



## Ant-induced soil modification and its effect on plant below-ground biomass

Petr Dostál<sup>a,\*</sup>, Magdalena Březnová<sup>b</sup>, Vladimíra Kozlíčková<sup>b</sup>,  
Tomáš Herben<sup>a,b</sup>, Pavel Kovář<sup>b</sup>

<sup>a</sup>*Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic*

<sup>b</sup>*Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 01, Praha 2, Czech Republic*

Received 10 April 2004; accepted 27 September 2004

### KEYWORDS

*Agrostis capillaris*;  
Bioturbation;  
Clonal plants;  
*Lasius flavus*;  
Potassium;  
Rhizome architecture

### Summary

*Lasius flavus* is a dominant mound-building ant species of temperate grasslands that significantly modifies soil parameters. These modifications are usually the result of workers' activities such as food accumulation and nest construction. An alternative hypothesis that could explain changes in soil is colony founding in areas of higher soil fertility.

In our study we investigated several soil parameters sampled in 10 ant nests and adjacent (control) plots in mountain grassland in Slovakia. The alternative hypothesis was tested by comparing occupied and abandoned mounds. While we found increased concentrations of available P and K in the nests, concentrations of total C, total N, Ca<sup>2+</sup> and Mg<sup>2+</sup> were lower there. We propose that differences found between the soil of nests and control plots are entirely a product of ant activity during mound occupancy and not due to initial soil differences during nest establishment. This was confirmed by the comparison of occupied and abandoned nests in which the soil fertility of abandoned nests was similar to conditions in the surrounding soil.

Along with the modification of soil chemistry, we recorded changes in soil physical properties and the vertical distribution of nutrients. Ant nests were characterized by the dominance of 0.02–0.1 mm particles and lower bulk density. In the same habitat, nutrient concentrations did not change along the vertical gradient in contrast to control plots where soil nutrients decreased and bulk density increased with depth. Root biomass followed the vertical pattern observed with nutrients: in control plots, most roots were concentrated in the uppermost layer (0–3 cm), whereas they were evenly distributed along the vertical gradient in the nests. We also found that rhizome

\*Corresponding author. Fax: +420 271015285.  
E-mail address: [dostal@ibot.cas.cz](mailto:dostal@ibot.cas.cz) (P. Dostál).

internodes of *Agrostis capillaris* were thinner and longer in plants from the mounds. Changes in soil physical properties, vertical distribution of nutrients and root biomass in the nests are most probably a consequence of mounding and soil mixing (bioturbation), which has been less reported on in ant-soil studies.  
© 2005 Elsevier GmbH. All rights reserved.

## Introduction

Ground ants together with earthworms and termites, belong to the principal groups of invertebrates that influence soil processes in terrestrial ecosystems (Lavelle et al., 1997). Ants change physical and chemical parameters of the soil by bioturbation and by accumulation of organic material. Due to the building of below-ground galleries, mounding and material mixing, the soil of ant nests is characterized by the impeded formation of soil horizons, increased porosity, drainage and aeration, reduced bulk density and modified texture and structure. Increased content of organic matter, P, N and K in the nests is due to food storage, aphid cultivation, and accumulation of faeces and ant remains (Hole, 1981; Paton et al., 1995; Lavelle et al., 1997; Folgarait, 1998).

Although food storage and nest construction are the most obvious explanations for soil changes (Petal, 1980; Dean et al., 1997; Folgarait, 1998), an alternative hypothesis suggests that differences in ant nest soil and the surrounding soil are a reflection of the initial soil conditions during nest establishment. This hypothesis assumes that fertilized queens initiate the construction of their nests selectively in patches with higher soil fertility. To date, there is very limited knowledge about the effect of soil properties on colony founding. Johnson (1998) tested the effect of soil type and moisture on colony establishment of two species of *Pogonomyrmex* and found a significant effect for both factors. However, in another study by Wagner et al. (2004), in an analysis of mounds of various ages, there was no evidence that colonies of *Pogonomyrmex barbatus* prefer more fertile soils during the establishment phase.

Whatever the cause of soil modification, ant-influenced soil environment affects the activity and abundance of other soil biota, such as soil microorganisms (fungi, bacteria, actinomycetes), nematodes and soil insects (Jacubczyk et al., 1972; Blomquist et al., 2000). Ultimately, soil abiotic and biotic changes generated by ants have an effect on the performance and diversity of vascular plants (King, 1977; Culver and Beattie, 1983; Rissing, 1986; Carlson and Whitford, 1991; Dean et al., 1997).

The effect of ant-induced soil modification on plant performance and vegetation pattern is well documented. Ant nests are known to enhance germination and seedling survival (Culver and Beattie, 1980; Dean and Yeaton, 1992), seed production (Rissing, 1986; Dean and Yeaton, 1993a) and plant growth (Dean and Yeaton, 1993b). Plant species associated with ant nests usually differ from species growing in adjacent areas (King, 1977; Culver and Beattie, 1983; Dean and Yeaton, 1993b; Dean et al., 1997; Folgarait et al., 2002).

In spite of the large body of knowledge on the effect of soil environment of ant nests on above-ground plant performance, surprisingly little information exists on its effect on plant below-ground parts (e.g. Blomquist et al., 2000). Typically, in the absence of ant disturbance, distribution and parameters of roots and rhizomes in grasslands follow vertical gradient of nutrients, water and bulk density; root and rhizome biomass decrease with depth (Fitter, 1994; Unger and Kasper, 1994; Pecháčková, 1997). The presence of ants, particularly of those building pronounced mounds, can substantially influence the biomass and architecture of roots and rhizomes through changes in the soil environment. Roots and rhizomes are morphologically highly plastic in response to abiotic conditions of the soil. They may respond by proliferation, architectural modification or changed nutrient uptake to changes in soil physical parameters (pore structure, compaction) and distribution of water and nutrients (Robinson, 1994; Hutchings and de Kroon, 1994).

There are several ant species that build pronounced mounds in European grasslands such as *Lasius niger*, *Lasius flavus*, several species of genus *Formica* or *Tetramorium caespitum*. However, *L. flavus* is the most common and dominant ant species that changes the character of the grasslands by building prominent and abundant soil nests (Woodell and King, 1991). Colonies of this ant species may build up to 2500 mounds ha<sup>-1</sup>. Soil nests of this species are long-lasting structures maintained by permanent soil transport to the surface (Jacubczyk et al., 1972). The effect of *L. flavus* on vegetation pattern has been repeatedly demonstrated (King, 1977; Dean et al., 1977; Kovář et al., 2001).

In this study, we demonstrated the effect of *L. flavus* on soil changes, including the effect of mounding and soil mixing during nest construction. We illustrated this effect by comparing soil from ten ant nests and ten adjacent control plots, including the vertical gradient of sampled habitats studied in four consecutive soil layers. In addition, we wanted to test the alternative hypothesis that links colony founding with increased soil fertility. Previous studies on *L. flavus* indicated that environmental factors control colony founding and survival, such as moisture, soil depth, vegetation type and management (Pontin, 1960; King, 1981; Dean et al., 1997; Blomquist et al., 2000). At the same time, there is no record of selective burrowing of the fertilized queen during colony establishment, except for occasional colonization of mole hills that may differ in soil characteristics (Woodell and King, 1991).

We had no data to analyze the selective placement of colonies at our study site, but we tested this alternative hypothesis by comparing soil properties of occupied and abandoned mounds. We assumed that if a colony was established in patches with distinct soil properties and ant activity was not an important source of soil modification, then the soil of abandoned mounds should not differ significantly from active nests.

We also investigated the effect of soil modification on below-ground plant biomass. We hypothesized that both modified chemistry and mounding during soil construction could have an effect on roots and rhizomes of existing vegetation. For that purpose we sampled ten mounds and ten control plots identical with those sampled for the analysis of soil properties and analyzed below-ground biomass and rhizome architecture in four (two respectively) consecutive soil layers.

Specifically, we wanted to address the following questions: (1) Do the mounds of *L. flavus* differ in the soil parameters from their surroundings? (2) Can these differences be explained by colony founding in more fertile sites? and (3) Do the soil characteristics of the mounds influence below-ground parameters of vegetation?

## Materials and methods

### Study site and species

This study was undertaken in the Slovenske Rudohorie Mts., Slovakia at an elevation of ca. 950 m a.s.l. near Obrubovanec Point (1020 m a.s.l.; 48°41'N, 19°39'E). This area is a mosaic of spruce and beech forests and mountain grasslands. Grass-

land vegetation is rather poor due to acidic bedrock (migmatites and amphibolites), with a dominance of grass species *Festuca rubra*, *F. pratensis*, *Agrostis capillaris* and *Dactylis glomerata*. It can be classified as the Nardo-Agrostion tenuis alliance Sillinger 1933. The grasslands are maintained by mowing and grazing. Only the latter management promotes the emergence of conspicuous ant mounds, which are formed mostly by yellow-ant *L. flavus* (Formicidae). This ant species is widespread and can be found in temperate regions of Europe, Asia and North America from lowlands to the mountains. It prefers open habitats like marshes, abandoned arable fields and grasslands. Colonies are formed by one queen and 8,000–25,000 workers (Waloff and Blackith, 1962). The ants live mostly underground in the soil mound and feed on the honeydew and young instars of root aphids (Pontin, 1978). The mound can be continuously occupied, one fertilized queen taking over from another. The size of the mounds increases over time, depending on the colony, territory size and the frequency of wet weather that is favorable for building (King, 1981). At our study site, colonies of this ant form on average 52 ant mounds per 100 m<sup>2</sup> and cover approximately 13% of the grassland area. Mounds can be up to 40 cm in height and 70 cm in diameter, but are usually smaller. Ant nests can be inhabited for more than 20 years (Kovář et al., 2001). The latter authors also found a clear distinction between plant species composition on the mounds and control plots. However, in terms of species diversity, mound and control plots were similar; both had approximately 10 species per 0.25 × 0.25 m<sup>2</sup> quadrat. Vegetation cover was usually sparser on the mounds than in their surroundings due to soil heaping (Petr Dostál, personal observation).

One of the dominant grass species *A. capillaris* was selected for the study of the effect of ants on below-ground plant parameters (see below). We chose this species because it is well represented on both mounds and control plots which enabled us to compare its performance between both microhabitats. *A. capillaris* is characterized by strong clonality, with fast growing rhizomes and strong branching. The closely related *A. stolonifera* was found to be phenotypically highly plastic in response to nutrient supply (Crick and Grime, 1987).

### Analysis of soil parameters and plant below-ground biomass

The same set of ten mounds and ten control plots (at 2 m distance from a mound edge) were used for

the analyses of bulk density, chemical parameters and below-ground plant parameters. Ant mounds were selected according to the following criteria: (i) at least 20 cm high, (ii) with minimum cover of *A. capillaris* of 40% (see below) and (iii) occupied by *L. flavus* only. Sampling was undertaken between October 23 and October 25, 1998, in an area of approximately 100 m × 100 m.

For the analysis of soil chemistry and below-ground plant parameters, blocks of soil 20-cm long, 7-cm wide and 18-cm deep were excavated from each of the ten mounds and ten control plots. These blocks were cut along their vertical axis into two sections of 10 cm × 7 cm × 18 cm. The first section was used for the analysis of chemical parameters whereas the second one served for the investigation of roots and rhizomes. Prior to transfer to the lab, each monolith was sliced into four horizontal layers: 0–3, 3–6, 6–12 and 12–18 cm.

Soil segments designated for the analysis of chemical parameters were transferred to the lab, air-dried, sieved through 2 mm mesh and homogenized. Total carbon and nitrogen were estimated by elemental analysis. Samples were ignited at 950 °C and the content of C and N was determined by thermo-conductometric detection in the elemental analyser Carlo Erba NC2500, CE Instruments, Milan, Italy (Ehrenberger and Gorbach, 1973). Concentrations of Ca<sup>2+</sup> and K<sup>+</sup> were assessed by flame atomic emission spectroscopy, Mg<sup>2+</sup> by flame atomic absorption spectroscopy after extraction with 1 M ammonium acetate (pH 7). Analyses were carried out using AAS Spectrometer Unicam 9200X, Unicam Ltd., Cambridge, UK (Moore and Chapman, 1986). Available phosphorus concentration was determined by colorimetric method with ammonium molybdenate–sulphuric acid reagent using the UV-Vis Spectrometer Unicam UV4-200, Unicam Ltd., Cambridge, UK (Olsen, 1954). pH of the soil solution was determined on samples in suspension after stirring using a Radiometer TT3 titrator. Both the actual and exchangeable pH were determined using distilled water and 0.1 M KCl, respectively.

The second core section was used for the study of architectural parameters of rhizomes and roots. Soil segments for below-ground plant biomass analysis were frozen before they were processed. Later, roots and rhizomes were extracted from each segment by washing. They were then dried at 80 °C for 24 h and weighed on analytical scales with a precision of 0.0001 g (METTLER 9908, Mettler-Toledo International Inc., Greifensee, Switzerland). Dry biomass was expressed per soil volume. Rhizomes of *A. capillaris* were separated from the rhizomes of other species before drying. The

following parameters were measured: (i) total rhizome length (length of all fragments in the soil segment), (ii) branching density (number of branches on all fragments in soil segment/total rhizome length), (iii) rhizome diameter (mean diameter of all fragments present in segment) and (iv) internode length (total rhizome length/number of nodes on all fragments).

Core samples for bulk density determinations were taken from vertical profiles exposed after above-ground sampling in both microhabitats. The cores were taken at depths of 0–7, 5–12 and 10–17 cm (a sample from 5 to 12 cm was side-shifted to avoid overlap) and after complete penetration it was pulled out carefully and promptly covered. Soil samples were dried at 105 °C for 24 h and weighed on analytical scales with a precision of 0.0001 g.

Soil texture was analyzed from samples taken from an additional set of blocks removed in July 1997. At that time, soil blocks of 10 cm × 10 cm × 10 cm were taken from each of ten randomly selected mounds and ten control plots. Soil blocks were transferred to the lab, air-dried and sieved through 5 mm mesh to remove litter and present biomass. Size distribution of soil particles was measured with sediment density meter (type ANALY-SETTE 20, Fritsch GmbH, Idar-Oberstein, Germany).

The intention of this study was to link soil parameters in consecutive soil layers with below-ground plant parameters in corresponding segments. Eighteen centimeter was the lowest depth of our sampling since below-ground biomass (roots) was almost always absent in deeper layers in control plots. The sampling design of the soil physical parameters to some extent deviated from this schema for several reasons. First, soil sampling for bulk density was limited by the diameter of the corer (7 cm) we had at our disposal. Therefore, we obtained samples that differed in size from samples taken for the analysis of soil chemistry and below-ground biomass. Moreover, we could not prevent partial overlap in sampled depths. The sampling for soil texture completely deviated from the above schema since it was included in this study only to illustrate soil differences between compared microhabitats, without ambitions to relate the results to the pattern of below-ground biomass.

### Comparison of occupied and abandoned ant nests

We analyzed the concentration of total carbon, total nitrogen, Ca<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, available P, C:N ratio and pH in soil samples taken from an additional set

of 17 mounds. Soil blocks of 10 cm × 10 cm × 10 cm were extracted from each of 17 mounds, processed and analyzed according to above protocol. Ten occupied and seven abandoned mounds were selected from a set of ant nests that were repeatedly checked for ant presence during four visits in 1979, 1981, 1988 and 1995 (Kovář et al., 2001). Most of the abandoned mounds (5 out of 7) had no record of *L. flavus* presence since 1988.

## Statistical analysis

The effect of microhabitat (mounds vs. control plots) was used as a between-subject factor and soil layer as within-subject factor in repeated measures ANOVAs with dependent variables of bulk density, nutrient concentrations, root and rhizome biomass and rhizome architecture. The Huynh-Feldt adjustment of the degrees of freedom was applied to correct for the dependence of soil layers. Data on below-ground plant biomass and rhizome architecture were log-transformed to meet the assumptions of normality.

The effect of microhabitat on the soil texture was analyzed with redundancy analysis. An unconstrained Monte-Carlo permutation test (199 simulations) was used to test for significance.

Redundancy analysis was run using CANOCO (ter Braak and Šmilauer, 1998) and all other analyses were performed using SPSS 11.5 for Windows, SPSS Inc., Chicago, USA.

## Results

### Soil parameters: mounds vs. control plots

The soil of ant mounds had a significantly lower bulk density than the soil of control plots (mound

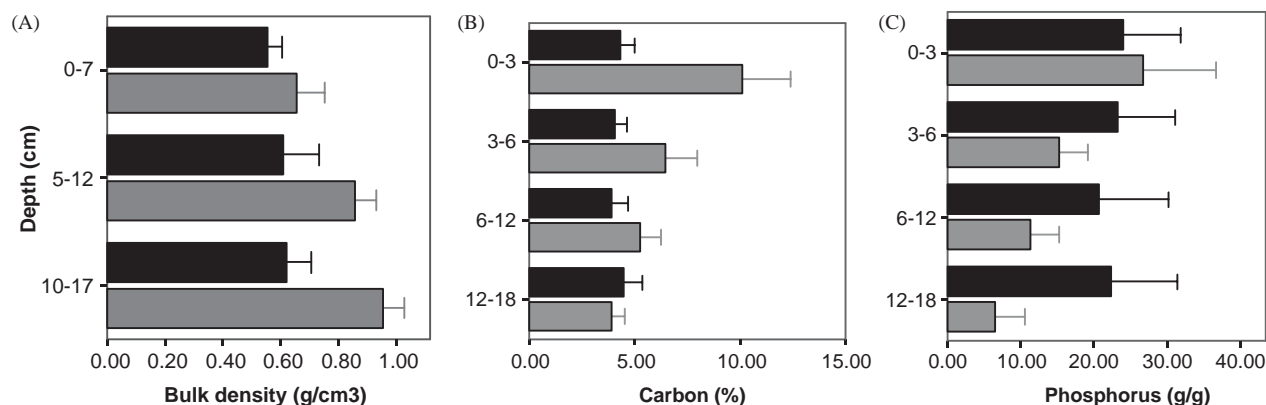
soil =  $0.60 \pm 0.09 \text{ g cm}^{-3}$  (mean  $\pm$  SD) vs. control soil =  $0.82 \pm 0.15 \text{ g cm}^{-3}$  (mean  $\pm$  SD);  $F_{1,18} = 89.9$ ;  $P < 0.001$ ). Bulk density increased with sampling depth ( $F_{2,36} = 60.00$ ;  $P < 0.001$ ). However, this change was much stronger in the control plots; interaction between microhabitat and sampling depth was significant ( $F_{2,36} = 24.4$ ;  $P < 0.001$ ; Fig. 1).

Surveyed microhabitats differed in the composition of soil particles. The mound soil had a smaller proportion of the coarsest particles (0.1–2 mm) but a higher proportion of 0.02–0.1 mm particles. Other size categories were similarly represented in both soils (Table 1). The differences observed between microhabitats in terms of soil particle composition were significant ( $F = 5.8$ ;  $P = 0.005$ ).

The mound soil was significantly different from the soil of control plots in all chemical parameters. There were greater concentrations of available P and  $\text{K}^+$  as well as higher pH ( $\text{H}_2\text{O}$ ) and C:N ratio in the mounds. In contrast, the soil from control plots had more C, N,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Table 2). Nutrient concentrations were also significantly different between successive soil segments. Concentrations decreased systematically with sampling depth; however, this pattern was typical for the soil from control plots only. In the mound soil, concentrations were similar between soil layers. Consequently, there was a significant interaction

**Table 1.** Particle size composition of soils from mounds and control plots

Particle size	Mound ( $n = 10$ ) (mean $\pm$ SD)	Control ( $n = 10$ ) (mean $\pm$ SD)
< 0.001 mm (%)	2.8 ( $\pm 1.8$ )	3.5 ( $\pm 2.7$ )
0.001–0.02 mm (%)	24.4 ( $\pm 6.4$ )	22.1 ( $\pm 6.5$ )
0.02–0.1 mm (%)	43.1 ( $\pm 4.8$ )	35.6 ( $\pm 7.8$ )
0.1–2 mm (%)	29.6 ( $\pm 5.6$ )	38.8 ( $\pm 6.4$ )



**Fig. 1.** (A) Bulk density, (B) carbon concentration, and (C) available phosphorus concentration (means  $\pm$  SD) in mounds (black bars) and control plots (gray bars) sampled at three and four depths, respectively.

**Table 2.** (A) Chemical parameters of soil from ant mounds and control plots (means from 0 to 18 cm), and (B) repeated measures ANOVA of the effect of microhabitat (mound vs. control), depth (0–3, 3–6, 6–12, 12–18 cm) and their interaction

	(A)		(B)		
	Mound ( $n = 10$ ) (mean $\pm$ SD)	Control ( $n = 10$ ) (mean $\pm$ SD)	Mound vs. control d. f. = 1, 18	Depth d. f. = 3, 54	Interaction d. f. = 3, 54
Total C (%)	4.21 ( $\pm 0.73$ )	6.44 ( $\pm 2.73$ )	55.3***	30.2***	30.1***
Total N (%)	0.30 ( $\pm 0.09$ )	0.58 ( $\pm 0.19$ )	80.3***	15.0***	15.9***
Ca <sup>2+</sup> ( $\mu\text{g g}^{-1}$ )	599.00 ( $\pm 149.10$ )	937.67 ( $\pm 652.87$ )	12.2**	41.2***	42.5***
Mg <sup>2+</sup> ( $\mu\text{g g}^{-1}$ )	113.70 ( $\pm 29.90$ )	167.13 ( $\pm 112.81$ )	16.4***	64.8***	59.5***
C/N ratio	15.24 ( $\pm 3.94$ )	10.92 ( $\pm 1.36$ )	30.9***	1.3 <sup>ns</sup>	2.0 <sup>ns</sup>
P ( $\mu\text{g g}^{-1}$ )	23.37 ( $\pm 8.67$ )	15.46 ( $\pm 9.93$ )	8.7**	15.8***	10.1***
K <sup>+</sup> ( $\mu\text{g g}^{-1}$ )	565.82 ( $\pm 316.73$ )	201.92 ( $\pm 156.10$ )	19.5***	1.5 <sup>ns</sup>	11.2**
pH (H <sub>2</sub> O)	5.13 ( $\pm 0.25$ )	4.73 ( $\pm 0.19$ )	35.1***	6.9**	4.0*

\*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns, not significant.

between microhabitat and sampling depth for all chemical soil parameters (Table 2; Fig. 1).

### Soil parameters: occupied vs. abandoned mounds

After abandonment, total C and N significantly increased whereas concentration of K<sup>+</sup> and pH decreased. This pattern was consistent with that found in the comparison of mounds and control plots. The only exception was the C:N ratio, which increased after abandonment. The Ca<sup>2+</sup>, Mg<sup>2+</sup> and available P concentrations did not differ between occupied and abandoned mounds (Table 3).

### Below-ground plant parameters

Below-ground plant biomass was higher in control plots than in the soil of the mounds. This difference was entirely due to the variation in the root biomass. Rhizome biomass of *A. capillaris* (and of the other species present) did not differ between microhabitats. Therefore, root/rhizome ratio was also significantly higher in control plots (Table 4; Fig. 2).

Along with the effect of microhabitat, root and rhizome biomass was significantly influenced by the sampling depth. In the soil from the control plots, total below-ground plant biomass and individual biomass of roots and rhizomes decreased (but increased in case of root/rhizome ratio) with sampling depth, in analogy to the pattern observed for the chemical parameters. In the mound soil, biomass did not change along the vertical profile (i.e. interaction of microhabitat and sampling depth was significant (Table 4; Fig. 2)).

Architecture traits of *A. capillaris* rhizomes were significantly different between mounds and control plots. Rhizomes in the mounds were thinner, had longer internodes and branched more frequently. Since the rhizomes were almost absent from the 6 to 12 cm and lower segments in the control plots, architecture traits were examined in the two uppermost layers only. The change of rhizome parameters along the vertical profile was closely related to the microhabitat. In control plots, length of internodes, branching density and total length decreased and rhizome diameter increased with sampling depth. In the mounds, the same parameters showed an opposite trend (except for branching density which did not change with depth). The effect of interaction of microhabitat and sampling depth on architecture traits was significant (Table 4; Fig 2)

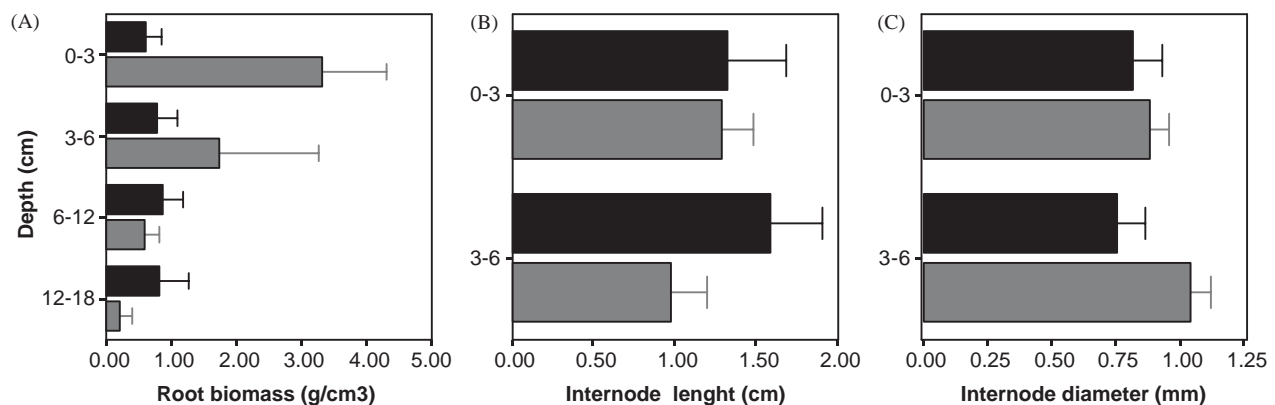
### Discussion

Two principal findings emerged from this study: (1) mound-building ants *L. flavus* change soil parameters of the mountain grassland investigated. We found significant differences between mounds and control plots in all nutrients we analyzed. We also documented that a change in the concentration of nutrients along the vertical gradient is different in the mounds than in control plots. In the soil surrounding the mounds concentrations of nutrients declined steeply and bulk density increased with depth whereas in the mounds these parameters remained unchanged in consecutive soil layers; (2) soil modification induced by ants influenced below-ground biomass of vegetation.

**Table 3.** (A) Chemical parameters of soil from occupied and abandoned mounds. Mound occupancy was checked by careful digging in the mounds; (B) one-way ANOVA of the effect of mound occupancy (occupied vs. abandoned)

	(A)	(B)	
	Occupied ( $n = 10$ ) (mean $\pm$ SD)	Abandoned ( $n = 7$ ) (mean $\pm$ SD)	Mound occupancy d. f. = 1, 15
Total C (%)	4.06 ( $\pm 0.33$ )	5.39 ( $\pm 1.19$ )	11.56**
Total N (%)	0.36 ( $\pm 0.03$ )	0.43 ( $\pm 0.07$ )	10.03**
Ca <sup>2+</sup> ( $\mu\text{g g}^{-1}$ )	497.01 ( $\pm 167.01$ )	368.62 ( $\pm 161.02$ )	2.50 <sup>ns</sup>
Mg <sup>2+</sup> ( $\mu\text{g g}^{-1}$ )	66.20 ( $\pm 15.46$ )	52.41 ( $\pm 26.19$ )	1.88 <sup>ns</sup>
C/N	11.31 ( $\pm 0.42$ )	12.33 ( $\pm 1.49$ )	4.26*
P ( $\mu\text{g g}^{-1}$ )	11.47 ( $\pm 4.70$ )	12.26 ( $\pm 4.65$ )	0.12 <sup>ns</sup>
K <sup>+</sup> ( $\mu\text{g g}^{-1}$ )	311.08 ( $\pm 63.75$ )	88.69 ( $\pm 15.41$ )	80.39***
pH (H <sub>2</sub> O)	4.60 ( $\pm 0.09$ )	4.21 ( $\pm 0.21$ )	24.93***

\*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns, not significant.

**Fig. 2.** (A) Root biomass, (B), internode length, and (C) internode diameter (means  $\pm$  SD) in ant mounds (black bars) and control plots (gray bars) sampled at four and two depths, respectively.

Our findings represent an example of ecosystem engineering (Jones et al., 1994) in which the activity of one group of organisms modulates the availability of resources and changes habitat properties for the another species. Both findings are discussed below in detail.

### Soil chemistry

Differences in nutrient concentrations between microhabitats can be ascribed to respective ant foraging and nest dwelling. Increased potassium in mound soil is derived from the honeydew of root aphids—one of *L. flavus*' principal food sources (Woodell and King, 1991). The higher concentration of available phosphorus can be explained by accumulation of food in the nests and an increased decomposition rate (Frouz et al., 1997). In contrast to increased concentrations of K<sup>+</sup> and P, lower concentrations of total carbon, total nitrogen and the exchangeable cations, Ca<sup>2+</sup> and Mg<sup>2+</sup>, in ant

mounds contradict previous findings (Peřtal, 1980; Folgarait, 1998). The decline of these nutrients in the mounds is probably due to the removal of organic matter from the nests by the ants. Alternatively or in addition the decline could be due to the replacement of the upper horizon with mound subsoil which is usually poor in organic matter (Levan and Stone, 1983).

Comparison of occupied and abandoned mounds confirmed that soil changes found in the mounds are due to ant activities during mound occupancy (Peřtal, 1980; Dean et al., 1997; Folgarait, 1998) and not due to soil differences during colony establishment. Changes in total carbon, nitrogen and potassium concentrations after abandonment was similar to the contrast in soil chemistry between mounds and control plots. This conclusion is consistent with findings of Wagner et al. (2004) who applied a different approach in the investigation of this question. They found, on the basis of extrapolations from regressions of soil chemical variables against colony age of *P. barbatus*, that an

**Table 4.** (A) Parameters of roots and rhizomes from mounds and control plots—biomass (means from 0–18 cm) and architecture of *Agrostis capillaris* (means from 0–6 cm) and (B) repeated measures ANOVA of the effect of microhabitat (mound vs. control), depth and their interaction on above parameters

	(A)		(B)							
	Mound (mean ± SD)	Control (mean ± SD)	Mound vs. control			Depth			Interactio	
			Residual d. f	d. f.	F	Residual d. f.	d. f.	F	d.f.	F
Biomass (g 100 cm <sup>-3</sup> soil)										
Total	1.01 (±0.39)	1.73 (±1.83)	17	1	9.86**	51	3	43.76***	3	43.87***
Roots	0.76 (±0.34)	1.45 (±1.51)	18	1	18.08***	54	3	40.05***	3	55.26***
Rhizomes of <i>A. capillaris</i>	0.18 (±0.15)	0.18 (±0.27)	18	1	0.17 <sup>ns</sup>	54	3	44.74***	3	18.32***
Rhizomes of other species	0.08 (±0.08)	0.10 (±0.19)	18	1	0.00 <sup>ns</sup>	54	3	55.88***	3	17.59***
Root/rhizome ratio	2.94 (±3.81)	11.14 (±13.36)	18	1	5.96*	18	1	4.73(*)	1	5.37*
ce:italic>A. capillaris architecture										
Internode length (cm)	1.46 (±0.36)	1.13 (±0.26)	18	1	8.17**	18	1	0.16 <sup>ns</sup>	1	28.78***
Internode diameter (mm)	0.79 (±0.11)	0.96 (±0.11)	18	1	21.62***	18	1	6.37*	1	32.49***
Branching density	0.17 (±0.08)	0.12 (±0.11)	17	1	4.88*	17	1	17.45**	1	8.89**
Rhizome length (cm cm <sup>-3</sup> soil)	0.61 (±0.33)	0.58 (±0.49)	18	1	0.36 <sup>ns</sup>	18	1	29.66***	1	37.90***

\*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ;  $P < 0.05$ ; (\*),  $0.05 < P < 0.10$ ; ns, not significant. Since rhizomes were almost absent from the 6–12 cm and lower segments in the control plots, the two uppermost layers were examined only for the analysis of the root/rhizome ratio and all architecture traits measured in rhizomes of *A. capillaris*.



increase in soil fertility is entirely a function of colony age and not of initial soil nutrient content.

Although concentrations of some critical nutrients were lower in the mound soil, a preliminary bioassay experiment elsewhere showed higher nutrient status of soil sampled from this habitat (Kozlíčková, unpublished data). In a greenhouse experiment she grew plants of five species (present at study site) in the soil from mounds and control plots. Both above- and below-ground biomass of plants growing in the mound soil was higher than of plants growing in control soil. Moreover, a higher root-to-shoot ratio of plants grown in control soil is evidence of nutrient shortages that are compensated for by higher allocation of biomass to roots at the expense shoot biomass (Chapin, 1980).

### Bioturbation and its effect on below-ground plant biomass

Concurrent to modification of soil chemistry, changes in soil physical parameters are evidence of the effect of nest construction and maintenance. Pronounced above-ground parts of ant nests are the result of intensive mounding (Hole, 1981) and serve to increase soil temperature needed for brood rearing (Woodell and King, 1991). To build and maintain mounds, ants must transport large amounts of the soil from deeper layers. King (1981) reported that one colony of *L. flavus* may transport more than 1 l of the soil annually. During the transfer of soil particles, ants are limited by their mandible range which is 600 µm (Haarlov, 1960), and consequently, we found particles of 0.02–0.1 mm to dominate in the mound soil. The loose soil structure of the mounds with their tunnels and chambers accounts for the observed decrease in bulk density in the mounds (King, 1977; Woodell and King, 1991; Blomquist et al., 2000).

In ant nests, soil homogenization was also demonstrated by the similar nutrient concentrations found between four consecutive layers down to a depth of 18 cm. In contrast, soil nutrients in the control plots decreased dramatically (and bulk density increased) with sampling depth (Lobry de Bruyn and Conacher, 1994; Paton et al., 1995; Folgarait, 1998).

Although the effect of soil chemistry modified by ants on plant growth is well known (e.g. Danin and Yom-Tov, 1990; Dean and Yeaton, 1993a; Dean et al., 1997), the effect of bioturbation on below-ground plant parameters is much less studied (e.g. Dean and Yeaton, 1993b). In the control plots, most of the root biomass was concentrated in the uppermost layer (0–3 cm) where we found the

highest amount of nutrients. In contrast, in the mounds with no gradient in nutrient concentrations, root biomass was similar in all four layers down to a depth of 18 cm. According to Robinson (1994), roots are able to proliferate in the nutrient-rich zone and thus increase nutrient uptake. The concentration of roots in the uppermost layer in the control plots is in accordance with this exploitation strategy.

The transport of large amounts of soil is an important source of disturbance in the nests. There were several indices on how mounding influenced below-ground parameters. First, total root biomass and root/rhizome ratio were smaller in the mounds than in the control plots. This can be either a direct effect of soil disturbance that prevents root development, or, a result of a lag in root colonization of newly heaped soil. Second, morphological characteristics of rhizomes in the mounds were similar to morphological responses to sand burial recorded in previous studies, such as elongated and thinner internodes (Disraeli, 1984; Seliskar, 1994). Elongation of internodes helps to bring assimilation organs to more favorable light conditions when buried under a layer of substrate.

To conclude, *L. flavus* created patches of soil that differed from the background soil in chemistry and physics. Bioturbation is one of the most important ant activities that homogenizes soil profile. Modified soil chemistry, soil homogenization and soil mounding all influence plant growth and below-ground plant parameters.

### Acknowledgments

We would like to thank the students who helped with soil sampling and soil analyses, namely Jana Raabová and Blanka Vlasáková. Dáša Pacovská is acknowledged for language revision. Dr. Marie Albrechtová kindly provided useful comments to the soil analyses. The Administration of Protected Landscape area Polana kindly gave permission to work in the study area. We appreciate the comments of Henrik Berg and of one anonymous reviewer of the earlier version of the manuscript. This study was supported by grants FRVŠ No. F 1699/1999 and GAUK No. 125/2000.

### References

- Blomquist, M.M., Olff, H., Blaauw, M.B., Bongers, T., van der Putten, W.H., 2000. Interactions between above- and belowground biota: importance for small-scale

- vegetation mosaics in a grassland ecosystem. *Oikos* 90, 582–598.
- ter Braak, C.J.F., Šmilauer, P., 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, Ithaca.
- Carlson, S.R., Whitford, W.G., 1991. Ant mound influence on vegetation and soils in a semiarid mountain ecosystem. *Am. Midl. Nat.* 126, 125–139.
- Chapin, F.S., 1980. The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst.* 11, 233–260.
- Crick, J.C., Grime, J.P., 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. *New Phytol.* 107, 403–414.
- Culver, D.C., Beattie, A.J., 1980. The fate of *Viola* seeds dispersed by ants. *Am. J. Bot.* 67, 710–714.
- Culver, D.C., Beattie, A.J., 1983. Effect of ant mounds on soil chemistry and vegetation patterns in a Colorado montane meadow. *Ecology* 64, 485–492.
- Danin, A., Yom-Tov, Y., 1990. Ant nests as primary habitats of *Silybum marianum* (Compositae). *Plant Syst. Evol.* 169, 209–217.
- Dean, W.R.J., Yeaton, R.I., 1992. The importance of harvester ant *Messor capensis* nest-mounds as germination sites in the southern Karoo, South Africa. *Afr. J. Ecol.* 30, 335–345.
- Dean, W.R.J., Yeaton, R.I., 1993a. The effects of harvester ant *Messor capensis* nest-mounds on the physical and chemical properties of soils in the southern Karoo, South Afr. *J. Arid Environ.* 25, 249–260.
- Dean, W.R.J., Yeaton, R.I., 1993b. The influence of harvester ant *Messor capensis* nest-mounds on the productivity and distribution of some plant species in the southern Karoo, South Africa. *Vegetatio* 106, 21–35.
- Dean, W.R.J., Milton, S.J., Klotz, S., 1997. The role of ant nest-mounds in maintaining small scale patchiness in dry grasslands. *Biodiv. Conserv.* 6, 1293–1307.
- Disraeli, D.J., 1984. The effect of sand deposits on the growth and morphology of *Ammophila breviligulata*. *J. Ecol.* 72, 145–154.
- Ehrenberger, F., Gorbach, S., 1973. Methoden der organischen Elementar- und Spurenanalyse. Verlag Chemie, Weinheim.
- Fitter, A.H., 1994. Architecture and biomass allocation as component of the plastic response of root systems to soil heterogeneity by plants. In: Caldwell, M.M., Pearcy, R.W. (Eds.), *Exploitation of Environmental Heterogeneity by Plants*. Academic Press, San Diego, pp. 305–323.
- Folgarait, P.J., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiv. Conserv.* 7, 1221–1244.
- Folgarait, P.J., Perelman, S., Gorosito, N., Pizzio, R., Fernández, J., 2002. Effects of *Camponotus punctulatus* ants on plant community composition and soil properties across land-use histories. *Plant Ecol.* 163, 1–13.
- Frouz, J., Šantrůčková, H., Kalčík, J., 1997. The effect of wood ants (*Formica polyctena* Forest.) on the transformation of phosphorus in a spruce plantation. *Pedobiologia* 41, 437–447.
- Haarløv, N., 1960. Microarthropods from Danish soils. *Oikos* (Suppl. 3), 1–176.
- Hole, F.D., 1981. Effects of animals on soil. *Geoderma* 25, 75–112.
- Hutchings, M.J., de Kroon, H., 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. *Adv. Ecol. Res.* 25, 159–238.
- Jakubczyk, H., Czerwiński, Z., Pętał, J., 1972. Ants as agents of the soil habitat changes. *Ekol. Polska* 20, 153–161.
- Johnson, R.A., 1998. Foundress survival and brood production in the desert seed-harvester ants *Pogonomyrmex rugosus* and *P. barbatus* (Hymenoptera, Formicidae). *Insectes Soc.* 45, 255–266.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 373–386.
- King, T.J., 1977. The plant ecology of ant-hills in calcareous grasslands. I. Patterns of species in relation to ant-hills in southern England. *J. Ecol.* 65, 235–256.
- King, T.J., 1981. Ant-hills and grassland history. *J. Biogeogr.* 8, 329–334.
- Kovář, P., Kovářová, M., Dostál, P., Herben, T., 2001. Vegetation of ant-hills in a mountain grassland: effects of mound history and of dominant ant species. *Plant Ecol.* 156, 215–227.
- Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., Dhillon, S., 1997. Soil functions in a changing world: the role of invertebrate ecosystem engineers. *Eur. J. Soil Biol.* 33, 159–193.
- Levan, M.A., Stone, E.L., 1983. Soil modification by colonies of Black Meadow Ants in a New York old field. *Soil Sci. Soc. Am J.* 47, 1192–1195.
- Lobry dr Bruyn, L.A., Conacher, A.J., 1994. The bioturbation activity of ants in agricultural and naturally vegetated habitats in semi-arid environments. *Aust. J. Soil Res.* 32, 555–570.
- Moore, P.D., Chapman, S.B., 1986. *Methods in Plant Ecology*. Blackwell Scientific Publications, Oxford.
- Olsen, R.S., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Dept. Agric. Stat.*, No. 939.
- Paton, T.R., Humphreys, G.S., Mitchell, P.B., 1995. *Soils—A New Global View*. Yale University Press, New Haven.
- Pecháčková, S., 1997. The dark side of the meadow. Ph.D. Thesis, Charles University Prague.
- Pętał, J., 1980. Ant populations, their regulation and effect on soil in meadows. *Ekol. Polska* 28, 297–326.
- Pontin, A.J., 1960. Field experiments on colony foundation by *Lasius niger* (L.) and *Lasius flavus* (F.) (Hym., Formicidae). *Insectes Soc.* 7, 227–230.
- Pontin, A.J., 1978. The numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). *Ecol. Entomol.* 3, 203–207.
- Rissing, S.W., 1986. Indirect effects of granivory by harvester ants: plant species composition and repro-

- ductive increase near ant nests. *Oecologia* 68, 231–234.
- Robinson, D., 1994. Tansley Review No. 73. The responses of plants to non-uniform supplies of nutrients. *New Phytol.* 127, 635–674.
- Seliskar, D., 1994. The effect of accelerated sand accretion on growth, carbohydrate reserves, and ethylene production in *Ammophila breviligulata* (Poaceae). *Am. J. Bot.* 81, 536–541.
- Unger, P.W., Kasper, T.C., 1994. Soil Compaction and Root Growth: A Review. *Agron. J.* 86, 759–766.
- Wagner, D., Jones, J.B., Gordon, D.M., 2004. Development of harvester ant colonies alters soil chemistry. *Soil. Biol. Biochem.* 36, 797–804.
- Waloff, N., Blackith, R.E., 1962. The growth and distribution of the mounds of *Lasius flavus* (Fabricus) (Hym: Formicidae) in Silwood Park, Berkshire. *J. Anim. Ecol.* 31, 421–437.
- Woodell, S.R.J., King, T.J., 1991. The influence of mound-building ants on British lowland vegetation. In: Huxley, C.R., Cutler, D.F. (Eds.), *Ant-Plant Interactions*. Oxford University Press, Oxford, pp. 521–535.