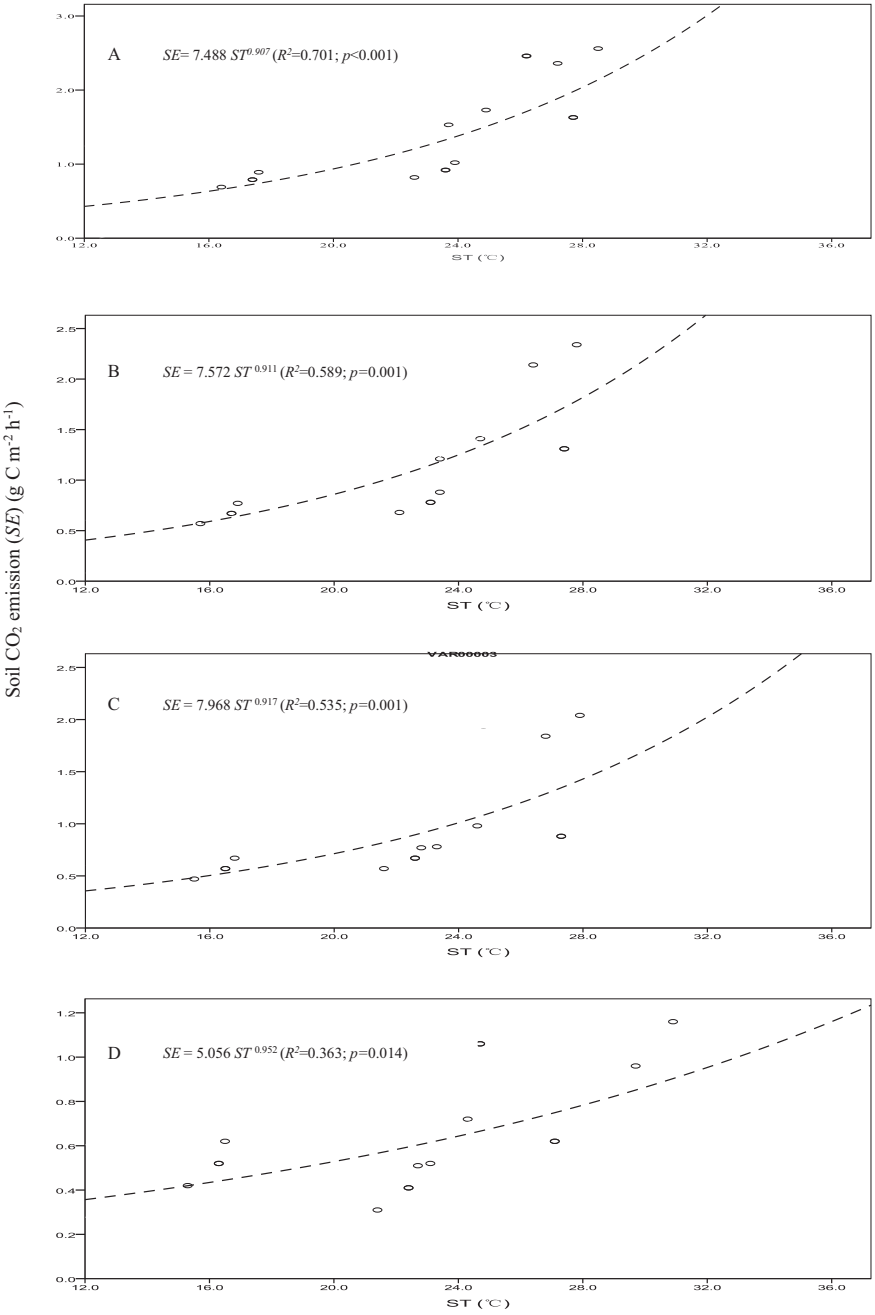

Fig. 2. Regression analyses of soil CO_2 emission (*SE*) and soil temperature (*ST*) at 5 cm soil depth in nests of three ant species: *Pheidole capellini*: A; *Odontoponera transversa*: B, *Pheidologeton affinis*: C and the reference soils: D in the tropical forest of Xishuangbanna.

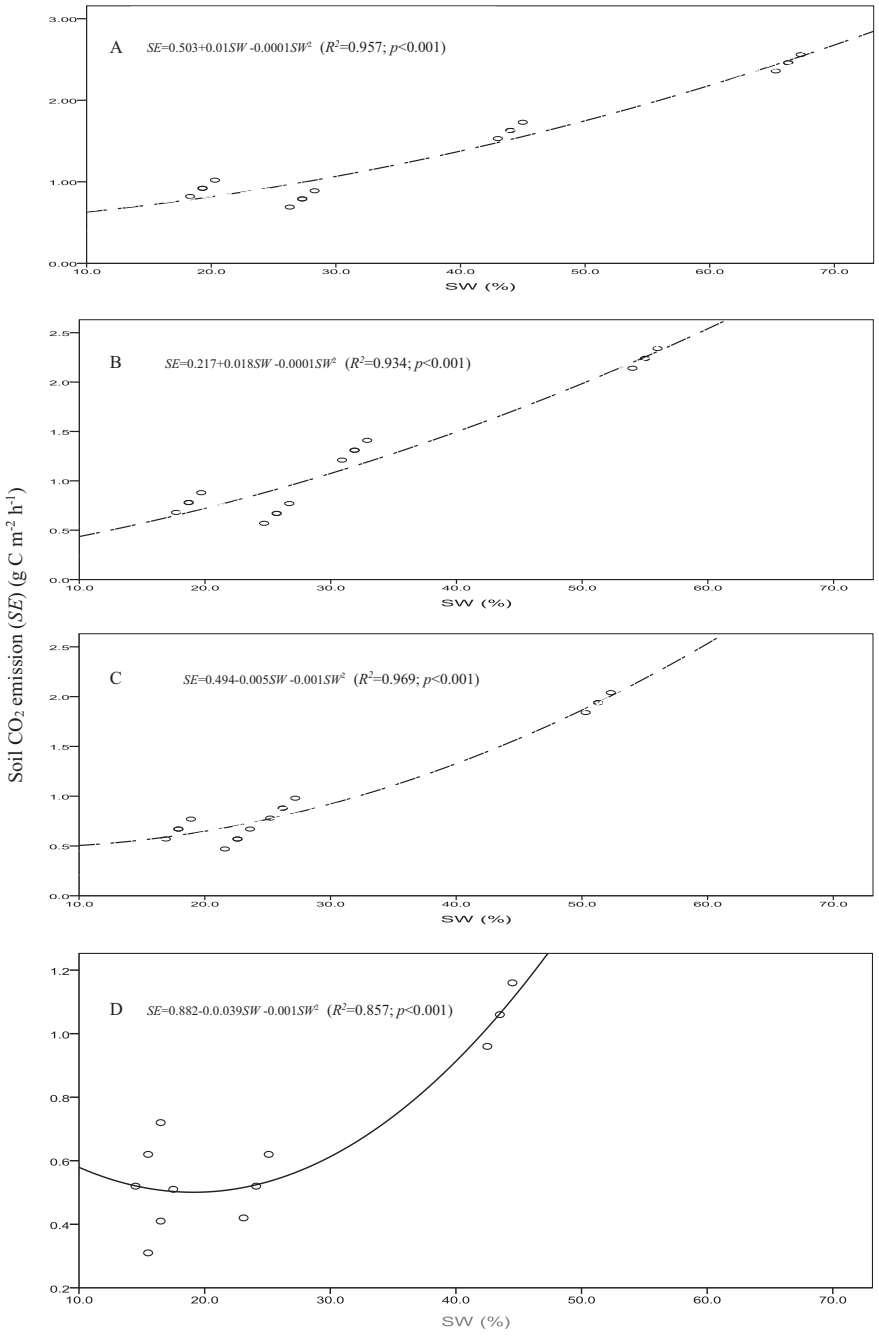

Fig. 3. Regression analyses of soil CO_2 emission (*SE*) and soil water (*SW*) at 5 cm soil depth in nests of three ant species: *Pheidole capellini*: A; *Odontoponera transversa*: B, *Pheidologeton affinis*: C and the reference soils: D in the tropical forest of Xishuangbanna.

3.3. Correlations of soil properties to CO_2 emission and C flux modified by ant nesting

Soil properties modified by ant nesting had a close association to soil CO_2 emission and C flux. The ST, SW, MBC, ROC and TOC were positively correlated with soil CO_2 emission and C flux, whereas pH and soil bulk density showed a negative relationship with soil CO_2 emission and C flux (Table 5). No significant correlations of soil CO_2 emission or C flux can be found to TN, DON, NH_4^+ and NO_3^- (Table 5).

4. Discussion

Our results confirmed that the underground ant nests were sources of CO_2 emissions in the tropical forest ecosystems, though the sizes of ant nests were markedly less than those wood ant nests composed by organic materials in temperate and boreal forests (Ohashi et al., 2005; Domisch et al., 2008; Jilkova et al., 2015a). These may be attributed to

Table 3
Contribution of three ant species to C flux compared to the surrounding reference forest soils in the tropical forest in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Values are Mean ± SE.

	C flux (kg C ha^{-1} a^{-1})	Contribution (%)
Pheidole capellini nests	261.59 ± 76	0.54 ± 0.09
Odontoponera transversa nests	4.94 ± 1.4	0.01 ± 0.008
Pheidologeton affinis nests	19.05 ± 3.6	0.04 ± 0.01
Reference soils	47,849.51 ± 1325	99.45 ± 0.12
Total soils	48,114.48	

Table 4
Soil properties (Mean ± SE) in nests of three ant species and the reference soils in the tropical forest in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Parameter	Reference soils	*Pheidole capellini*	*Odontoponera transversa*	*Pheidologeton affinis*
ST	22.4 ± 2.12^{a}	22.5 ± 2.23^{a}	21.9 ± 2.14^{a}	21.8 ± 2.13^{a}
SW	23.9 ± 9.74^{d}	38.2 ± 14.35^{a}	31.8 ± 12.13^{b}	28.5 ± 10.56^{c}
pH	3.38 ± 0.45^{d}	3.67 ± 0.82^{a}	3.54 ± 0.68^{b}	3.49 ± 0.57^{c}
Bulk density ($g\ cm^{-3}$)	1.37 ± 0.34^{a}	1.01 ± 0.11^{d}	1.24 ± 0.26^{b}	1.19 ± 0.19^{c}
MBC ($g\ kg^{-1}$)	0.61 ± 0.18^{d}	1.84 ± 0.44^{a}	1.65 ± 0.31^{b}	0.74 ± 0.26^{c}
TOC ($g\ kg^{-1}$)	12.16 ± 2.31^{d}	24.02 ± 7.29^{a}	17.05 ± 5.23^{b}	14.73 ± 3.44^{c}
ROC ($g\ kg^{-1}$)	0.55 ± 0.09^{d}	1.21 ± 0.34^{a}	0.83 ± 0.22^{b}	0.67 ± 0.16^{c}
TN ($g\ kg^{-1}$)	1.22 ± 0.25^{c}	1.49 ± 0.17^{b}	1.47 ± 0.16^{b}	1.59 ± 0.33^{a}
DON ($mg\ kg^{-1}$)	19.38 ± 2.25^{c}	26.9 ± 3.36^{b}	27.6 ± 3.44^{b}	30.02 ± 5.32^{a}
NH_4^+ ($mg\ kg^{-1}$)	16.85 ± 1.97^{d}	27.9 ± 4.71^{b}	23.24 ± 3.65^{c}	29.8 ± 7.53^{a}
NO_3^- ($mg\ kg^{-1}$)	21.42 ± 4.14^{c}	24.2 ± 4.26^{c}	26.2 ± 6.57^{b}	31.2 ± 8.31^{a}

The different letters were significantly difference ($p < 0.05$). ST: soil temperature; SW: soil water; Bulk density: BD; MBC: microbial biomass carbon; TOC: total organic carbon; ROC: readily oxidizable organic carbon; TN: total nitrogen; DON: dissolved organic nitrogen; NH_4^+: NH_4–N; NO_3^-: NO_3–N.

large population size of three feeding-behaviors ant species supported by diversity of food resource in the tropical forests (Wang et al., 2016a, 2016b), which exerted a pronounced effect on CO_2 emissions. Furthermore, the honeydew sustained *Pheidole capellini*, and the relevant surplus phloem were spread inside the nest, which might nourish a more CO_2 releasing microflora (Domisch et al., 2009). Probably, CO_2 releasing microflora in nests was also fed by the prey or scavenger or the digested residues brought into by *Odontoponera transversa* and *Pheidologeton affinis*. In this study, the average CO_2 efflux values (0.91–1.35 g C m^{-2} h^{-1}) from the nests of three ant species were 1.4–2.9 times higher in comparison to the surrounding reference soils. The results were lower than the other studies in an alpine forest and a meadow which reported 3.5–12.4 times higher CO_2 efflux from ant mounds compared to surrounding soil in Switzerland (Risch et al., 2005). However, they were similar with the ones of Dauber and Wolters (2000) that observed 1.7–2.7 times higher CO_2 efflux from ant mounds in Germany. Therefore, the underground-nesting ant species in the tropical forest can have considerably contribute to soil CO_2 emissions, though their nest were smaller than the mounds of aboveground nesting ants in temperate and boreal ecosystems. Ants can become the major source of CO_2 emission in ant nests in the tropic, because of the larger population size and nesting activity of the ants.

In this tropical forest, the contributions of ants to soil CO_2 emission varied among ant species. *Pheidole capellini*, a honeydew harvester species, had higher nest CO_2 emission compared to *Odontoponera transversa* and *Pheidologeton affinis*. *P. capellini* ants were observed to have the greatest population and input of nesting materials (e.g. petals) per nest, also larger nest diameter and more complex architecture (Table 1), which exerted a greater effect on soil CO_2 emission from the nests. *O. transversa* with the lowest ant abundance in each nest had second of CO_2 emission, which may owe to bigger individual size. The results indicated that the difference in nest CO_2 emission of three feeding-habits ant species may be mainly determined by the different abundance and body size of ants, diverse inputs of nest materials, and more complicated nest structure in the tropical environment.

The temporal variations in soil CO_2 emissions were significantly affected by soil temperature and soil water modified by ant nesting. In this study, ant nesting significantly increased soil water, and not soil temperature. These resulted in greater effect of soil water on CO_2 emissions compared to soil temperature. Jilkova and Frouz (2014) found that CO_2 emission in the wet nests was much higher (three times) than that in dry soils. The wet conditions inside ant nest strongly stirs microbial activity of decomposition in nests (Frouz, 1996, 2000; Jilkova and Frouz, 2014), which has a significant effect on soil CO_2 production.

Microbial respiration affected by ants can contribute substantially to overall CO_2 emission in soil (Laakso and Setälä, 1997; Stadler et al., 2006). In this study, total microbial biomass is about 1.2–3 times higher in nest soil than in the reference soil, indicating a higher decomposition potential and a higher amount of resources in ant nests than in the reference soils. Simultaneously, we observed significant correlations between microbial biomass and CO_2 emission from nests. Therefore, soil CO_2 produced in the nests can be derived by microbial respiration.

The foraging activities of ants can promote the CO_2 emission through regulating microbial decomposition and respiration in nests, because ants incorporate plenty of honeydew and other organic materials into the nests (Dighton, 1978; Michalzik and Stadler, 2000; Domisch et al., 2009). In this study, the ant effect on CO_2 emission varied with species, which may be closely associated with their different feeding strategies. *P. capellini*, a honeydew harvester species (Wang et al., 2017), had higher nests CO_2 emission than *O. transversa* and *P. affinis*. Readily degradable sugars in the honeydew of aphids diffuse through the soil/nest substrate and activate microbial decomposition and respiration (Joergensen and Scheu, 1999; Seeger and Filser, 2008). Ants are known to accelerate microbial decomposition by their direct physical effects on litter and by their indirect trophic impacts on microorganisms (Stadler et al., 2006).

The microbial activity in forest soils can be limited by many other factors, such as poor physical conditions, low nutrient availability and low pH (Amador and Gorres, 2007). However, the activity of microbial organisms could be enhanced in ant nests, because ants increase nutrient availability and also pH (Joergensen and Scheu, 1999; Jilkova et al., 2012) in their nests, especially when higher water content is maintained in the nests (Frouz, 2000). In this study, ant nesting increased the soil moisture and the availability of carbon and nutrient in nest soils. Especially, pH was significantly elevated in nests of three ant species compared to lower substrate pH value (3.38 ± 0.57). We also observed a significant association of soil water, soil bulk, pH, total organic carbon and readily oxidizable organic carbon to CO_2 emission. However, soil N contents (i.e., TN, DON, NH_4^+ and NO_3^-) had no significant effects on soil CO_2 emission or C flux, which may attributed to no

Table 5
Correlations of selected soil properties with soil CO_2 emission and C flux. Values are Pearson correlation coefficients. $^{**}p < 0.01$, $^{*}p < 0.05$.

Variables	ST	SW	pH	Bulk density	MBC	TOC	ROC	TN	DON	NH_4^+	NO_3^-
Soil CO_2 emission	-0.67^{*}	-0.93^{**}	-0.86^{*}	-0.89^{*}	0.92^{**}	0.88^{*}	0.89^{*}	0.64	0.76	0.68	0.53
C flux	-0.72^{*}	-0.94^{**}	-0.88^{*}	-0.85^{*}	0.97^{**}	0.91^{**}	0.90^{**}	0.73	0.79	0.57	0.42

ST: soil temperature; SW: soil water; Bulk density: BD; MBC: microbial biomass carbon; TOC: total organic carbon; ROC: readily oxidizable organic carbon; TN: total nitrogen; DON: dissolved organic nitrogen; NH_4^+: NH_4–N; NO_3^-: NO_3–N.

N limited but rather C limited in tropical soils. As a result, the ant nests in this study represent favourable micro-environment for decomposition and thus for the release of CO_2 into the forest floor.

We estimated the total annual C flux from underground ant nests to be 0.01–0.54 % of the forest floor efflux, which was much lower than the estimates by Risch et al. (2005), but was similar with the ones reported by Ohashi et al. (2012). In our study, the ant nests do not seem to be an important factor for calculating regional, national, or global CO_2 budgets. However, more research on underground nesting ant species is demanded, especially in the tropics, where ant biomass and diversity are much higher than in temperate and subtropical ecosystems (Wang et al., 2017) and their contribution to the heterogeneity of soil CO_2 emissions may be larger.

5. Conclusion

In this study, these underground nests of three ant species were sources for CO_2 emission, which increased the heterogeneity of soil C flux in the tropical forest ecosystems. These increases may mainly be attributed to the large population size of three feeding-behaviors ant species supported by diversity of food resource in the tropical forest. The diverse contribution among these three ant species to soil CO_2 emission was closely associated with their abundance and body size, and diverse inputs of nest materials by feeding-behaviors. *Pheidole capellini*, a honeydew harvester species, had higher nest CO_2 emission compared to *O. transversa* and *P. affinis*. Furthermore, ants differ in their effects on soil CO_2 emission may be due to the different modification on soil micro-environment (i.e., temperature and moisture), microbial (i.e., MBC) and physico-chemical (i.e., pH, Bulk density, TOC and ROC) properties in the tropical forest soils.

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