The effect of wood ants (*Formica s. str.*) on soil chemical and microbiological properties

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Abstract
The wood ants are very important for boreal forest ecosystems. They substantially affect soil properties, both in their nests and in the nest surroundings. In this study we focused especially on changes in soil pH and activity of microorganisms. Samples were taken from eight ant nests in each of the two types of forest, both in eastern Finland. Samples were taken at four sampling locations, i. e., from the bottom of the nest, the top, the rim and the control (> 3m from each nest). Soil respiration, pH, water content and organic matter content were measured. Soil respiration was higher in ant nests, although there was lower moisture. This could be explained by the import of easily available substances in prey and honeydew into the nest. pH values were higher in ant nests. This is most probably caused by enhanced contents of basic cations that are usually found in ant nests. Differences in organic matter content were found between individual nest sampling locations, which could be caused by differences in ant nest construction. The differences between the two types of forest show that the influence of very similar ant species differs in dependence on environmental conditions.

Key Words
Birch forest, pine forest, humus layer, soil mineral layer, *Formica aquilonia*, *Formica rufa*.

Introduction
Wood ants (*Formica s. str.*, Hymenoptera: Formicidae) are very important for temperate and boreal forest ecosystems because they affect many soil properties (Dlusskij 1967; Frouz and Jilkova 2008). They are considered to be ecosystem engineers (Jones *et al.* 1994; Jouquet *et al.* 2006). They affect soil pH in their nests and their surroundings by mixing soil layers and bringing food into their nests (Frouz *et al.* 2003). Through their activities they maintain stable temperature regime (Frouz and Finer 2007) and they also affect the composition of soil microorganisms in their nests (Czerwiński *et al.* 1971; Petal *et al.* 2003). Through these effects, soil respiration in ant nests could be different from that of the nest surroundings. In this study we focused especially on differences in soil respiration, soil pH, water content and organic matter content between individual sampling locations in ant nests and between ant nests and their surroundings.

Methods
Sampling sites and study design
This study was conducted in two types of forest in eastern Finland in August 2009. The first type was a birch (*Betula spp.*) dominated forest and the second type was a pine (*Pinus sylvestris*) dominated forest. We selected 8 ant nests in each type of forest (a birch forest – *Formica aquilonia*, a pine forest – *Formica rufa*). Soil samples were taken at four sampling locations at each nest, i.e., from the top of the nest, the bottom, the rim, and the control (>3 m away of the nest). Samples were taken from the humus layer and from the soil mineral layer at control locations. Soil samples were then stored in a refrigerator for measuring soil respiration and water content, and then they were dried at 70°C for measuring pH and organic matter content.

Analyses of soil samples
For soil respiration, 10g of soil sample was incubated in a 100ml bottle for two days with 3 mL of 1N NaOH at 25°C and then NaOH was titrated with HCl (Page 1982). Water content was determined after drying for 12h at 105°C. Soil pH was measured in a 1:5 soil: water suspension by glass electrode. Organic matter content was determined based on ignition loss after 5 hours in 600°C.

Statistics
The data were analysed using Statistica 8.0. Split-plot design ANOVA with the nest as a random factor was used for determination of effects of the forest type and sampling location on each soil property. Post-hoc test (Tukey HSD) was used to determine significant differences between individual sampling locations.

Results

The type of forest has significant effect on soil pH ($F_{1,56}$=7.62, p<0.05) and water content ($F_{1,56}$=14.59, p<0.05), whereas sampling location has significant effect on all measured soil properties, i.e., soil respiration ($F_{4,56}$=27.37, p<0.05), soil pH ($F_{4,56}$=68.23, p<0.05), water content ($F_{4,56}$=94.60, p<0.05), and organic matter content ($F_{4,56}$=165.34, p<0.05) (Table 1). Interaction between the type of forest and sampling location has also significant effect on all measured soil properties, i.e., soil respiration ($F_{4,56}$=7.86, p<0.05), soil pH ($F_{4,56}$=6.21, p<0.05), water content ($F_{4,56}$=7.21, p<0.05), and organic matter content ($F_{4,56}$=6.50, p<0.05) (Table 1).

Table 1. Influence of a type of forest, a sampling location and interaction between these two factors on soil respiration, pH, water content (WC), and organic matter content (OMC) of 80 samples taken from 16 ant nests in total. Significant interactions <0.05, non-significant interactions ns. Split-plot design ANOVA.

	Respiration	pH	WC	OMC
(1) type of forest	ns	$F_{1,56}$=7.62 <0.05	$F_{1,56}$=14.59 <0.05	ns
(2) sampling location	$F_{4,56}$=27.37 <0.05	$F_{4,56}$=68.23 <0.05	$F_{4,56}$=94.60 <0.05	$F_{4,56}$=165.34 <0.05
(1x2) interaction	$F_{4,56}$=7.86 <0.05	$F_{4,56}$=6.21 <0.05	$F_{4,56}$=7.21 <0.05	$F_{4,56}$=6.50 <0.05

In the birch forest, soil respiration in ant nests was higher in comparison with the control and significantly differed from that of the soil mineral layer, but not from that of the humus layer, except of the top of ant nests (Figure 1a). Soil respiration was the highest at the top of ant nests and significantly differed from that of the bottom but not the rim of ant nests. In the pine forest, soil respiration in ant nests was also higher in comparison to the control, but significantly differed from that of the humus and the soil mineral layer only at the bottom and the rim of ant nests (Figure 1a). There were no significant differences between the top, the bottom, and the rim of ant nests. Between the two types of forest, there was a significant difference in soil respiration only between the tops of ant nests.

Soil pH in ant nests in the birch forest differed from that of the humus layer, with higher values in ant nests, but not from the soil mineral layer (Figure 1b). There were no differences in pH between individual sampling locations in ant nests, but there was a difference in pH between the humus and the soil mineral layer. In the pine forest, soil pH in ant nests differed from that of the humus layer, with higher values in ant nests, but not from the soil mineral layer, except of the top of ant nests (Figure 1b). There were also differences between the top and the bottom of ant nests, with higher values at the bottom, and the humus and the soil mineral layer. Between the two types of forest, there were no significant differences in pH between individual sampling locations.

In the birch forest, water content in ant nests was significantly lower than that of the humus layer, but does not differed from that of the soil mineral layer, except of the top of ant nests (Figure 1c). There were differences between the top of ant nests and the bottom and the rim, with higher values at the top. There was also a significant difference between the humus and the soil mineral layer, with higher values in the humus layer. In the pine forest, there were only differences in water content between ant nests and the soil mineral layer and the humus layer, with higher values in the humus layer (Figure 1c). Between the two types of forest, there was only a significant difference between the tops of ant nests.

Organic matter content in ant nests in the birch forest significantly differed from that of the soil mineral layer and that of the humus layer, except of the top of ant nests (Figure 1d). In ant nests, there were differences between all sampling locations, with the highest values at the top of ant nests, and there was also a difference between the humus and the soil mineral layer, with higher values in the humus layer. In the pine forest, organic matter content in ant nests significantly differed from that of the humus layer and that of the soil mineral layer, except of the rim of ant nests (Figure 1d). In ant nests, there were differences only between the top of ant nests and the bottom and the rim, with higher values at the top. There was a difference between the humus and the soil mineral layer, with higher values in the humus layer. Between the two types of forest, there were significant differences between the bottoms of ant nests and the humus layer.

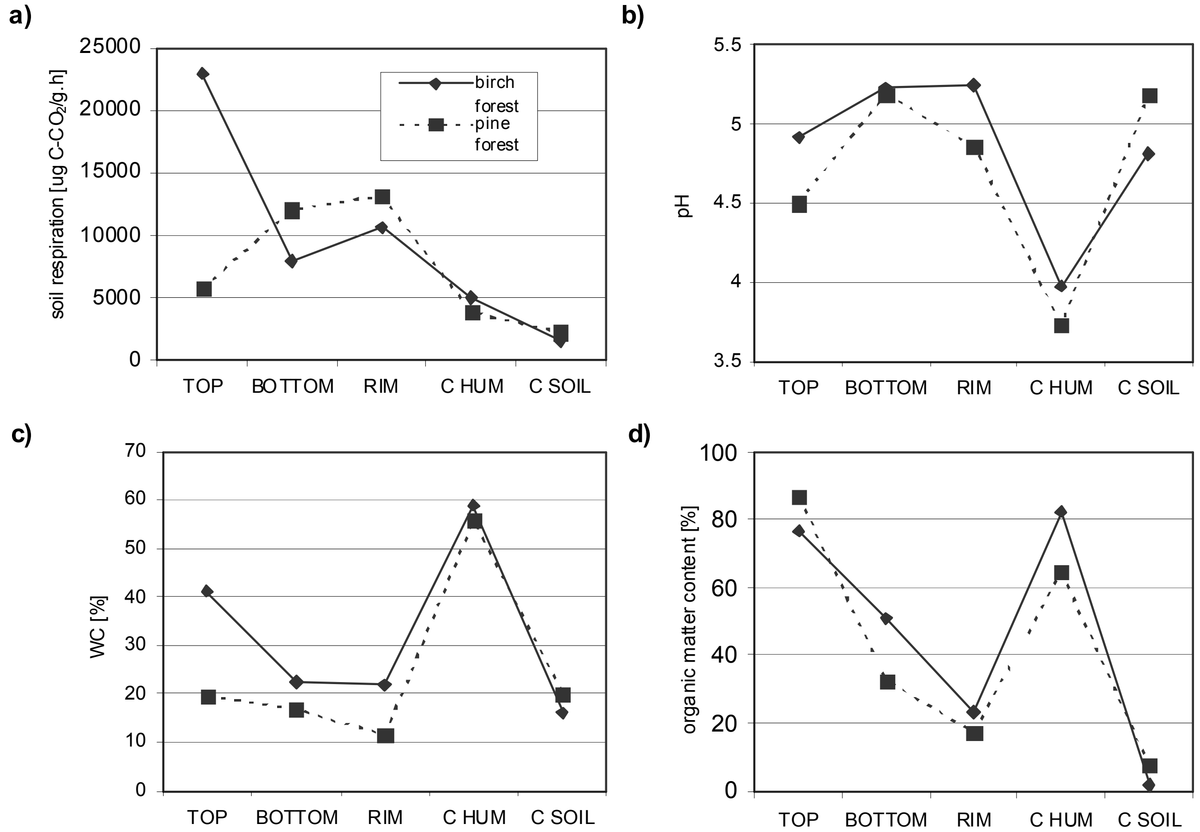

Figure 1. The effect of sampling locations on a) soil respiration, b) soil pH, c) water content, and d) organic matter content in a birch and a pine forest. TOP, BOTTOM, and RIM mean sampling locations in ant nests, C HUM and C SOIL mean control sampling locations in the humus and the soil mineral layer. Data are based on 80 soil samples in total (split-plot design ANOVA).

Discussion

Soil respiration quite differed between the two types of forest. In the birch forest, the highest respiration rates were found at the top of ant nests, which values differed significantly from that of the humus layer. In the pine forest, the highest respiration rates were found at the bottom and the rim of ant nests. These differences may be caused by differences in soil pH, water content and the quality of organic matter content. These properties are known to be responsible for the changes in soil respiration (Amador and Görres 2007; Lenoir *et al.* 2001). The nest respiration strongly depends on nest moisture but here, drier nests had higher respiration rates probably due to the import of easily available substances in prey and honeydew into the nests (Horstmann 1974). Higher respiration rates in ant nests are in agreement with Laakso and Setälä (1998). In that study, ant nests are considered as hot spots for decomposers.

Higher pH values were found in ant nests, especially in comparison to the humus layer. This is not a surprising result given that the forest humus layer is typically highly acidic (Brady and Weil 2001). pH is strongly correlated with organic matter content (Frouz *et al.* 2003). At the top of ant nests, higher pH values were found in comparison to the humus layer, and at the bottom and the rim, higher pH values were found in comparison to the soil mineral layer, although high organic matter content was found in ant nests. These results show that there are also other factors influencing soil pH. One of them could be higher contents of basic cations in ant nests (Frouz *et al.* 2003). In agreement with previous studies, ant nests were drier in comparison to the surroundings of the nests (Dlusskij 1967; Frouz and Jilkova 2008; Jurgensen *et al*. 2008). The highest water contents were found at the tops of ant nests, which is in agreement with Frouz (1996).

The differences in organic matter content between the tops of ant nests and the bottoms and the rims of ant nests could be most probably explained by differences in ant nest construction. The bottom and the rim of an ant nest is built by excavation of the deeper soil profiles that do not contain much organic matter, whereas the top of an ant nests is mainly built of needles and twigs from the nest surroundings (Dlusskij 1967). The differences between the two types of forest show that the influence of even very similar ant species differs in dependence on environmental conditions (Holec 2006)

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