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Interactive effects of soil-dwelling ants, ant mounds and simulated grazing on local plant community composition

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Abstract

Interactions between aboveground vertebrate herbivores and subterranean yellow meadow ants (*Lasius flavus*) can drive plant community patterns in grassland ecosystems. Here, we study the relative importance of the presence of ants (*L. flavus*) and ant mounds under different simulated grazing regimes for biomass production and species composition in plant communities. We set up a greenhouse experiment using intact soil cores with their associated vegetation.

We found that plant biomass production in the short term was affected by an interaction between simulated grazing (clipping) and ant mound presence. Clipping homogenized production on and off mounds, while in unclipped situations production was higher off than on mounds. During the experiment, these differences in unclipped situations disappeared, because production on unclipped mounds increased. Plant species richness was on average higher in clipped treatments and patterns did not change significantly over the experimental period. Plant community composition was mainly affected by clipping, which increased the cover of grazing-tolerant plant species. The actual presence of yellow meadow ants did not affect plant community composition and production.

We conclude that the interaction between ant mounds and clipping determined plant community composition and biomass production, while the actual presence of ants themselves was not important. Moreover, clipping can overrule effects of ant mounds on biomass production. Only shortly after the cessation of clipping biomass production was affected by ant mound presence, suggesting that only under low intensity clipping ant mounds may become important determining plant production. Therefore, under low intensity grazing ant mounds may drive the formation of small-scale plant patches.

Zusammenfassung

Die Interaktionen zwischen oberirdisch aktiven, herbivoren Wirbeltieren und der unterirdisch aktiven Gelben Wiesenameise (*Lasius flavus*) können die Muster von Pflanzengemeinschaften in Graslandökosystemen bestimmen. Wir untersuchten die relative Bedeutung des Vorhandenseins von Ameisen und Ameisenhöfen bei unterschiedlichen simulierten Beweidungssystemen für die Biomasseproduktion und Artenzusammensetzung. Wir installierten ein Gewächshausexperiment mit intakten Bodenkernen und ihrer jeweiligen Vegetation.

Wir fanden, dass die Biomasseproduktion der Pflanzen kurzfristig von der Interaktion zwischen simulierter Beweidung (Schnitt) und dem Vorhandensein von Ameisenhöfen beeinflusst wurde. Der Schnitt glich die Produktion auf Ameisenhöfen

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und abseits davon einander an, während bei unbeschnittenen Proben die Produktion abseits der Hügel höher war als auf diesen. Während des Experiments verschwanden diese Unterschiede in den unbeschnittenen Behandlungen, weil die Produktion auf unbeschnittenen Ameisenhügeln anstieg.

Der Artenreichtum der Pflanzen war im Durchschnitt höher in den beschnittenen Behandlungen, und diese Muster änderten sich nicht wesentlich während der Dauer des Experimentes. Die Zusammensetzung der Pflanzengemeinschaft wurde hauptsächlich durch das Beschneiden beeinflusst, wodurch der Deckungsgrad beweidungstoleranter Arten zunahm. Die tatsächliche Anwesenheit der Gelben Wiesenameise beeinflusste die Zusammensetzung und Produktion der Pflanzengemeinschaft nicht.

Wir schließen, dass die Interaktion zwischen Ameisenhügeln und Beschneiden die Zusammensetzung und Produktion der Pflanzengesellschaft beeinflusste, während die Anwesenheit von Ameisen nicht wichtig war. Darüberhinaus kann das Beschneiden den Einfluss von Ameisenhügeln auf die Biomasseproduktion übertönen. Schon kurz nach Beendigung des Beschneidens wurde die Biomasseproduktion durch das Vorhandensein von Ameisenhügeln beeinflusst, was nahelegt, dass Ameisenhügel nur bei geringer Beschneidungsintensität einen wichtigen Einfluss auf die Pflanzenproduktion nehmen. Deshalb könnten Ameisenhügel bei geringer Beweidung die Bildung kleinräumiger Pflanzenformationen steuern.

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Keywords: Aboveground–belowground interactions; Herbivores; *Lasius flavus*; Vegetation composition; Simulated grazing; Multivariate analysis; Principle response curves (PRC)

Introduction

Aboveground and belowground components of terrestrial ecosystems are linked by plants. Consequently, the interaction between aboveground and belowground organisms is an important driver of plant community composition and structure (Wardle et al. 2004). Aboveground and belowground herbivores directly reduce plant biomass, thereby altering the competitive ability of plant species and hence plant community composition (van der Putten & Peters 1997; Olf & Ritchie 1998). Moreover, aboveground–belowground interactions modify soil nutrient cycling which feeds back to plant growth and community composition (Bardgett & Wardle 2003).

The interaction between aboveground vertebrate herbivores and subterranean yellow meadow ants (*Lasius flavus*) has been identified as an important determinant of plant community composition and heterogeneity in grassland ecosystems (e.g. King 1977a, 1977b; Blomqvist, Olf, Blaauw, Bongers, & van der Putten 2000). Yellow meadow ants are ecosystem engineers that modify biotic and abiotic soil properties (Jones, Lawton, & Shachak 1994). They dig up soil from deeper layers to build nest mounds and thereby create open, competition-free space that can be colonized by plants (King 1977b). Moreover, digging modifies abiotic soil conditions (such as pH, organic matter content, temperature and water availability), changes soil community composition and reduces abundances of plant-parasitic pathogens (Blomqvist et al. 2000; Dauber & Wolters 2000; Dostal, Breznova, Kozlickova, Herben, & Kovar 2005). Also, *L. flavus* ‘farm’ root aphids (which are root sap suckers) in their nest mounds, from which they harvest honey dew excretion (Pontin 1978), and which they consume as well. This can be viewed as an obligate symbiosis. The honeydew serves as an important food source for yellow meadow ants, in return the ants tend these aphids in their nests by protecting them from enemies, cleaning them from

microbes and moving them to suitable root sites (Pontin 1978).

All the changes in biotic and abiotic environmental conditions induced by yellow meadow ants affect plant species directly and indirectly and can alter the plant community composition on mounds (e.g. King 1977a, 1977b; Dean, Milton, & Klotz 1997; Blomqvist et al. 2000; Frouz & Jilková 2008). Both King (1977a, 1977b) and Blomqvist et al. (2000) showed that yellow meadow ants interact indirectly with aboveground vertebrate grazers. When tall grasses become dominant in the absence of vertebrate grazing, ants build higher mounds to keep up with the vegetation height. This is probably done to maintain sufficient solar radiation reaching the soil surface, thus to regulate the temperature in the mound. In turn, altered ant activity and mound structure may influence plant growth.

It has been acknowledged that plant community changes on mounds of yellow meadow ants are driven by changes in biotic and abiotic soil conditions as the result of soil heaping by the ants (King 1977a, 1977b; Blomqvist et al. 2000; Frouz & Jilková 2008). The relative importance of the actual presence of the ants themselves on the changes in plant community composition has not been quantified. However, ants could potentially induce changes in plant growth and plant species composition by tending root aphids, which are belowground sap-suckers. Therefore, the aim of our study is to understand whether plant community changes induced by yellow meadow ants are mainly driven through presence of mounds or through the actual presence of the ants themselves. In addition, we investigated how the presence of mounds and of ants themselves interacted with the removal of aboveground plant biomass. We address the following questions (1) do ants alter plant community production and species composition predominantly by building nest mounds (and their associated effects on soil properties) or does the actual presence of the ants themselves affect the plant community as well (for example by tending root aphids)? and (2) how does the

interaction between ants and aboveground biomass removal affect production and species composition of a grassland plant community?

The hypothesis is that the presence of ant mounds is important for determining plant community composition, because ants have been reported to change biotic and abiotic soil conditions (King 1977a, 1977b; Blomqvist et al. 2000; Frouz & Jilková 2008). In addition, the actual presence of the ants themselves will contribute to alter plant community composition and production as well, because ant activity will keep the soil aerated. Moreover, the actual presence of ants affects the abundance, performance and herbivory intensity of root aphids (Pontin 1978), which in turn may affect plant growth and performance. Finally, we hypothesize that aboveground grazing, i.e. the removal of aboveground plant biomass, interacts with the effects of yellow meadow ants (King 1977a, 1977b; Blomqvist et al. 2000). Therefore, we expect the strongest influence on plant community composition when ant mounds, ants and aboveground grazing are all present.

We set up a greenhouse experiment using intact soil cores and their associated plant communities from the field. In a full factorial design, we studied effects of simulated grazing (clipping), ant mound presence and the actual presence of ants themselves (ants were removed or not by poison addition) on changes in plant community production and species composition. During 9 months we measured biomass production and plant community composition.

Methods

Study site

We set up a greenhouse experiment using soil cores from Junner Koeland, a 100-ha nature reserve along the river Overijsselse Vecht (52°32'N, 6°36'E). In this study site it has been found that yellow meadow ants have an important influence on plant community composition in the field (Blomqvist et al. 2000). However, whether these changes were caused by the presence of mounds or the presence of ants themselves has not been addressed yet. Therefore, our greenhouse experiment is a direct follow-up of the field study by Blomqvist et al. and information on biotic and abiotic characteristics of mound and matrix soil can be obtained from their paper (Blomqvist et al. 2000).

The Junner Koeland consists of a 50-ha floodplain area and of a heathland area. The soil type of the floodplain is alluvial, loamy sand. The floodplain area was flooded regularly until 1910 when the river Vecht was canalized. Nowadays, the site is flooded on an irregular basis (once every few years). The area has been used as common grazing land by farmers from the village Junne, and has been grazed extensively by livestock for centuries. Nowadays, it is managed as a nature reserve by the National Forestry Service (Staatsbosbeheer), and is grazed by cattle (*Bos taurus*) from April

to November at stocking rates of about 0.3 animals per ha. The most abundant natural vertebrate herbivores in the area are European rabbits (*Oryctolagus cuniculus*) and common voles (*Microtus arvalis*). Dominant plant species in the grasslands are the graminoids *Agrostis capillaris*, *Festuca rubra*, *Holcus lanatus*, and *Luzula campestris*. Frequently occurring dicots are *Rumex acetosa* and *Trifolium repens*. Also, the grasslands locally host several endangered plant species, such as *Dianthus deltoides*, *Succisa pratensis*, *Thymus serpyllum* and *Mentha pulegium* (nomenclature follows van der Meijden 2005).

Yellow meadow ants (*L. flavus*) are abundant subterranean invertebrates in the floodplain areas of Junner Koeland nature reserve. The density of ant mounds in the floodplain grasslands where we carried out our experiment is approximately 0.5 mounds per m². The ants build conspicuous nest mounds by digging up fresh sand from deeper soil layers thereby changing biotic and abiotic soil properties (e.g. Dean et al. 1997; Blomqvist et al. 2000; Frouz & Jilková 2008).

Experimental set up

In the Junner Koeland nature reserve we laid out a randomized block design for the collection of soil samples, consisting of 6 blocks of 5 m × 5 m in the cattle-grazed floodplain grassland. In September 2006 we collected 4 pairs of soil cores of approximately 10 L with a diameter of approximately 25 cm, totaling 48 soil cores (6 blocks × 4 pairs). One of the soil cores consisted of a whole ant mound and the other of matrix soil. Mound and matrix soil cores were collected at a distance of at least 1 m around, because foraging tunnels of yellow meadow ants can extend to 1 m around the ant nests (Woodell & King 1991). The cores were left intact and put into 10-L pots. We used the soil cores to set up a full factorial greenhouse experiment testing three factors. (1) Clipping of aboveground biomass. In half of our pots aboveground biomass was clipped homogeneously every 2 weeks to a height of approximately 4 cm to simulate repeated cattle grazing during the growing season. This vegetation height corresponds to the vegetation height in the field (Veen et al., unpublished data). Hence, our clipping treatment represents the stocking density of approximately 0.3 animals per ha. In the other half biomass was clipped every 3 months to prevent pots becoming limited for plant growth. (2) Presence of ant mounds. Half of the pots contained soil cores that consisted of matrix soil with their associated vegetation. The other half of the soil cores collected in the field contained an ant colony. We collected small, probably young mounds, to be able to maintain complete ant colonies with its associated vegetation in the 10-L pots. We could not check whether the queen was present in these colonies without destroying the mounds. Therefore, we collected three additional mounds of the same size which were destructively sampled. In all of these mounds we located a queen; hence, we are confident that we used complete, functioning

colonies in our experiment. (3) Presence of ants. To half of the ant mounds we added ant poison baits (organophosphate Trichlorfon 0.1%, Kruidvat, The Netherlands). For this we placed five 15-ml plastic cups on top of the soil at 2 and 4 weeks after the start of the experiment. The ants came to collect the poison themselves, consuming it and feeding it to their offspring. Therefore the poison specifically killed the ants without affecting other soil fauna directly. The ant bodies remained in the nest and were not removed, because this would have destroyed the ant mounds. To control for potential effects of ant poison on other soil organisms, we also added poison baits to half the pots containing matrix soil cores. To check whether our ant removal treatment was successful, we collected soil cores of the top 5-cm of the soil at the end of the experiment and separated all insects from the soil using a Tullgren apparatus (van Straalen & Rijninks 1982). In these samples we counted the number of ants (Appendix A). We did not find any ants in pots that originally contained an ant mound and received poison bait, while all pots with mounds that did not receive poison bait still did contain ants. However, we also found a few pots that originally contained matrix soil but were colonized by yellow meadow ants. Regarding digging activity in the pots, colonization seemed very recent and happened probably towards the end of the experiment.

Our full-factorial set up resulted in 8 different treatments, each replicated 6 times (i.e. 6 random blocks): (a) not clipped + no mounds + no poison, (b) not clipped + no mounds + poison, (c) not clipped + mounds + no poison, (d) not clipped + mounds + poison, (e) clipped + no mounds + no poison, (f) clipped + no mounds + poison, (g) clipped + mounds + no poison, (h) clipped + mounds + poison.

The experiment was maintained in the greenhouse for 9 months at a L15:D9 photoperiod and 23/17 °C. Pots were watered automatically with demineralized water. Pots with soil cores originating from the same random block were kept together in the greenhouse. To control for differences in microclimate in the greenhouse the spatial arrangement of the pots was randomized once every 2 weeks.

Measurements

Clipped plant material was dried at 70 °C for 24 h and weighed. Plant community composition was measured in weeks 4, 6, 8, 10, 12, 14, 22, 28, and 39 of the experiment using a point quadrat method (Levy & Madden 1933; Goodall 1952). A frame with 52 holes was placed over the 10-L pots. Through each hole a metal pin was lowered slowly onto the vegetation. The first plant that was touched by the pin was recorded. Point quadrat measures may underestimate species with a lower height (Jain, Kuriakose, & Balakrishnan 2010). Therefore, in addition to the point quadrat measures we also estimated the percent cover of each plant species at 6 and 9 months after the start of the experiment. We neither find large

differences in cover estimations of species, nor in the number of species. Therefore, in our experiment, the point quadrat measure gave a good representation of the plant cover. Initial plant community composition was determined immediately at the collection of the soil cores by estimating the percent cover of each plant species.

Data analysis

We calculated cumulative biomass production over the first 3 months and over the whole experimental period of 9 months by adding up all biomass measures for these periods respectively. Plant species richness was calculated as the number of plant species per pot using the point quadrat data. We calculated plant species richness after 3 and 9 months. Treatment effects on cumulative biomass production and plant species richness were tested using a general linear model with clipping, presence of ant mounds and poison addition as fixed factors. Block was used as a random factor. Data were distributed normally (Shapiro-Wilk normality test) and had homogeneous variances (Levene's test). To determine the effects of our fixed factors we used a likelihood ratio analysis to compare models with and without the variable of interest using a chi-square test statistic (Crawley 2007). Analyses were performed using R version 2.10.0. We used the lme4 package to perform general linear mixed models (Bates & Maechler 2009).

We used redundancy analysis (RDA) and principle response curves (PRC) to analyze initial plant community composition and the extent and direction of plant community changes over time (Van den Brink & ter Braak 1999; Leps & Smilauer 2003). RDA is a constrained form of principle component analysis (PCA). RDA and PCA find the best set of predictor variables (summarized in ordination axes) that explain a multivariate data set. In contrast to PCA, in RDA the ordination axes are constrained to linear combinations of predictor (environmental) variables. We used RDA analysis to test the effect of our experimental treatments (clipping, mound presence and poison addition) on plant community composition and development. Initial plant community composition was analyzed using RDA analysis with presence of mounds, clipping treatment and poison treatment as explanatory variables.

To analyze the development of plant communities over time, we used an RDA analysis with treatment × sampling time combinations as explanatory (i.e. environmental) variables. Sampling time and block were used as covariables. This allowed us to test whether our treatments significantly explained plant community changes over time. Additionally, we used three separate RDA analyses to test the explanatory power of the separate treatment levels on temporal changes in plant community composition, i.e. of clipping, mounds and poison. In each of these RDA analyses we used the treatment × time combinations for one of our treatments as environmental variable, and treatment × time combinations

for the other two treatments as covariables. This allowed us to test how much variation was explained by each of the separate treatments, i.e. clipping, mound and poison in addition to the other two treatments.

We used principle response curves (PRCs) to graphically present plant community changes over time for each of our experimental treatments (Van den Brink & ter Braak 1999). In PRC analysis one of the treatments is used as reference treatment against which the changes in the other treatments are plotted. We used clipped pots without mounds and poison as a reference, because this corresponds to the dominant situation in the Junner Koeland nature reserve. PRC analysis is based on a partial RDA where each treatment \times time combination (i.e. our full-factorial combination of all treatment levels), except for the reference treatment, is used as explanatory variable. Sampling time was added as covariable. The vertical scores of the first-order PRCs are based on the scores of environmental variables on the first axis of the RDA. In the PRC diagram we included a 1-D plot that shows the plant species scores on the first RDA axis. A positive score on the 1-D plot indicates that a species increases in abundance over time; a negative score indicates that a species decreases in abundance over time. A combination of the PRCs with the 1-D species plot illustrates changes in species abundance in the different treatments over time.

The significance of the RDA axes was tested using Monte Carlo permutation tests with 999 permutations. Within blocks permutations were restricted by a split-plot design that represented the temporal structure of the data. We allowed whole plots (data that were collected in the same pot at the same time) to permute at random, while split-plots (different pots at different times) were not permuted. To test the significance of higher-order ordination axes we added the lower-order axes as covariables. We only present results for the first ordination axes, because in all cases higher-order axes were found to be not significant ($P > 0.05$).

RDA and PRC analyses were carried out using CANOCO version 4.5 for Windows (ter Braak & Smilauer 2002).

Results

Biomass

Cumulative biomass production over 3 months was affected by an interaction between clipping and the presence of ant mounds (Table 1, Fig. 1A). Only in pots without mounds that were left unclipped biomass production was higher than in all other treatments. Under clipping there was neither an effect of the presence of mounds nor of poison on biomass production, while in the absence of clipping it did matter whether mounds were present or not. Both clipping (Table 1) and the presence of ant mounds (Table 1) reduced biomass production. The addition of ant poison did not affect biomass production after 3 months (Table 1, Fig. 1A). After 9 months, cumulative biomass

Table 1. Biomass production and species richness. Results from the general linear models on biomass production and species richness in the different experimental treatments. Bold values are significant.

	3 months		9 months	
	χ^2 -value	<i>P</i> -value	χ^2 -value	<i>P</i> -value
Biomass production				
Clipping	8.253	0.004	15.28	<0.001
Mound	14.31	<0.001	0.036	0.850
Poison	1.017	0.313	1.107	0.293
Clipping x mound	4.162	0.041	0.819	0.366
Clipping x poison	2.307	0.129	1.046	0.306
Mound x poison	0.071	0.790	0.041	0.840
3-Way interaction	0.092	0.762	0.037	0.847
Species richness				
Clipping	9.268	0.002	5.864	0.015
Mound	0.106	0.744	0.617	0.432
Poison	1.561	0.211	0.202	0.653
Clipping x mound	3.963	0.047	2.451	0.063
Clipping x poison	0.020	0.887	0.015	0.904
Mound x poison	0.138	0.711	2.425	0.119
3-Way interaction	0.708	0.400	0.218	0.641

Degrees of freedom (df) were 1 for all analyses.

production was affected by clipping only. In clipped pots less biomass was produced after 9 months (Table 1, Fig. 1B). Neither the presence of ant mounds (Table 1), nor of poison (Table 1) affected total biomass. Biomass production over the study period was faster on ant mounds than off ant mounds (Table 2, Fig. 1C), leading to similar amounts of biomass produced on and off unclipped mounds at the end

Table 2. Change in biomass production and species richness. Results from general linear models on the change in biomass production and species from month 3 to month 9 of the experiment. Bold values are significant.

	χ^2 -value	<i>P</i> -value
Change production		
Clipping	2.280	0.131
Mound	10.47	0.001
Poison	1.694	0.193
Clipping x mound	1.471	0.225
Clipping x poison	1.649	0.199
Mound x poison	0.489	0.485
3-Way interaction	0.013	0.910
Change species richness		
Clipping	0.028	0.868
Mound	0.342	0.559
Poison	2.093	0.148
Clipping x mound	0.136	0.712
Clipping x poison	0.052	0.819
Mound x poison	1.999	0.157
3-Way interaction	0.227	0.634

Degrees of freedom (df) were 1 for all analyses.

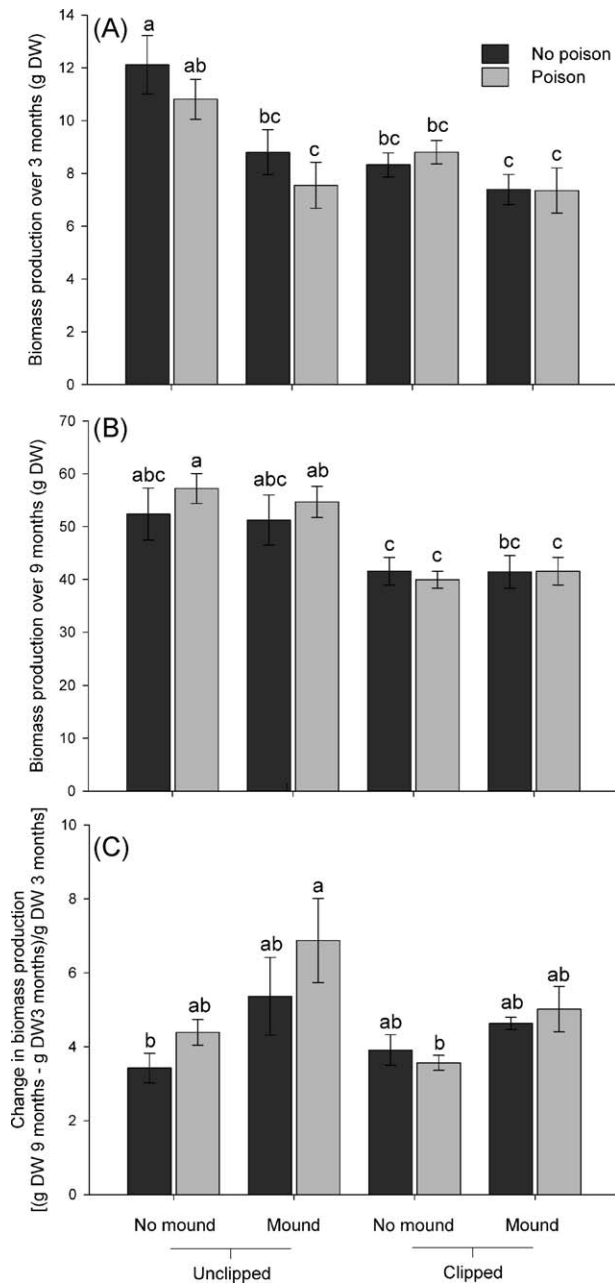


Fig. 1. Cumulative biomass production (g dry weight – g DW) per treatment over (A) 3 months and (B) 9 months and (C) the change in biomass production over the study period. Treatments represent a full-factorial design with three factors: aboveground biomass was left or removed by clipping (unclipped vs. clipped), ant mounds were absent or present (no mound vs. mound), and ants were left or removed (no poison vs. poison). Error bars display standard errors. Different letters above bars indicate significant differences at $P < 0.05$.

of the study period (Fig. 1B). This explains that the effect of the interaction between mounds and clipping in biomass production after 3 months disappeared, and only the clipping treatment affected the amount of biomass produced after 9 months.

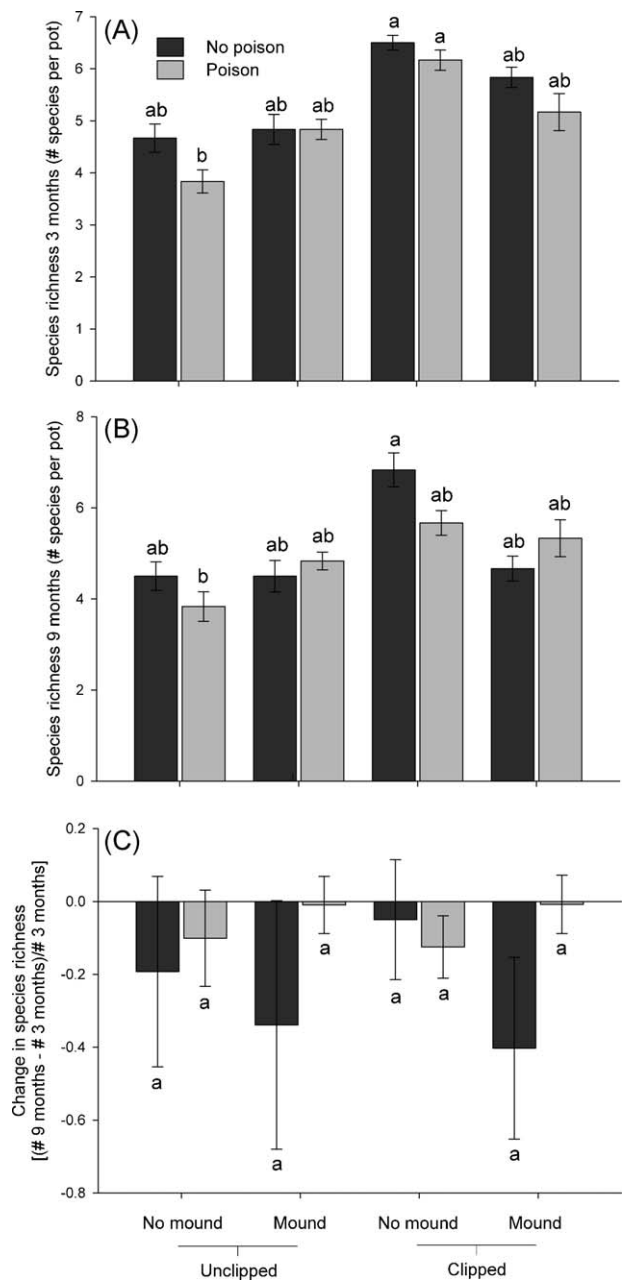


Fig. 2. Plant species richness (number of plant species per pot) after (A) 3 months and (B) 9 months and (C) the change in plant species richness over the study period. Treatments represent a full-factorial design with three factors: aboveground biomass was left or removed by clipping (unclipped vs. clipped), ant mounds were absent or present (no mound vs. mound), and ants were left or removed (no poison vs. poison). Error bars display standard errors. Different letters above bars indicate significant differences at $P < 0.05$.

Plant species richness

After 3 months species richness was affected by an interaction between clipping and the presence of ant mounds (Table 1, Fig. 2A). Only in pots with clipping and without mounds we found a higher number of species. Clipping

significantly increased plant species richness (Table 1, Fig. 2A) from 4.5 species in unclipped pots to 5.9 species on average in clipped pots. Neither the presence of ant mounds, nor the actual presence of the ants themselves (Table 1, Fig. 2A) affected plant species richness after 3 months. After 9 months we found a very similar pattern. The interaction between clipping and the presence of mounds tended to affect species richness (Table 1, Fig. 2B), because species richness was higher in the pots with clipping and without mounds only. Again, clipping increased species richness from 4.4 species in unclipped pots to 5.6 species clipped pots (Table 1, Fig. 2B). Neither the presence of ant mounds, nor of poison affected species richness (Table 1, Fig. 2B). These results show that clipping is the main determinant of the number of plant species, because the effect of the interaction between clipping and mound was weak and we did not find consistently different effects of mounds under certain clipping treatments. The change in plant species richness over the study period was not affected by the experimental treatments (Table 2, Fig. 2C).

Plant community composition

Initial plant community composition was neither affected by the clipping (1st axis: $F = 0.792$, $P = 0.538$), nor by the poison treatment (1st axis: $F = 1.31$, $P = 0.338$). The presence of ant mounds did influence initial plant community composition (1st axis: $F = 4.19$, $P < 0.001$) and explained 9.3% of the variation in plant species composition. On cores with ant mounds the cover of *Cerastium arvense*, *Festuca rubra*, and *Stellaria graminea* was increased, while on cores from matrix soils the cover of, for example, *Rumex acetosa*, *Taraxacum spec* and *Trifolium repens* was higher (Fig. 3).

Our experimental treatments significantly explained plant community changes over time (1st axis: $F = 33.80$, $P < 0.001$, 11.6% of the variation in the first 2 RDA axes). Detailed analyses showed that this was due to the effect of clipping on temporal plant community patterns (1st axis: $F = 34.89$, $P = 0.036$). Neither the presence of mounds (1st axis: $F = 16.06$, $P = 1.000$) nor the addition of poison (1st axis: $F = 5.91$, $P = 0.555$) could significantly explain plant community development. Hence, Fig. 4 shows that the principle response curves (PRCs) only separate the clipped from the unclipped treatments, while there is little effect of the presence of ant mounds or poison addition. PRCs of clipped pots do not differ much from the reference treatment that was clipped as well, while unclipped pots have a divergent community composition. Clipped treatments had higher abundances of grazing-tolerant plant species such as *Festuca rubra* and *Agrostis capillaris* while unclipped pots had higher abundances of plant species that become dominant in ungrazed situations, such as *Holcus lanatus* and *Rumex acetosa* (Fig. 4). For an overview of the plant abundances in clipped and unclipped treatments, on and off ant mounds, averaged over the whole study period see Appendix A.

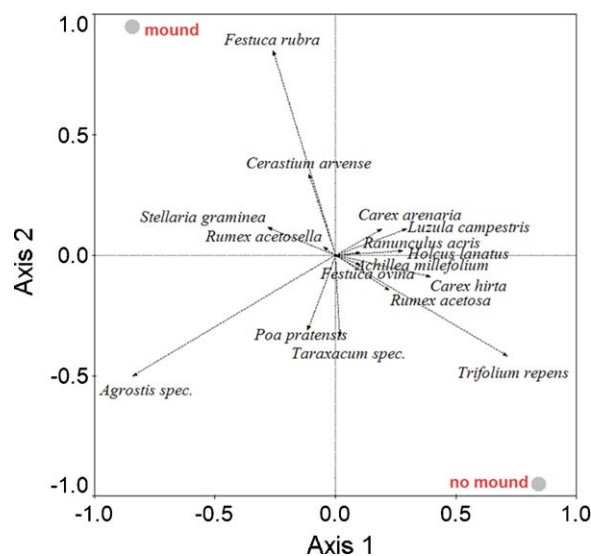


Fig. 3. Ordination plot based on redundancy analysis (RDA) showing initial plant community composition on the collected soil cores. Arrows show the response of the plant species. Grey dots represent whether mounds were present (mound) or absent (no mound). The clipping and poison treatment are not plotted, because they did not affect initial plant community composition ($P \gg 0.05$).

Discussion

We found that an interaction between yellow meadow ant mound presence and simulated grazing was more important driving biomass production and species richness in the plant community than the actual presence of yellow meadow ants themselves. Simulated grazing overruled effects of ant mounds on small-scale plant community production in the short term, because it homogenized biomass production on and off mounds. Hence, differences in biomass production on and off mounds became expressed in the absence of simulated grazing only. In the long term, biomass production was affected by simulated grazing, and not by the presence of ant mounds, because the influence of ant mounds in unclipped situations disappeared due to a larger change in biomass production on unclipped ant mounds.

Our study confirms that aboveground grazers (e.g. Milchunas & Lauenroth 1993; Olf & Ritchie 1998; Proulx & Mazumder 1998) can affect plant species richness, plant community composition and plant production. In productive ecosystems, removal of aboveground biomass by (simulated) grazing enhances light availability and relaxes competition for light between plant species (Olf et al. 1997). As a result, grazers promote the cover of subordinates (Glenn & Collins 1990; Olf et al. 1997; Knapp et al. 1999; Veen, Blair, Smith, & Collins 2008) and hence plant species diversity (Bakker 1989; Collins, Knapp, Briggs, Blair, & Steinauer 1998; Bakker, Ritchie, Olf, Milchunas, & Knops 2006). In real ecosystems grazers also affect the plant community indirectly through the deposition of excreta and via trampling (Hobbs 1996; Bardgett & Wardle 2003). Since such effects

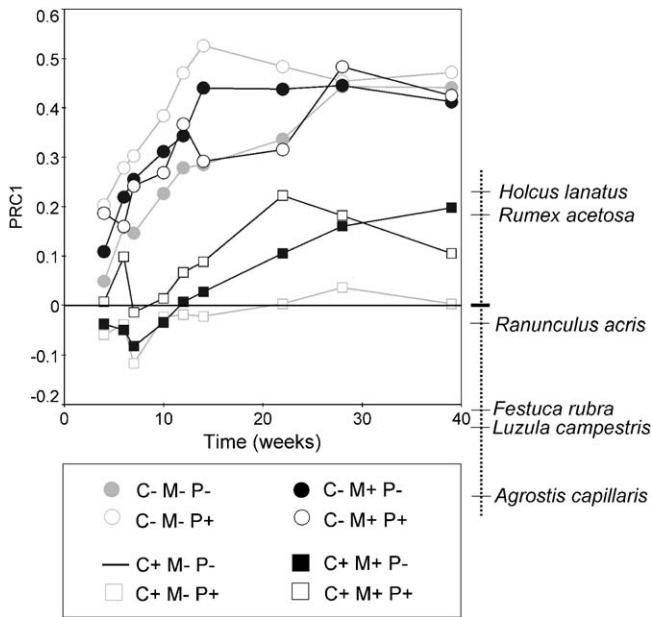


Fig. 4. Principle response curves showing the extent and direction of plant community changes in our greenhouse experiment along the first RDA axis (PRC1: $F = 33.704$, $P = 0.002$). Curves are plotted relative to changes in C+M–P– (clipping, no mound, no poison), which serves as the reference treatment (straight line). Different symbols represent the different treatments. Unclipped treatments are represented by circles, clipped treatments by squares; treatments without ant mounds grey symbols, treatments with mounds by black symbols; treatments without poison bait by filled symbols and treatments with poison bait by open symbols. The vertical 1-D plot represents the scores the plant species on the first RDA axis. A positive value means that a species increases as compared to the control treatment (C+M–P–); while a negative value means a decrease. For clarity, only species with the best fit to the first ordination axis are included in the diagram (“Lower axis minimum fit” was set to 4).

of grazing play out over longer time scales they are expected to have less influence on plant community characteristics in our experiment than the actual removal of biomass.

Effects of yellow meadow ants on the plant community were mediated by the presence of mounds, and not ants themselves, and depended on the simulated grazing regime. Altered biotic and abiotic soil properties in ant mounds may drive changes in plant production and species composition (e.g. Blomqvist et al. 2000; Dauber & Wolters 2000; Dostal et al. 2005). However unexpectedly, we found a lower plant production on unclipped ant mounds, which have been identified previously as microbial hot-spots (Dauber & Wolters 2000) with high nutrient availability (Blomqvist et al. 2000). The lower plant production on unclipped mounds in our study may result from reduced levels of soil moisture (Blomqvist et al. 2000) or increased herbivory by e.g. root aphids in ant mounds (Pontin 1978). We also found that plant species richness was lower on than off ant mounds, which confirmed other studies (Dean et al. 1997; Lenoir 2009). Plant species that are

sensitive to soil heaping, increased herbivory by root aphids, or lower soil moisture levels may decline on ant mounds, resulting in a lower number of plant species on mounds as compared to off mounds. Still, ant mounds may enhance overall diversity in the plant community over larger spatial scales (King 1977a, 1977b; Blomqvist et al. 2000), because they serve as competition- (more bare soil) and pathogen-free (less plant-feeding nematodes) zones (King 1977a, 1977b; Blomqvist et al. 2000). Thereby they provide a refuge to different plant species that cannot establish in the matrix vegetation (Dean et al. 1997; Lenoir 2009).

Our results show that clipping can overrule effects of yellow meadow ants. Ant mounds did not affect biomass production in clipped treatments. Moreover, in the long term the clipping treatment was the only determinant of biomass production, species richness and species composition. Clipping may have exerted such a strong control on plant species that only grazing-tolerant species became abundant in the plant community, regardless of other factors such as ant mounds and plant–soil feedback effects (Veen, Geuverink, & Olf 2011). Indeed, we found a strong increase in grazing-tolerant plant species like *Festuca rubra* and *Agrostis capillaris* in the clipped pots, both on and off ant mounds, while in unclipped pots tall grasses that are not tolerant to aboveground grazing, such as *Holcus lanatus*, increased on and off mounds. However, at the start of our experiment we found that ant mound effects on plant production became expressed. This suggests that under conditions of more extensive grazing ant mounds may drive plant community patterns (Dean et al. 1997; Lenoir 2009).

Grazing by root herbivores can have major consequences for plant community composition (van der Putten & Peters 1997; Wardle et al. 2004). Therefore, we expected that the actual presence of ants would affect plant production and species composition via their influence on aphid performance and herbivory (Pontin 1978). However, we did not find effects of ants themselves on the plants. Maybe, ant effects on root aphids have interacted with ant effects on belowground plant pathogens (Blomqvist et al. 2000), or the nutrient input of dead ant bodies in the poison treatment overruled negative effects of root aphids, or changes in aphid performance play out over longer time scales. To mechanistically show how the actual presence of ants can influence plant patterns via their effects on root aphids, it will be necessary to set up controlled experiments where ant and aphid presence are manipulated and monitored.

Even though we did not find strong effects of ant mounds on plant species composition during the experiment, we would have expected to find large differences on and off mounds at the start of our experiment (Dean et al. 1997; Blomqvist et al. 2000). However, the presence of ant mounds explained only a small, but significant, part of the initial differences between mound and matrix vegetation. On the one hand, ants can have highly variable effects on soil characteristics and plants, even within soil types (Cammeraat & Risch 2008). On the other hand, the mounds that we collected in the field

were very small and ant-induced changes in for example biotic soil properties take time to develop (Dauber & Wolters 2000). However, it has been shown that soil heaping of yellow meadow ants in young mounds smothers existing vegetation and leads to the invasion of a new set of plant species immediately (King 1977b). Therefore, it seems that cattle grazing in the field may have homogenized the local plant community (Glenn, Collins, & Gibson 1992; Adler, Raff, & Lauenroth 2001; Bakker 2003) by locally exerting a strong grazing pressure, thereby overruling effects of yellow meadow ants on plant species composition from the start of our experiment.

Conclusion

The interaction between ant mounds and simulated above-ground grazing determined plant production and species composition on and off mounds of yellow meadow ants. The actual presence of ants themselves was of less importance. Our results show that shortly after the cessation of grazing biomass production is different on and off ant mounds, while in intensively grazed or ungrazed situations effects of yellow meadow ants and ant mounds were not expressed in plant community production and species composition. Therefore, in situations of extensive vertebrate grazing, differences in biomass production on and off mounds may become expressed and mounds of yellow meadow ants, rather than ants themselves, will than be important drivers of small-scale plant community production and species composition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baec.2011.10.001.

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