

Respiration of wood ant nest material affected by material and forest stand characteristics

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Abstract: We studied differences in respiration of materials from different parts of wood ant nest (top, bottom, and rim) and from the nest surroundings (humus layer and mineral soil). Samples were taken from 8 wood ant (*Formica aquilonia*) nests in each of the two types of forest (birch and pine) in eastern Finland. The differences were related to material and forest stand characteristics (i.e., moisture, pH, carbon content, and C:N ratio). As a result, the highest respiration per g DW was measured at the top of ant nests in the birch forest. However, respiration did not significantly differ between the parts of ant nests in the pine forest. Respiration of the humus layers in both forest stands was on average higher, whereas respiration of the mineral soils in both forest stands was lower in comparison with respiration of the nest materials. The respiration per g C did not show any significant differences between different parts of nests and surrounding soil. The most important factors influencing respiration of the materials appeared to be moisture, carbon content, and pH. In conclusion, respiration of wood ant nest material is affected by the specific material and forest stand characteristics.

Key words: *Formica aquilonia*; birch forest; pine forest; CO₂; moisture; carbon content; pH

Introduction

Wood ants (*Formica* s. str., Hymenoptera: Formicidae) include species of ants that are dominant in temperate and boreal forest ecosystems (Dlusskij 1967). They are widespread from the temperate zone of Europe and Asia to the North-Palaeartic area, even north of the Arctic Circle (Czechowski et al. 2002; Puntila & Kilpeläinen 2009). Wood ant species are mainly associated with coniferous and mixed forests, although they occur also in deciduous forests (Czechowski et al. 2002).

Due to their foraging and building activities they affect especially nutrient cycling (Frouz et al. 2005; Frouz & Jílková 2008). Recently, their nests were found to be “hot spots” for CO₂ production (Ohashi et al. 2005; Risch et al. 2005; Domisch et al. 2006). This CO₂ originates from several sources: ant respiration, decomposer community respiration, and respiration of roots penetrating into the belowground parts of the nest (Lenoir et al. 2001; Risch et al. 2005). In our study, we focused on the decomposition activity of organisms (i.e., CO₂ efflux), which is an important part of the carbon cycle (Steubing 1970). The knowledge of CO₂ sources and their dependence on environmental characteristics is important to the understanding of ecosystem carbon balance in forest ecosystems.

Respiration of the decomposer community can be influenced by many material properties, such as mois-

ture, acidity, carbon content, C:N ratio, or the relation between cellulose and lignin (Edwards et al. 1970; Steubing 1970; Paul & Clark 1996). Although the ant nest material is reported to be drier in comparison to the nest surroundings, decomposition processes are rather favoured there (Frouz et al. 1997; Laakso & Setälä 1997; Lafleur et al. 2002). Since the water content differs between the parts of the nest, the moisture is highest in the upper part of the nest where there was also measured the highest temperature (Coenen-Staß et al. 1980). Thus, microbial activity and decomposition processes are encouraged especially in moist surface layers of ant nests (Laakso & Setälä 1998; Dauber et al. 2001; Frouz et al. 2003). Wood ant nests and their close vicinity are usually reported to be less acidic in comparison with the wider surroundings (Jílková et al. 2011, 2012), which could also favour the activity of bacteria and actinomycetes, not only fungi typical for acidic soils (Paul & Clark 1996). Because of foraging and building activities of ants, amount of organic matter (i.e. carbon content) is enhanced in ant nests (Frouz et al. 1997), but the quality of organic matter (C:N ratio and the relation between cellulose and lignin) depends on the source of organic matter and thus on the forest stand (Edwards et al. 1970).

In this study we examined material respiration in different parts of ant nests in two forest stands and in the humus layer and mineral soil from the surroundings

Table 1. Properties of materials from the respective sampling locations at the birch and the pine forest.

Sampling location	Moisture (%)		pH		Carbon content (%)		C:N ratio	
	$F_{(4,56)} = 5.04 P < 0.05$		$F_{(4,56)} = 6.16 P < 0.001$		$F_{(4,56)} = 6.79 P < 0.001$		$F_{(4,56)} = 15.76 P < 0.001$	
	Birch	Pine	Birch	Pine	Birch	Pine	Birch	Pine
TOP	41.15 ± 1.85a	19.55 ± 1.40w	4.92 ± 0.14a	4.50 ± 0.05w	43.08 ± 0.71a	52.30 ± 1.11w	20.97 ± 0.13a	67.70 ± 6.08w
BOTTOM	22.50 ± 1.43b	16.80 ± 1.33w	5.23 ± 0.08a	5.19 ± 0.05x	32.58 ± 0.88b	25.40 ± 5.44x	19.22 ± 0.78a	30.27 ± 2.81x
RIM	21.80 ± 5.00b	11.46 ± 1.22w	5.24 ± 0.07a	4.86 ± 0.06y	12.07 ± 4.14c	9.14 ± 0.26y	19.05 ± 0.42a	30.12 ± 0.15x
HUMUS	59.01 ± 2.18c	51.19 ± 4.97x	3.97 ± 0.07b	3.73 ± 0.07z	45.44 ± 1.01a	29.74 ± 2.55x	22.50 ± 0.80a	31.23 ± 0.35x
SOIL	16.15 ± 1.82b	20.00 ± 0.75w	4.81 ± 0.12a	5.18 ± 0.03x	0.63 ± 0.03d	2.23 ± 0.16y	30.78 ± 3.08b	36.07 ± 1.46x

Explanations: TOP refers to the top of the ant nests, BOTTOM to the base of the nests, RIM to the periphery of the nests, and HUMUS and SOIL to the humus layer and mineral soil, respectively, as controls. Mean ± SEM and results of three-way ANOVAs are shown. Statistically homogeneous groups of values of the same material property are marked by the same letter (one-way ANOVA, Tukey HSD post hoc test).

as controls. We related the differences to microsite and forest stand characteristics. Our hypotheses were that (1) material respiration is the highest in the top of the nests, (2) the differences in material respiration are explained by material properties (e.g., moisture, pH, carbon content), and (3) the forest stand characteristics are responsible for the differences in material respiration, especially due to carbon content of the litter.

Material and methods

Study sites

The study was conducted in August 2009 in two forest stands, birch (*Betula pendula* Roth) and pine (*Pinus sylvestris* L.), in eastern Finland near Joensuu (62°39'28" N, 29°39'38" E, birch stand; 62°41'20" N, 29°44'33" E, pine stand). The birch forest was a 47-year old stand with a tree density of 2,376 trees per ha and an ant nest density of 9.9 nests per ha. Ant nests in the birch forest were constructed mainly from birch twigs, bark, and leaves. However, a small portion of the pine needle material was also included since the birch forest stand was situated ca. 100 m from the pine forest stand. The pine forest was a 37-year old stand with a tree density of 2,235 trees per ha and an ant nest density of 6.3 nests per ha. Ant nests in the pine forest were constructed mainly from pine twigs and needles, with a considerable amount of conifer resin.

Sampling design

Samples of materials were taken with a shovel from 8 mounds of *Formica aquilonia* Yarrow, 1955 ants in both forest stands. Sampling locations were the top (20 cm below the surface), the bottom (the centre of the mounds on the level of the forest floor) and the soil rim of the mounds (the periphery of the mounds), and the humus layer and mineral soil approximately 10 m from an adjacent mound as controls. In total, 80 material samples were taken. Fresh samples were immediately transported to the laboratory for analyses.

Material analyses

Respiration of the materials, moisture, pH, and content of carbon and nitrogen were measured. Respiration was established using 5 g of the fresh materials after a two-day incubation at 20°C by titration of NaOH according to Page (1982). Moisture was determined as a difference between fresh mass and dry mass after drying at 105°C for 12 h. pH was measured in a 1:10 material:water suspension with a glass electrode. The organic matter content was assessed

based on loss-on-ignition after 5 h in 600°C. The content of carbon and nitrogen was analyzed in samples of dry crushed soil using elemental analyzer EA 1108 (Carlo Erba Instruments).

Statistical analyses

Three-way ANOVA with forest stand, sampling location and nest as categorical variables, with nest as a random effect nested in forest stand, were used to analyze differences in material properties. If the interaction between forest stand and sampling location was significant, one-way ANOVA and post hoc test (Tukey HSD) was performed to assess differences between the sampling locations. All computations were made using the program Statistica 10.

Results

Material respiration per g DW soil differed significantly between the sampling locations and forest stands (Fig. 1). Differences were more pronounced in the birch forest than in the pine forest. The highest respiration was measured at the top of ant nests in the birch forest, where it was seven times higher than respiration at the top of ant nests in the pine forest. The bottom and rim in both forest stands, as well as the top of ant nests in the pine forest were not markedly different in material respiration. Respiration of the humus layers in both forest stands was significantly higher than respiration of mineral soils; it was also higher than respiration at the bottom and rim of ant nests in both forest stands, whereas respiration of the mineral soils was lower. When we calculated respiration per g C, the differences between individual parts of nests and nest surroundings were not significant.

The other material properties also considerably differed between the two forest stands (Table 1). Ant nests in the birch forest were on average moister than ant nests in the pine forest with the highest moisture at the top of ant nests. Material pH was similar in both forest stands and was lower at the tops of ant nests in comparison with the bottoms and rims. The carbon content was the highest at the tops of ant nests in both forest stands with greater differences between sampling locations in the pine forest. C:N ratio was on average higher in the pine forest with the highest value at the

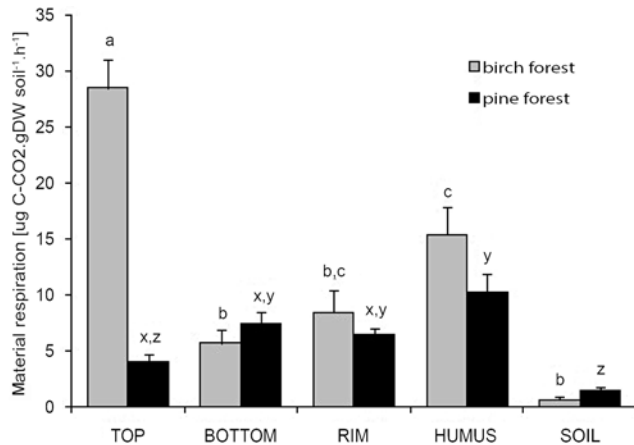


Fig. 1. Respiration of materials per g DW soil from sampling locations in the birch and pine forests (three-way ANOVA, $F_{4,56} = 22.35$, $P < 0.001$). TOP refers to the top of the ant nests, BOTTOM to the base of the nests, RIM to the periphery of the nests, and HUMUS and SOIL to the humus layer and mineral soil, respectively, as controls. Columns with different letters are statistically different (one-way ANOVA, Tukey HSD post hoc test). Means \pm SEM are shown.

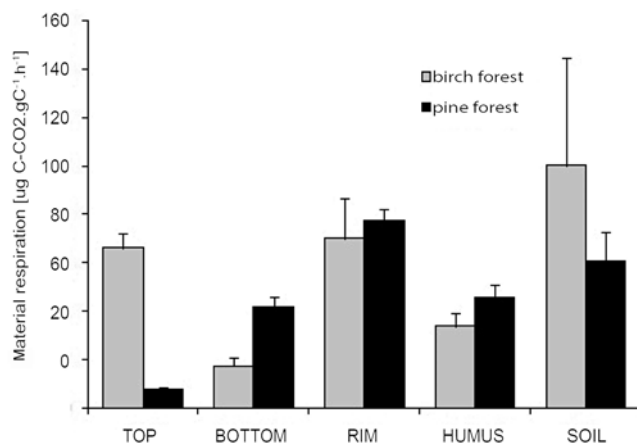


Fig. 2. Respiration of materials per g carbon from sampling locations in the birch and pine forests (three-way ANOVA, $F_{4,56} = 1.42$, $P = 0.24$). TOP refers to the top of the ant nests, BOTTOM to the base of the nests, RIM to the periphery of the nests, and HUMUS and SOIL to the humus layer and mineral soil, respectively, as controls. Means \pm SEM are shown.

top of ant nests. In the birch forest, C:N ratio did not differ between the sampling locations.

Discussion

In agreement with our hypotheses, material respiration per g DW differed between the sampling locations and, also, between the two forest stands. This confirms earlier findings that the same species can affect soil properties differently in different ecosystems (Frouz et al. 2003; Holec & Frouz 2006). Respiration of the material from the top of ant nests in the birch forest was significantly higher than respiration in the other parts of ant nests. Since litter decomposition is a complex process dependent on moisture, temperature, or litter

quality (Edwards et al. 1970; Steubing 1970; Paul & Clark 1996), this significant difference could be most probably explained by higher moisture of the material from the top of ant nests together with quite low C:N ratio in the birch forest in comparison with the pine forest. However, in the pine forest the respiration of the nest materials from the different parts of ant nests was similar. The reason for missing significant differences between microsites within ant nests could be similar moisture in all the materials and C:N ratio in the material from the top twice as high as in the bottom and rim of ant nests. Despite the low pH of the humus layers in both forest stands, the humus layer respiration was higher than in the bottoms and rims of ant nests, which could be caused by higher moisture and carbon content. Apparently, the moisture, C:N ratio, and carbon content, separately or in combination, had an important effect on material respiration in our samples.

Even though the temperature is considered to be the most important factor influencing the rate of organic matter turnover, its overall effect is complicated by the interaction between temperature and moisture (Edwards et al. 1970). In addition to higher temperatures recorded inside ant mounds (Coenen-Staß et al. 1980), an increase of moisture was found stimulating decomposition processes of mound material (Frouz 1996; Lenoir et al. 2001). But as mounds are usually drier than the surrounding forest floor due to ant constructing activities (Seifert 1996; Lafleur et al. 2002), decomposition processes can be lowered there (Lenoir et al. 2001; Domisch et al. 2008). Nevertheless, decomposition in ant nests in the pine forest was not decreased due to lower moisture content, which suggests that there are other properties affecting this process, e.g. carbon availability. On the other hand, materials from ant nests in the birch forest had on average higher moisture than in the pine forest, which means that the litter decomposition was promoted there probably due to higher moisture content. This result is in accordance with results of Coenen-Staß et al. (1980), who found higher moisture inside ant nests, and thus increased microbial activity.

Nevertheless, the material respiration was affected not only by moisture, but also by the C:N ratio, which was found to be lower in the birch forest and thus to be another factor stimulating decomposition in this forest stand. C:N ratio at the top of ant nests in the pine forest was twice as high in comparison with the bottom and the rim. Mound material at the top of ant nests in the pine forest consists predominantly of needles, whereas the bottom and the rim consist of the mixture of needles and mineral soil (Kristiansen & Amelung 2001). Needles from coniferous trees contain resistant constituents such as cellulose and lignin, and often have large C:N ratio (Steubing 1970). Hence, the C:N ratio at the top of ant nests in the pine forest was affected by the high C:N ratio of needles.

However, since the availability of carbon is very important for the respiration of organisms (Schulze et al. 2000), we calculated the respiration per g C and

it showed different results than the respiration per g DW. The respiration increased in ant nests, especially in comparison to the humus layers. The most important properties that probably affected the respiration were pH of the material and availability of carbon. Higher pH favors the activity of bacteria more than that of fungi (Paul & Clark 1996). Since bacteria have higher rate of metabolism, it can thus lead to higher CO₂ production. In ant nests, ants accumulate vast amounts of food, mainly honeydew, which consists of simple sugars easily available to microorganisms. Microorganisms have enough energy for decomposition processes and that also leads to higher CO₂ production (i.e., priming effect).

In conclusion, our data showed that material decomposition, and thus CO₂ efflux, in our forest stands are affected mainly by moisture, carbon content and availability, C:N ratio, and pH. Although we examined ant mounds of the same species (*Formica aquilonia*) in both forest stands, material properties of ant nests differed between the birch and the pine forests. From this we can conclude that nest material properties are affected not only by the ant species present and its way of life, feeding and nest construction (Petal 1978), but also by the forest stand characteristics.

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