



## Short Communication

Species-specific effects of belowground-nesting ants on soil N<sub>2</sub>O emissions in a tropical forestShaojun Wang<sup>a,b,\*</sup>, Qianbin Cao<sup>a</sup>, Qianqian Zuo<sup>a</sup>, Ping Wang<sup>a</sup>, Bo Yang<sup>a</sup>, Shuang Zhao<sup>a</sup>, Minkun Chen<sup>a</sup>, Run Cao<sup>a</sup><sup>a</sup> Department of Ecology and Environment, 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

## ARTICLE INFO

## Keywords:

Belowground-nesting ants

Mediated effect

N<sub>2</sub>O flux

Predator

Tropical forest

## ABSTRACT

Most studies on the contribution of ants to N<sub>2</sub>O emissions focus on above-ground species. However, it is uncertain how belowground-nesting ants affect such gaseous N emissions. Here, we tested the species-specific effects on N<sub>2</sub>O emissions in a tropical forest, of three ant species, namely *Pheidole capellini* (a honeydew harvester), *Odontoponera transversa* (a predator), and *Pheidologeton affinis* (a scavenger). We observed an approximately three-to six-fold increase in N<sub>2</sub>O emissions from nests compared to reference soils. Soil temperature and water increased in ant nests and via regression models explained 54–70% and 78–90% of the emissions respectively. N<sub>2</sub>O emissions were closely associated with mediated effects of ants on carbon and nitrogen pools. The emissions from predatory *O. transversa* nests were about twice as high as from the nests of honeydew-harvester *P. capellini* and saprophagous *P. affinis*. We postulate that these variations in N<sub>2</sub>O emissions among ant species are due to the different extents of modification of soil variables in tropical forest by them.

Atmospheric N<sub>2</sub>O is a major greenhouse gas with a global-warming potential approximately 300 times higher than CO<sub>2</sub> (Battaglia and Joos, 2018; IPCC, 2006). Tropical forest soils can contribute 14–23% of N<sub>2</sub>O emissions to the global budget from natural sources (IPCC, 2007), but it is uncertain how the emissions are regulated in tropical forest soils.

Ants affect N<sub>2</sub>O fluxes through accumulation of food or building materials, deposition of excreta or residues, and modification of soil heterogeneity, as a result of the construction of above- and belowground nests (Cammeraat and Risch, 2008; Wang et al., 2018). Aboveground nests are hot spots for N release, producing 2–17 times higher N<sub>2</sub>O than surrounding soils (Bender and Wood, 2003; Wu et al., 2013). However, little is known about the emissions modulated by belowground ant nests in tropical forest soils.

Ants significantly affect N<sub>2</sub>O effluxes through modifying the properties of nest soils (Wang et al., 2018). Changes in nest temperature, humidity and bulk density alter the oxic and anoxic conditions that affect N<sub>2</sub>O emissions (Wu et al., 2015). The decreased pH, and increased C and N pools in ant nests can impinge on electron donors and acceptors,

as well as diversity of bacterial functional groups (e.g., nitrifying, denitrifying, and N<sub>2</sub>-fixing symbiotic bacteria) that regulate the process of N<sub>2</sub>O emissions (Boots et al., 2012; Frouz and Jilkova, 2008; Lan et al., 2014). In particular, ant species differ in modifications on C and N accumulations due to different feeding and nesting types, which diversely affects N<sub>2</sub>O emissions (Wang et al., 2017).

Ants are often quite abundant with diverse foraging habits due to disparate food resources and micro-climates in tropical forests (Wang et al., 2016). Here, we utilized three belowground-nesting ant species (honeydew-harvester *P. capellini*, predator *O. transversa*, and scavenger *P. affinis*) (Sonthichai et al., 2006) to identify the species-specific effects on N<sub>2</sub>O emissions in a Xishuangbanna tropical forest. We hypothesized that N<sub>2</sub>O emissions vary with ant species due to their different extent of modifications on soil variables.

Within each of three randomly assigned sub-plots (10 m × 10 m; 20 m apart) in three replicated sites (60 m × 50 m, 200 m apart) in *Musa acuminata* communities (Table S1), we surveyed nesting traits (Table 1) for three ant species using a bait method (Wang et al., 2017). In each subplot, N<sub>2</sub>O emissions from five nests of each ant species and five pairs

\* Corresponding author. Department of Ecology and Environment, Southwest Forestry University, 300 Bailongsi, Kunming, 650224, PR China.

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

**Table 1**

The trait of colonies and nests for three ant species in Xishuangbanna tropical forest

Ant species	Abundance (ind. nest <sup>-1</sup> )	Body size (mm)	Nest number per plot	Nest density (nests ha <sup>-1</sup> )	Nest diameter (cm)	Nest Depth (cm)	Nest architecture Above- and belowground
<i>Pheidole capellini</i>	340 ± 32 <sup>a</sup>	2.3 ± 0.2 <sup>b</sup>	38 ± 9 <sup>a</sup>	3800 ± 231 <sup>a</sup>	10.6 ± 2.5 <sup>a</sup>	9.2 ± 1.6 <sup>a</sup>	4 cm piled petals for nesting; 4 chambers in first layer (4 cm depth), 1 chambers in second layer
<i>Odontoponera transversa</i>	47 ± 11 <sup>c</sup>	7.8 ± 0.4 <sup>a</sup>	11 ± 4 <sup>c</sup>	1100 ± 84 <sup>c</sup>	8.8 ± 0.2 <sup>c</sup>	10.3 ± 2.2 <sup>a</sup>	Few scattered soils; 3 chambers in first layer (5 cm depth), 2 chamber in second layer
<i>Pheidologeton affinis</i>	210 ± 18 <sup>b</sup>	1.0 ± 0.1 <sup>c</sup>	24 ± 6 <sup>b</sup>	2400 ± 106 <sup>b</sup>	9.8 ± 1.7 <sup>b</sup>	8.1 ± 1.0 <sup>a</sup>	Few scattered soils; 3 chambers in first layer (3.5 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. SE: standard error. Different lowercase letters indicate significant differences ( $p < 0.05$ ) between the ant species.

of reference soils were measured tri-monthly from March 2017 to June 2018, using a static chamber technique (Bender and Wood, 2003; Jílková et al., 2015). We explored the association of N<sub>2</sub>O emissions to soil variables. The details of the study methods are presented in the supplementary material.

The belowground nests emitted 2.7–6 times higher N<sub>2</sub>O than nearby reference soils (Fig. 1A, Table S2). The emissions were distinctly higher than the values recorded for aboveground nests in subtropical (1.5 times) (Wu et al., 2013) and Mexico rainforest (3 times) (Majeed et al., 2018). In contrast, they were lower than those in a bermudagrass pasture (16.5 times) (Bender and Wood et al., 2003). Relatively high nest emissions in the tropical forest may result from substantial N input by large ant populations favored by disparate food resources and micro-climates (Wang et al., 2017). The incorporation of food (remnants) into the nests by ants might greatly affect microbial development, thus increasing the emissions (Roxo et al., 2010).

The presence of ants increased nest temperature and moisture compared with reference soils (Fig. 1B and C), thereby stimulating the emissions of N<sub>2</sub>O. Soil water contributed more (78–90%) to explanation of the temporal variations in N<sub>2</sub>O than soil temperature (54–70%) (Figs. 2 and 3). Higher level of soil water is beneficial for nitrification as well as denitrification (Cammaraat and Risch, 2008; Wu et al., 2013). In particular, a higher N<sub>2</sub>O emission from *O. transversa* nests may have been due to structurally more complex belowground nests with resultant holding higher water content in vertical tunnels and horizontal chambers, thus favoring soil microorganisms (Frouz and Jílková, 2008).

Ant activity decreased bulk density and increased microbial carbon in belowground nests (Table S3;  $p < 0.05$  or 0.01), resulting in a significant increase of N<sub>2</sub>O emissions. The decrease in bulk density is likely to increase soil permeability, which stirs the microbial nitrification and denitrification (Dambreville et al., 2008; Xiong et al., 2015). The increased microbial carbon (~3 times) hinted a higher microbial activity in ant nests, which induced a higher potential of increasing the emissions (Gu et al., 2018).

The size of soil C and N pools was also elevated in nest soils (Table S3;  $p < 0.05$  or 0.01). Carbon and nitrogen input into ant nests are the most critical factor for acceleration of N<sub>2</sub>O emissions, as low nutrients availability can limit microbial processes of nitrification and denitrification (He et al., 2018). In particular, the inorganic N components, i. e., NH<sub>4</sub><sup>+</sup> (2.4–3.8 times) and NO<sub>3</sub><sup>-</sup> (2.9–4.2 times), were observably

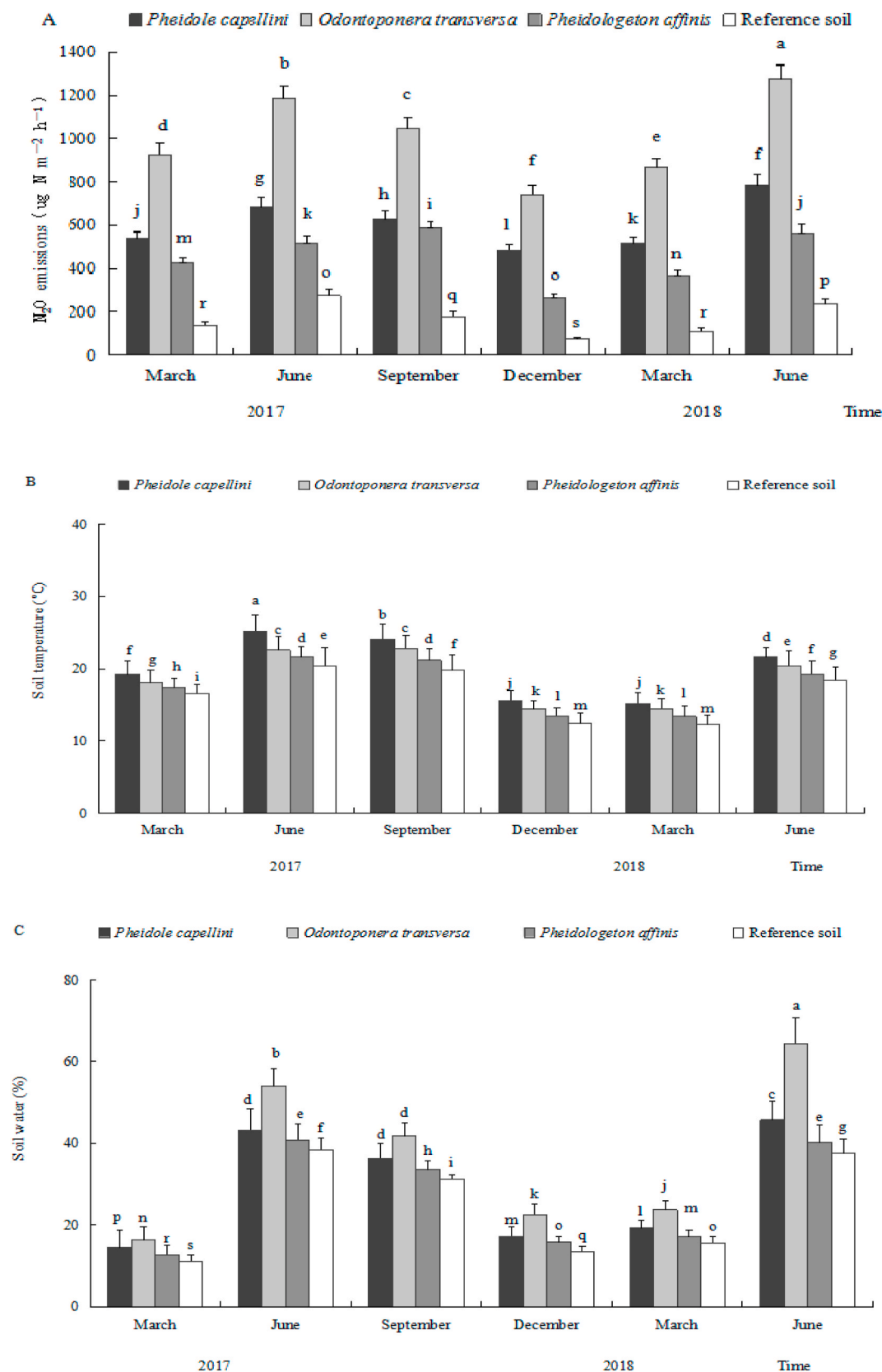
increased in ant nests compared with reference soils (Table S3;  $p < 0.05$  or 0.01). A higher NH<sub>4</sub><sup>+</sup> concentration can stimulate N<sub>2</sub>O emissions through increasing nitrification rate, while a higher level of NO<sub>3</sub><sup>-</sup> increases the emissions via denitrification (Zhu et al., 2015).

The contribution of belowground nests to N<sub>2</sub>O emissions varied with ant species. *O. transversa* ants had the lowest abundance and nest density, but exhibited the highest emissions. This was closely correlated with higher level of microbial carbon and C and N pools in their nests (Table S4). The broad-spectrum diet of these predatory ants, including invertebrate prey and seed elaiosomes (Hölldobler and Wilson, 2008), exhibits a high N concentration, serving as N-rich substrate for potential microbial nitrification and denitrification (Kotova et al., 2015; Majeed et al., 2018). The higher C diets of honeydew-harvester *P. capellini* ants could nourish additional N<sub>2</sub>O-releasing microbiota (Benckiser, 2010; Thompson et al., 2016), their nests thus had the second highest emissions. This might have been due to a higher population that integrated greater C materials; however, this ant species could not supply sufficient N substrates to meet the needs of microorganisms. Scavenger *P. affinis* had a smaller size of C and N pool, which ranked the lowest emissions among three ant species.

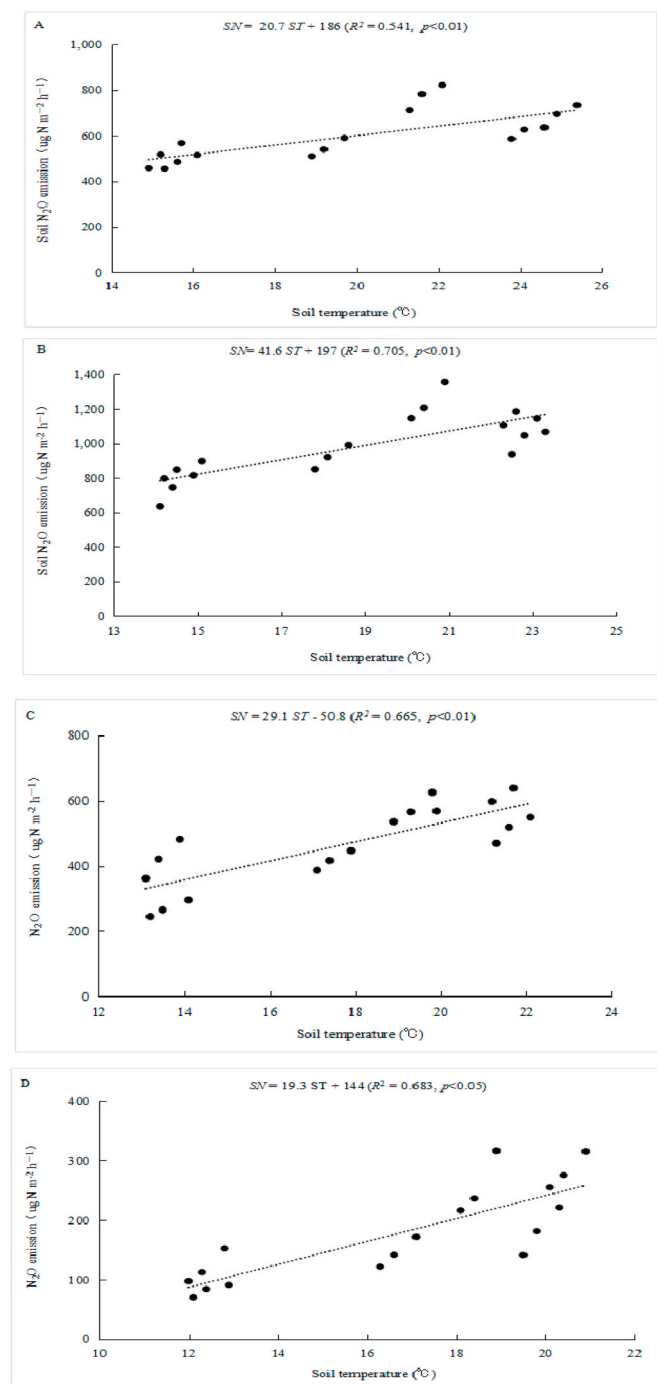
The belowground-nesting ants contributed 0.4–1.2% of the total forest floor efflux (Table S5), which was comparable with the values recorded for aboveground nests in an American pasture (1%). They had similar contribution to N efflux as aboveground-nesting ants, though the size of these belowground nests was smaller. This may be due to the higher ant populations that incorporated richer C and N substrates into their nests to increase the emissions. In particular, N<sub>2</sub>O emissions seemed to have a close association with feeding habits of ants that affected the nature of the food incorporated into nests. In the future studies, replicated feeding habits of these ant species would be utilized to test the hypothesis about what role the different feeding habits have.

#### Declaration of competing interest

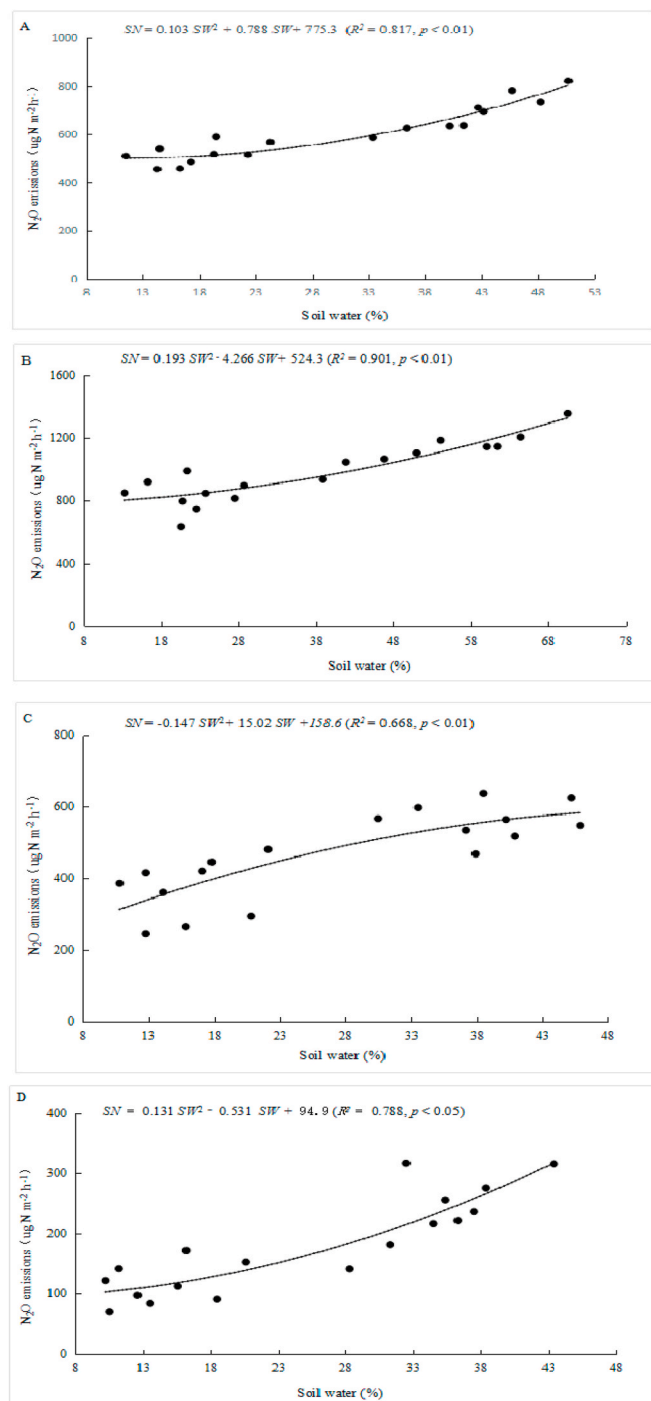
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



**Fig. 1.** Temporal variations in  $\text{N}_2\text{O}$  emissions (A), temperature (B), and water (C) in the nests of three ant species and their reference soils in Xishuangbanna tropical forest. Bars are mean  $\pm$  SE. SE: standard error. Treatments with the different letters are significantly different (ANOVA with Tukey's Honest Significant Difference,  $p < 0.05$ ).



**Fig. 2.** Regression analyses of soil  $N_2O$  emissions (SN) and soil temperature (ST) at 15 cm depth in the nests of three ant species (i.e., *Pheidole capellini*: A, *Odontoponera transversa*: B, and *Pheidologeton affinis*: C), and in the reference soils (D) in Xishuangbanna tropical forest.



**Fig. 3.** Regression analyses of soil  $N_2O$  emissions (SN) and soil water (SW) at 15 cm depth in the nests of three ant species (*Pheidole capellini*: A, *Odontoponera transversa*: B, and *Pheidologeton affinis*: C), and in the reference soils (D) in Xishuangbanna tropical forest.

## Acknowledgments

This research was supported by the National Nature Science Foundation of China (31660191 and 41461052), China 948 Program of the National Forestry Bureau (2015-4-39). We appreciate the anonymous reviewer who made a number of very insightful points and suggestions for improvement of iterations of this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.108020>.

## References

- Battaglia, G., Joos, F., 2018. Marine N<sub>2</sub>O emissions from nitrification and denitrification constrained by modern observations and projected in multi-millennial global warming simulations. *Global Biogeochem Cycles*. 32, 92–121.
- Bender, M.R., Wood, C.W., 2003. Influence of red imported fire ants on greenhouse gas emissions from a piedmont plateau pasture. *Commun Soil Sci Plant Anal*. 34, 1873–1889.
- Benckiser, G., 2010. Ants and sustainable agriculture. A review. *Agron Sustain Dev*. 30, 191–199.
- Boots, B., Keith, A.M., Niechoj, R., Breen, J., Schmidt, O., Clipson, N., 2012. Unique soil microbial assemblages associated with grassland ant species with different nesting and foraging strategies. *Pedobiologia* 55, 33–40.
- Cammeraat, E.L.H., Risch, A.C., 2008. The impact of ants on mineral soil properties and processes at different spatial scales. *J. Appl. Entomol.* 132, 285–294.
- Dambreville, C., Morvan, T., Germon, J.C., Dambreville, C., Morvan, T., Germon, J.C., 2008. N<sub>2</sub>O emission in maize-crops fertilized with pig slurry, matured pig manure or ammonium nitrate in brittany. *Agric Ecosyst Environ*. 123, 201–210.
- Frouz, J., Jilkova, V., 2008. The effect of ants on soil properties and processes (*Hymenoptera: Formicidae*). *Myrmecol News*. 11, 191–199.
- Gu, L., Huang, B., Lai, C., Xu, Z., He, H., Pan, X., 2018. The microbial transformation of <sup>17</sup>β-estradiol in an anaerobic aqueous environment is mediated by changes in the biological properties of natural dissolved organic matter. *Sci Total Environ*. 641, 631–632.
- He, T., Li, Z., Xie, D., Sun, Q., Xu, Y., Ye, Q., Ni, J.P., 2018. Simultaneous nitrification and denitrification with different mixed nitrogen loads by a hypothermia aerobic bacterium. *Biodegradation* 29, 1–12.
- Hölldobler, B., Wilson, E.O., 2008. *The Superorganism: the Beauty, Elegance, and Strangeness of Insect Societies*. W.W. Norton and Company, New York City, p. 576.
- Ipcc, 2006. In: Eggleston, H.A., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.), *IPCC Guidelines for National Greenhouse Gas Inventories*. Prepared by the National Greenhouse Gas Inventories Programme. IGES, Japan.
- Ipcc, 2007. *Climate Change, 2007, the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.
- Jilkova, V., Cajthaml, T., Frouz, J., 2015. Respiration in wood ant (*Formica aquilonia*) nests as affected by altitudinal and seasonal changes in temperature. *Soil Biol. Biochem.* 86, 50–57.
- Kotova, A.A., Umarov, M.M., Zakalyukina, Y.V., 2015. Features of nitrogen and carbon transformation in nests of soil ants. *Moscow Univ. Soil Sci. Bull.* 70, 25–28.
- Lan, T., Han, Y., Roelcke, M., Nieder, R., Cai, Z., 2014. Sources of nitrous and nitric oxides in paddy soils: nitrification and denitrification. *J Environ Sci*. 26, 581–592.
- Majeed, M.Z., Miambi, E., Barois, I., Bernoux, M., Brauman, A., 2018. Characterization of N<sub>2</sub>O emissions and associated microbial communities from the ant mounds in soils of a humid tropical rainforest. *Folia Microbiologica* 63, 381–389.
- Roxo, E., Campos, A.E.C., Alves, M.P., Couceiro, P.M.R., Harakava, R., Melo, F.A.F., 2010. Ants' role (*Hymenoptera: Formicidae*) as potential vectors of Mycobacteria dispersion. *Arq. Inst. Biol.* 77, 359–362.
- Sonthichai, S., Gavinjan, N., Suwannaratana, S., Jaitrong, W., 2006. A comparison of ant populations in restored forest of different ages and adjacent natural vegetation in northern Thailand. *Witthayasan Kasetsat*. 40, 882–889.
- Thompson, K.A., Bent, E., Abalos, D., Wagner-Riddle, C., Dunfield, K.E., 2016. Soil microbial communities as potential regulators of in situ N<sub>2</sub>O fluxes in annual and perennial cropping systems. *Soil Biol Biochem.* 103, 262–273.
- Wang, S.J., Wang, H., Li, J.H., 2016. Distribution characteristics of ant mounds and correlating factors across different succession stages of tropical forests in Xishuangbanna. *Biodivers. J.* 24, 916–921.
- Wang, S.J., Wang, H., Li, J.H., 2017. Ants can exert a diverse effect on soil carbon and nitrogen pools in a Xishuangbanna tropical forest. *Soil Biol Biochem.* 113, 45–52.
- Wang, S., Li, J., Zhang, Z., Cao, R., Chen, M., Li, S., 2018. The contributions of underground-nesting ants to CO<sub>2</sub> emission from tropical forest soils vary with species. *Sci Total Environ*. 630, 1095–1102.
- Wu, H.T., Lu, X.G., Wu, D.H., Song, L.H., Yan, X.M., Liu, J., 2013. Ant mounds alter spatial and temporal patterns of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions from a marsh soil. *Soil Biol Biochem.* 57, 884–891.
- Wu, H.T., Lu, X.G., Tong, S.Z., Batzer, D.P., 2015. Soil engineering ants increase CO<sub>2</sub> and N<sub>2</sub>O emissions by affecting mound soil physicochemical characteristics from a marsh soil: a laboratory study. *Appl. Soil Ecol.* 87, 19–26.
- Xiong, Z.Q., Li, S.C., Yao, L., Liu, G.H., Zhang, Q.F., Liu, W.Z., 2015. Topography and land use effects on spatial variability of soil denitrification and related soil properties in riparian wetlands. *Ecol. Eng.* 83, 437–443.
- Zhu, T., Zhang, J., Ping, H., Long, S., Cong, W., Ding, W., Meng, L., Zhou, K., Hu, Z., 2015. N<sub>2</sub>O emissions from banana plantations in tropical China as affected by the application rates of urea and a urease/nitrification inhibitor. *Biol. Fertil. Soils*. 51, 673–683.