Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture

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Abstract. Large herbivores can influence plant and soil properties in grassland ecosystems, but especially for belowground biota and processes, the mechanisms that explain these effects are not fully understood. Here, we examine the capability of three grazing mechanisms—plant defoliation, dung and urine return, and physical presence of animals (causing trampling and excreta return in patches)—to explain grazing effects in *Phleum pratense–Festuca pratensis* dairy cow pasture in Finland. Comparison of control plots and plots grazed by cows showed that grazing maintained original plant-community structure, decreased shoot mass and root N and P concentrations, increased shoot N and P concentrations, and had an inconsistent effect on root mass. Among soil fauna, grazing increased the abundance of fungivorous nematodes and *Aporrectodea* earthworms and decreased the abundance of detritivorous enchytraeids and *Lumbricus* earthworms. Grazing also increased soil density and pH but did not affect average soil inorganic-N concentration. To reveal the mechanisms behind these effects, we analyzed results from mowed plots and plots that were both mowed and treated with a dung and urine mixture. This comparison revealed that grazing effects on plant attributes were almost entirely explained by defoliation, with only one partly explained by excreta return. Among belowground attributes, however, the mechanisms were more mixed, with effects explained by defoliation, patchy excreta return, and cow trampling. Average soil inorganic-N concentration was not affected by grazing because it was simultaneously decreased by defoliation and increased by cow presence. Presence of cows created great spatial heterogeneity in soil N availability and abundance of fungivorous nematodes. A greenhouse trial revealed a grazing-induced soil feedback on plant growth, which was explained by patchiness in N availability rather than changes in soil biota. Our results show that grazing effects on plant attributes can be satisfactorily predicted using the effects of defoliation, whereas those on soil fauna and soil N availability need understanding of other mechanisms as well. The results indicate that defoliation-induced changes in plant ecophysiology and the great spatial variation in N availability created by grazers are the two key mechanisms through which large herbivores can control grassland ecosystems.

Key words: dung and urine soil amendment; earthworms; Elymus repens; *enchytraeid; Finnish dairy-cow pasture; herbivore; nematodes; nitrogen;* Phleum pratense–Festuca pratensis *grassland; soil feedback; spatial variation; trampling.*

INTRODUCTION

Ecological linkages between aboveground and belowground organisms and their role in the structure and functioning of terrestrial ecosystems are getting increasing interest among ecologists (Van der Putten et al. 2001, Wardle 2002, Wardle et al. 2004). In particular, the ability of herbivores to initiate changes in plant community structure and plant growth that further propagate in aboveground and belowground food webs has been actively discussed (Masters et al. 1993, Bardgett et al. 1998b, Bardgett and Wardle 2003). In

grassland ecosystems, large mammalian herbivores are known to influence plant community structure (Coppock et al. 1983, Cid et al. 1991, Collins et al. 1998, Chase et al. 2000, Hellström et al. 2003), primary production (McNaughton 1976, Frank and McNaughton 1993), and growth and turnover of plant roots (Pandey and Singh 1992, Johnson and Matchett 2001, Frank et al. 2002). Grazers have also been shown to affect microbes and animals that control decomposition of dead organic matter in soil (Holt 1997, Bardgett et al. 2001, Sankaran and Augustine 2004, Patra et al. 2005), with consequences on soil nutrient mineralization (Hassink 1992, Frank and Groffman 1998, Frank et al. 2000). However, in most variables the effects appear to vary from negative to positive in different studies (Milchunas and Lauenroth 1993, Wardle 2002, Bardgett

and Wardle 2003), and the reason for this is not fully understood.

Predicting and understanding grazing effects is complicated because grazing is a combination of several factors simultaneously affecting plants and their environment (Frank and McNaughton 1993). Defoliation, i.e., removal of plant shoot tissue, has effects on plant growth and carbon allocation (Briske et al. 1996, Ferraro and Oesterheld 2002), which can influence root carbon exudation (Paterson and Sim 1999) and the rhizosphere organisms that rely on the carbon released from plant roots (Hamilton and Frank 2001). Defoliation also reduces input of aboveground litter to soil, thus decreasing the amount of coarse organic matter in the soil (Burke et al. 1999). On the other hand, inputs of animal urine and dung create patches in the upper layers of soil that are rich in nitrogen and organic matter (Afzal and Adams 1992, Bogaert et al. 2000). These patches provide resources for soil microbes (Bardgett et al. 1998a, Peacock et al. 2001, Bittman et al. 2005) and animals (Curry 1976, Forge et al. 2005) and can support vigorous plant production (Day and Detling 1990, Steinauer and Collins 2001). The physical contact with grazers also has consequences as animal treading damages plant foliage and decreases the amount and size of soil pores (Greenwood and McKenzie 2001). Earlier experiments have aimed at separating the effects of defoliation, excreta return and trampling on pasture herbage production (e.g., Curll and Wilkins 1983, Lobry de Bruyn and Kingston 1997), but to our knowledge no study has examined the relative importance of these mechanisms in the effects of grazing on belowground organisms and soil nutrient availability.

Here we present results from a three-year grazing study established on a dairy cow pasture in Finland. The experimental site consisted of replicated treatment plots of different combinations of three grazing mechanisms: (1) defoliation, (2) dung and urine return, and (3) physical presence of dairy cows (causing trampling and excreta return in patches). The purpose of our study was to clarify the relative roles of these mechanisms in the effects of grazing on plant and soil properties in the pasture. Moreover, since it has been shown that defoliation-induced changes in the soil can feed back to plants (Hamilton and Frank 2001, Mikola et al. 2005a, Sørensen et al. 2008), we collected soil from different treatment plots in the field and followed the growth and N uptake of grass seedlings planted into these soils in a greenhouse. This allowed us to examine whether the different grazing mechanisms can create changes in the soil that feed back on plant growth and nutrient uptake, and thus partly explain the effects of grazing on plants observed in the field. We expected grazing to affect most of the measured plant and soil variables, both in the field and in the feedback test, but hypothesized that the mechanisms explaining these effects will differ among the variables. We assumed that (1) the effect of grazing on plants will mostly be explained by defoliation and dung and urine return, because these mechanisms will directly affect plant nutrient availability, plant growth, and plant resource allocation (Ferraro and Oesterheld 2002, Bazot et al. 2005, Ilmarinen et al. 2007). We also expected (2) the effects of grazing on soil microfauna (nematodes in our study) to be mostly explained by defoliation and dung and urine return, as these microscopic animals are closely associated with plant roots (Griffiths 1994) and can readily respond to changes in plant growth and resource allocation (Mikola et al. 2001, 2005b). For soil abiotic attributes and bigger soil animals (enchytraeids and earthworms in our study), we assumed (3) the physical presence of cows also to have a significant role in explaining the grazing effects, as cow trampling will alter soil structure (Greenwood and McKenzie 2001). Finally, we assumed that (4) defoliation (due to reducing C supply to soil decomposers and removing nutrients from the pasture), dung and urine return (due to returning nutrients to the pasture), and cow presence (due to affecting large animals and creating patchiness in nutrient return) all will explain the grazing-induced soil feedback on plant growth and nutrient uptake in the greenhouse trial.

METHODS

Field site description

The study was carried out in a 2.0-ha pasture at the research station of MTT Agrifood Research Finland, Animal Production Research, Maaninka, Finland (63°10′ N, 27°18′ E). In Finland, pastures typically follow a four-year rotation, which includes one year for establishment (year 1999 in our case) and three years for cow grazing (years 2000–2002 in our case). The site we used had one pasture rotation before our experiment, preceded by several years of ley-arable crop rotations. To reduce the abundance of weeds, such as *Elymus repens*, a new pasture rotation begins with herbicide application and plowing in the last autumn of the previous rotation (in our case, glyphosate was applied at 1.44 kg/ha in the autumn of 1998). Glyphosate applications are not known to have long-term harmful effects on earthworms (Dalby et al. 1995, Mele and Carter 1999) or nematodes (Sanderson et al. 1999), but there is evidence that shortly after application, glyphosate can interfere with earthworm reproduction (Casabé et al. 2007). The new pasture is established in the spring following the herbicide application (in our case 1999) using a seed mixture of preferred grasses and barley, *Hordeum vulgare* L., which serves as a temporary cover plant that is harvested later in the summer. For our pasture, a seed mixture (70:30 mass : mass) of timothy (*Phleum pratense* L. cv Tuukka) and meadow fescue (*Festuca pratensis* Huds. cv Antti), respectively, was used with a total seeding rate of 20 kg/ha.

At the study area, growing seasons typically extend from the beginning of May to the beginning of October

and have a cumulative, >5°C temperature sum (calculated by summing up for the whole growing season those degrees of daily mean temperature that exceed 5°C) of around 1200 (data collected at a meteorological station 300 m from the pasture). During study years 2000–2002 the growing seasons started earlier, lasted longer (except in 2002), and had a higher cumulative temperature sum than on average (Appendix A). In 2000 the monthly mean temperatures deviated little from the long-term means, but the summers of 2001 and 2002 were warmer than average (Appendix A). Precipitation each year was ~10% lower than the long-term mean and spread differently over the months in different years: in 2001 each month was equally rainy, whereas in 2000 June and in 2002 June and July were significantly rainier than the other months (Appendix A). Plant-available soil moisture, measured using gypsum blocks (Model 5201, Soilmoisture Equipment Corporation, Santa Barbara, California, USA) at the depth of 20 cm at two points of the experimental area, differed considerably between the study years: during the six-week period before harvesting, soil moisture was variable in 2000, low in 2001 and high in 2002 (Appendix B). The soil organic-matter content (0–40 cm profile) varied between 3% and 5.9% at the site, and the mineral part of the soil consisted of silt (41%), fine sand (35%), clay (20%), and coarse sand (4%). The soil had a pH (H_2O) of 6.2, and exchangeable K and soluble P concentrations, measured using acid (pH 4.65) ammonium acetate solvent (Vuorinen and Mäkitie 1955), of 118 and 12.9 mg/L soil, respectively. The pasture was fertilized with 215 kg N, 10 kg P, and 36 kg K per hectare per year, divided into three applications each year.

Setup and treatments in the field experiment

The setup of the field experiment consisted of four different types of treatment plots: (1) Control plots with normal NPK fertilization only (C plots), (2) Mowed plots (M plots), (3) plots that were Mowed but also received mixture of cow Dung and Urine (MDU plots), and (4) plots that were Grazed by cows (G plots). For establishing the plots, a homogeneous area of 68 × 13.5 m (later called "experimental area") was selected along a fenced perimeter of the pasture in the spring of 2000 (see Appendix C for a depiction of the area and the arrangement of treatment plots within the area). At each of the three open sides of the experimental area, 6-m-wide zones were allocated to cow grazing, while the remaining inner area (56 × 7.5 m) was fenced and allocated to other treatments. The experimental area was then divided longitudinally into 10 blocks and four treatment plots (each 1.75 × 2.20 m) were established in each block—the C, M and MDU plots inside and the G plot outside the fence—giving 10 replicates for each treatment. Inside the fence, the three plots were placed 1 m away from each other (giving 1-m buffer zones between plots of different treatments) and 1 m from the fence. Treatments were then randomly allocated to the

plots. The imaginary G plot was placed 5 m away from the fence to avoid the area of excessive animal trampling near the fence. Finally, three sampling plots (each 30 × 30 cm, situated cornerwise to each other [checkerboard style], with a 20-cm distance between corners) were established in the middle of each treatment plot, with 50-cm-wide buffer zones against the edges of the treatment plot. One of these three plots was sampled each year. The effect of current sampling on further samplings was reduced by replacing the removed plant-soil monolith with a similar monolith taken from additional treatment plots established for this purpose (i.e., the removed and the substitute material had an identical treatment background).

Cows grazed the pasture in a rotational manner (four to five times each year), with the number and timing of grazing rotations depending on yearly herbage production. To determine the number of cow × grazing days needed for each grazing rotation, the estimate of total available herbage in the pasture (kilograms of dry matter per hectare) was divided by daily herbage allowance adjusted to 23 kg dry matter per day per cow. Mowing and addition of the dung–urine mixture to the treatment plots were timed according to grazing rotations: i.e., at each rotation, M plots were mowed on the same day as cows started grazing the pasture, and during the second and fourth rotation, the dung-urine mixture was added to MDU plots. At each mowing, plant shoots in M and MDU plots were cut to 6-cm stubble height and the harvested material was removed from the plot using a Haldrup Forage Plot Harvester (J. Haldrup, Løgstør, Denmark). Cow dung and urine were collected during milking and stored separately in covered containers for 6 to 12 h until mixed together and applied to the field. The yearly load of dung and urine per MDU plot was estimated using earlier information of (1) the mean herbage production per hectare in a similar pasture, (2) the mean amount of supplementary forage needed to cover the energy and protein requirements of milk production by the cows, and (3) the mean digestibility of the grazed herbage and the supplementary forage. Dung and urine were applied in two portions: the first application during the second grazing rotation contained two fifths of the mixture and the second application during the fourth rotation contained three fifths of the estimated total addition (in 2002 only the first portion was applied because the last sampling took place before the second application). At the end of the growing seasons in 2000 and 2001 the validity of the estimation of needed dung and urine load was checked using information gathered during the season, and if needed, the applied amounts were corrected in the first application of the following year. As a whole, the five applications (2 + 2 + 1) of dung-urine mixture returned 872 g dry matter, 258 g C, 61 g N (of which 45 g was soluble), 6.1 g P, 71 g K, 6.5 g Mg, and 11 g Ca per square meter to the MDU plots.

Sampling procedure

Each year one of the 30 × 30 cm sampling plots was harvested one day before the fourth grazing rotation, i.e., 31 July in 2000, 20 August in 2001, and 7 August in 2002. All aboveground plant material was first removed using scissors, dried in a forced-air oven at 60°C for 20 h and weighed. Concentrations of C and N in shoot mass were analyzed by a Leco 2000 analyzer (Leco Corporation, Saint Joseph, Michigan, USA) and P concentrations using the wet-ashing method and ICP-OES analyzer (see Luh Huang and Schulte 1985). To compare total shoot production by the fourth grazing rotation in C, M, and MDU plots (i.e., the sum of mowed and harvested material), shoot material >6 cm above the soil surface was collected from one sampling plot in each M and MDU treatment plot at the first, second, and third grazing rotation before the treatment plots were mowed with the Haldrup harvester. Plant community structure was determined at the last sampling in 2002 by visually estimating the proportion of different species in the shoot mass.

For measuring root biomass, three soil cores (diameter 5.6 cm, depth 10 cm) were collected from each sampling plot. The depth of 10 cm was chosen for root and soil sampling as the majority of grass roots, for instance over 80% of *P. pratense* roots (Garwood and Sinclair 1979), occur at this soil layer. Roots were washed over a sieve, dried at 70°C for 48 h, weighed and their C, N, and P concentrations were analyzed using the same methodology as for shoots. After collecting soil cores for root measurements, the remaining 30 × 30 cm wide and 10 cm deep soil layer (around 13 kg fresh mass) was dug up for measuring soil animal abundances and soil attributes. The soil was first carefully sieved by hand to collect earthworms, which were then kept amidst moist kitchen paper at 15°C for 24 h to clean their gut, sorted into *Aporrectodea* and *Lumbricus* species, and weighed. The soil was then mixed and nematodes and enchytraeids were extracted from 30 and 100 g (fresh mass) subsamples of soil, respectively, using wet funnel devices (O'Connor 1962, Sohlenius 1979). The total number of nematodes was counted live and later, using preserved samples, around 70 specimens per sample were identified to genus and allocated into trophic groups according to Yeates et al. (1993). Enchytraeids were counted and their length measured live and the fresh biomass estimated according to Abrahamsen (1973). Together the investigated animal groups (nematodes, enchytraeids, and earthworms) cover all size classes of soil animals (i.e., micro-, meso- and macrofauna) and comprise all trophic groups of the animals (i.e., bacterivores, fungivores, herbivores, omnivores, detritivores, and predators).

Soil water content was measured by drying 100-g subsamples of soil at 70°C for 2 d, and soil density by dividing the dry mass of root samples by their volume. Soil pH was measured from a mixture of 5 g air-dried soil and 25 mL distilled water 4 h after mixing. For measuring concentrations of KCl-extractable NH_4-N and NO_2+NO_3-N in soil, a 700-g subsample of soil was passed through a 4-mm mesh sieve (to remove roots and to mix the soil properly) and 2 g (fresh mass) of soil was used for the analysis. In the last sampling year 2002, this soil was also used for establishing the feedback test.

The soil feedback test

To find a soil feedback on plant growth that is mediated by decomposers and soil nutrient availability, plant growth has to become nutrient limited during the feedback test. Small microcosms were therefore established for the test, each consisting of a plastic pot (height 9 cm, diameter 8 cm), 250-g treatment-plot soil (dry mass equivalent), and one *P. pratense* seedling. One microcosm was established for each treatment plot (40 microcosms in total), so the treatments and the number of replicates correspond to those in the field experiment. The soil was passed through a 4-mm mesh sieve to remove roots that could confound the results when decaying in the soil, and also to remove any earthworms possibly left in the soil as these were considered too large for the microcosm environment. Otherwise, the soil community was not deliberately altered. To estimate the ability of soil organisms in each soil to release nutrients from dead organic matter for plant uptake, 1.5 g of dry ^{15}N-labeled *P. pratense* shoot material was mixed with the soil before adding the soil into the pots. The labeled shoot material was produced by irrigating *P. pratense* seedlings in a sand culture with $^{15}NH_4 ^{15}NO_3$-enriched nutrient solution prepared according to Ingestad (1979). Shoot material was dried and cut into pieces of 15 mm long and had a total N concentration of 2.06% of dry mass and ^{15}N atom% of 13.6. The moisture content of the soil–litter mixture was adjusted to 30% (dry-mass basis) and one 4-week-old *P. pratense* seedling was planted in the middle of each microcosm. During the feedback test, soil moisture was maintained constant by regular irrigations and the microcosms were kept in a growth chamber with a daily cycle of 20 h light (photosynthetic photon flux density 240 µmol·m^{-2}·s^{-1} at the level of plant shoots, temperature 20°C) and 4 h dark (temperature 14°C).

The microcosms were destructively harvested 74 d after establishment. Plant shoots were first removed, dried at 70°C for 48 h, and weighed. Soil was then cut vertically at the point of the shoot base and one half was used for root analyses. Roots were washed over a sieve, dried at 70°C for 48 h and weighed. The concentrations of C and N of shoot and root mass were analyzed using a CHNS-O analyzer (EA 1110; Thermo Finnigan/CE Instruments, Milan, Italy), while the ^{15}N atom% of shoot mass was determined at Iso-Analytical Limited (Sandbach, Cheshire, UK). The excess ^{15}N in shoot mass (calculated by subtracting background values from measured values) was used to calculate the amount of total N transferred from litter to plant. To evaluate root colonization rates of AM fungi, a 0.2–0.5 g subsample of

fresh roots was preserved in 50% alcohol. Samples were later bleached in 10% KOH overnight, acidified in 1% HCl, and stained at 90°C for 1 h with 0.01% methyl blue (Phillips and Hayman 1970, Grace and Stribley 1991). Colonization rates were then estimated under a dissecting microscope using the following percentage scale: 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% of a root colonized.

Statistical analyses

Multivariate statistics.—To explore general relationships among the response variables and to analyze relationships between the response variables, treatments, and years, multivariate statistical analyses were conducted using CANOCO 4.5 (ter Braak and Smilauer 1998). The predictor variables (i.e., treatments and years) were related to overall patterns in the data using overlays in principal-component analysis (PCA) graphs. Single-variable effects (marginal and conditional), independent of field blocks and years, were tested by computing partial redundancy analyses, RDA (the canonical constrained extension of PCA). In RDA, Monte Carlo tests were performed with restricted random permutations of samples reflecting the experimental design. PCA and RDA calculations were based on correlation matrices in order to standardize the variables of varying scales and magnitudes. Because simultaneous use of many variables requires strict data consistency, the few missing values in the response variables were replaced by the average of their treatment × year combination. Total shoot production was excluded from all analyses due to missing values in G plots.

To evaluate whether the observed responses of plants and soil animals to the treatments could be indirect, i.e., mediated by treatment effects on other variables, interactions between four groups of response variables (soil attributes, plant attributes, nematode trophic groups and annelid worms; see Table 2) were analyzed using partial Mantel tests (Mantel 1967) and the zt program (Bonnet and Van de Peer 2002). To reveal spurious correlations between the response variables (i.e., interactions mediated by third parties, as far as these were included in the measured variables), two groups were related at a time and the effect of a third group was controlled. All possible combinations of the four groups were tested by calculating the standardized Mantel statistic r_M as a measure of "effect size" (McCune and Grace 2002), based on 1000 randomizations. To calculate the dissimilarity matrices, the Euclidean distance was used as a distance measure for soil and plant parameters, while the Bray-Curtis coefficient (Faith et al. 1987) was used for soil animal groups.

Univariate statistics.—To further test the effects of grazing on plant and soil variables and to reveal the mechanisms explaining these effects, the field variables were analyzed individually in two parts using the SPSS

statistical package (SPSS 2002). The effect of year and the dependence of treatment effects on year (i.e., the year × treatment interaction) were first tested using repeated-measures ANOVA, with degrees of freedom of F statistics corrected using Greenhouse-Geisser ε. To reveal the relative role of different mechanisms, four a priori selected treatment effects were then tested using contrast tests. The general effect of "grazing" was first tested by contrasting results from G plots with those from C plots (contrast G vs. C); the effect of "defoliation" was then tested by contrasting results from M plots with those from C plots (M vs. C); the effect of "dung and urine return" was tested by contrasting results from MDU plots with those from M plots (MDU vs. M); and finally, the effect of "physical presence of cows" was tested by contrasting results from G plots with those from MDU plots (G vs. MDU). Contrasts were tested using entire three-year data sets when no significant year × treatment effect appeared in the repeated-measures ANOVA, whereas when such effect was found, contrasts were tested separately for each year. Homogeneity of variances was tested using Levene's test and if necessary, a logarithmic transformation was applied to the response variables.

RESULTS

Multivariate analyses of the field data

When contrasted in a PCA (principal-component analysis) of all available field data (except for shoot production), the M (mowed), MDU (mowed, plus dung and urine addition), and G (grazed by cows) plots differ clearly from the C (control) plots in reducing most plant attributes except for shoot P and N concentrations (Fig. 1a, Table 1). This different response of plant attributes to the treatments determines the main data variation in the PCA ($R^2 = 0.19$), with axis 1 dominated by plant shoot and root biomass and shoot P and root N concentrations together with *Aporrectodea* earthworms and soil water content (Table 1). Another dimension of the PCA (axis 2, $R^2 = 0.14$) is mainly explainable by root P concentration, soil density, *Lumbricus* earthworms, and herbivorous nematodes (Fig. 1a, Table 1). PCA axis 3 ($R^2 = 0.11$) summarizes the variation in nematodes, while axis 4 ($R^2 = 0.09$) describes the variation in soil pH and N concentrations of soil and plant shoots (Fig. 1b, Table 1). When the years, instead of the treatments, are contrasted in the PCA, year 2001 differs clearly from years 2000 and 2002 (Fig. 2). This difference is mainly explainable by lower soil moisture and *Aporrectodea* earthworm biomass and higher root dry mass and soil inorganic N concentration in 2001 than in 2000 and 2002 (Fig. 2).

To derive multivariate patterns of the variation restricted to treatments only, i.e., to control for the effects of field blocks and years, a partial redundancy analysis (RDA) was conducted (Fig. 3). The four RDA axes (i.e., the four explanatory variables) explain 17.4%
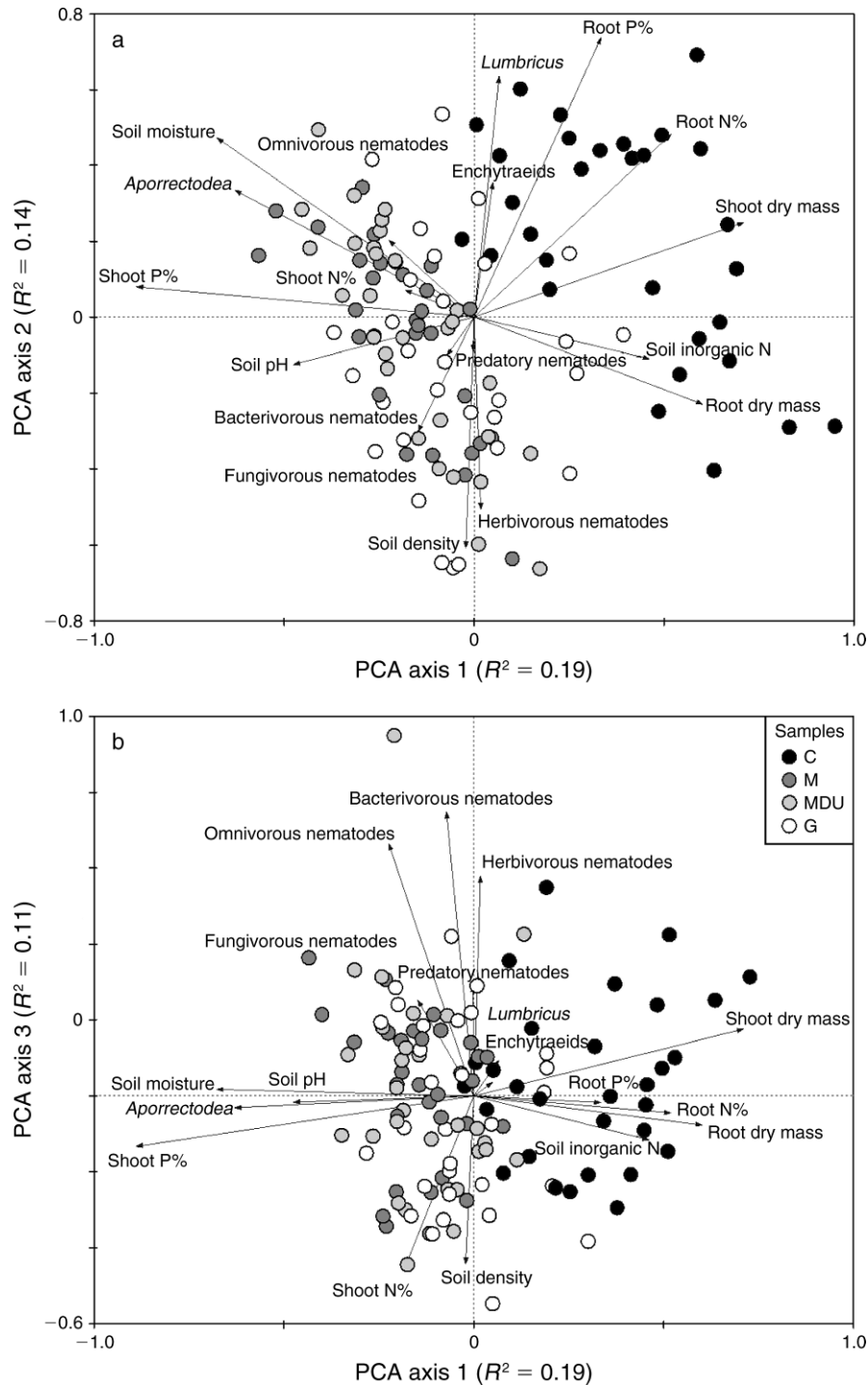
Fig. 1. Principal-component analysis (PCA) graph of field data (except for shoot production) in (a) axis 1 × axis 2 and (b) axis 1 × axis 3 ordination planes with the four pasture treatments (C = control, M = mowing, MDU = mowing combined with addition of dung and urine, G = cow grazing) as an overlay. The response variables are drawn as standardized vectors that indicate the direction in the ordination plane to which their values increase. The angle between the vectors is inversely proportional to the correlation between the variables. R^2 values in the axis labels give the percentage of variance of the data explained by the respective axis.

of the total variance in the data, and all their marginal (independent) effects on the extraction of RDA axes are significant (for MDU, $R^2 = 0.03$, $P = 0.001$; for M, $R^2 = 0.03$, $P = 0.001$; for G, $R^2 = 0.04$, $P = 0.001$; and for C, $R^2 = 0.14$, $P = 0.001$ when included in this order). Like the PCA, the RDA reveals the difference between control and other treatments, with control plots associated with higher values of shoot mass, root N and P concentrations, and *Lumbricus* biomass and with lower values of shoot P concentration, *Aporrectodea* biomass, soil moisture and soil pH than the other treatment plots (Fig. 3). Grazing treatment also clearly differentiates from the other three, being positively associated with fungivorous nematodes and soil density and negatively associated with enchytraeids (Fig. 3).

The partial Mantel tests found a significant association between the soil and plant variables only (Table 2). The relationship between the soil parameters and the annelids is also noteworthy for $r_M = 0.090$, but is not highly significant even after the effect of nematodes is controlled for.

Univariate analyses of treatment effects and explaining mechanisms in the field data

Plant shoot attributes.—In comparison to control, cow grazing decreased total standing shoot mass on average by 56% and increased shoot P concentration by 65% (Fig. 4a, e, Table 3). The effect on shoot N concentration reversed during the years: grazing decreased shoot N concentration by 33% in 2000, but had an opposite effect of similar magnitude in 2001 and 2002 (Fig. 4c, Table 3). Relative to control, the effects of mowing on shoots were parallel and of similar size to those of grazing (Fig. 4a, c, e, Table 3). Adding dung and urine into mowed plots raised shoot N concentration by 11% in 2002, but had no other effects on plant shoots (Fig. 4a, c, e, Table 3). The presence of cows led to 44% higher standing shoot mass in 2002 than mowing combined with dung and urine addition, but no other effects on plant shoots were found (Fig. 4a, c, e, Table 3). Shoot production, which comprises both harvested and mowed shoot mass (not estimated in grazed plots), was not affected by mowing or dung and urine addition (Fig. 5, Table 3).

Plant root attributes.—In comparison to control, cow grazing increased root mass by 106% in 2000, had an insignificant effect in 2001 and decreased root mass by 28% in 2002 (Fig. 4b, Table 3). Root N and P concentrations were decreased by grazing on average by 23% and 22%, respectively (Fig. 4d, f, Table 3). The effects of mowing, when compared to control, were parallel but stronger than those of grazing whenever the two effects co-occurred; e.g., mowing decreased root mass by 54% in 2002 and decreased root N concentration on average by 37% (Fig. 4b, d, f, Table 3). Unlike grazing, mowing had no effect on root mass in 2000 and already reduced root mass in 2001 by 42% (Fig. 4b, Table 3). Adding dung and urine into mowed plots had

TABLE 1. Variable scores from a PCA of field-experiment data (containing all years and all variables except for shoot production).

Variable	PCA axis			
	1	2	3	4
Shoot dry mass	**0.711**	0.250	0.176	−0.208
Root dry mass	**0.602**	−0.299	−0.077	−0.114
Shoot N concentration	−0.182	0.072	−0.455	**0.554**
Shoot P concentration	**−0.889**	0.080	−0.133	0.135
Root N concentration	**0.518**	0.482	−0.045	0.195
Root P concentration	0.335	**0.738**	−0.018	−0.186
Bacterivorous nematodes	−0.072	−0.101	**0.749**	0.276
Fungivorous nematodes	−0.147	−0.302	0.252	0.305
Herbivorous nematodes	0.018	**−0.507**	**0.580**	0.012
Omnivorous nematodes	−0.224	0.203	**0.666**	−0.008
Predatory nematodes	−0.003	−0.091	0.292	0.329
Enchytraeids	0.049	0.356	0.036	0.073
Aporrectodea earthworms	**−0.630**	0.335	−0.032	−0.113
Lumbricus earthworms	0.066	**0.636**	0.094	0.152
Soil density	−0.022	**−0.607**	−0.443	0.073
Soil water content	**−0.677**	0.473	0.017	0.296
Soil inorganic-N concentration	0.462	−0.110	−0.116	**0.678**
Soil pH	−0.475	−0.125	−0.016	**−0.560**
Eigenvalue for the axis	0.192	0.143	0.110	0.091

Notes: The scores are directly proportional to the correlations between the response variables and the PCA axes. Score values $\geq |0.5|$ are in bold type and indicate a large contribution of the variable to the properties of the respective PCA axis.

no effects on roots, while the presence of cows, when compared to the MDU plots, led to 60% higher root mass in 2001–2002 and increased root N concentration by 22% (Fig. 4b, d, f, Table 3).

Plant community structure.—Plant species composition was significantly affected by cow grazing (measured only in the last study year): while the percentage of the sown species, *P. pratense* and *F. pratensis*, was 14% and that of the weed *Elymus repens* 80% of total shoot mass in C plots, the percentages were 82% and 16%, respectively, in G plots (Fig. 6). The effects of mowing were parallel to those of grazing, i.e., significantly lower percentages of *E. repens* and higher percentages of sown species were found in M than C plots (Fig. 6). Adding dung and urine increased the percentage of *E. repens* ($P = 0.023$ in MDU vs. M contrast), but did not affect the percentage of sown species (Fig. 6). Similarly, relative to MDU plots, the presence of cows increased the percentage of *E. repens* ($P = 0.015$) but did not affect the percentage of sown species (Fig. 6). The percentage of dicots in total shoot mass did not differ between treatment plots, and graminoid species other than *P. pratense*, *F. pratensis*, or *E. repens* appeared in M (on average 2% of shoot mass) and MDU plots only (4%) (Fig. 6).

Abundance of soil animals.—Relative to control plots, cow grazing increased the abundance of fungivorous nematodes 3.6-fold, but did not affect the abundance of other nematode trophic groups (the response of predators was not statistically analyzed as predators were found in 37% of samples only) (Fig. 7a–e, Table 4).
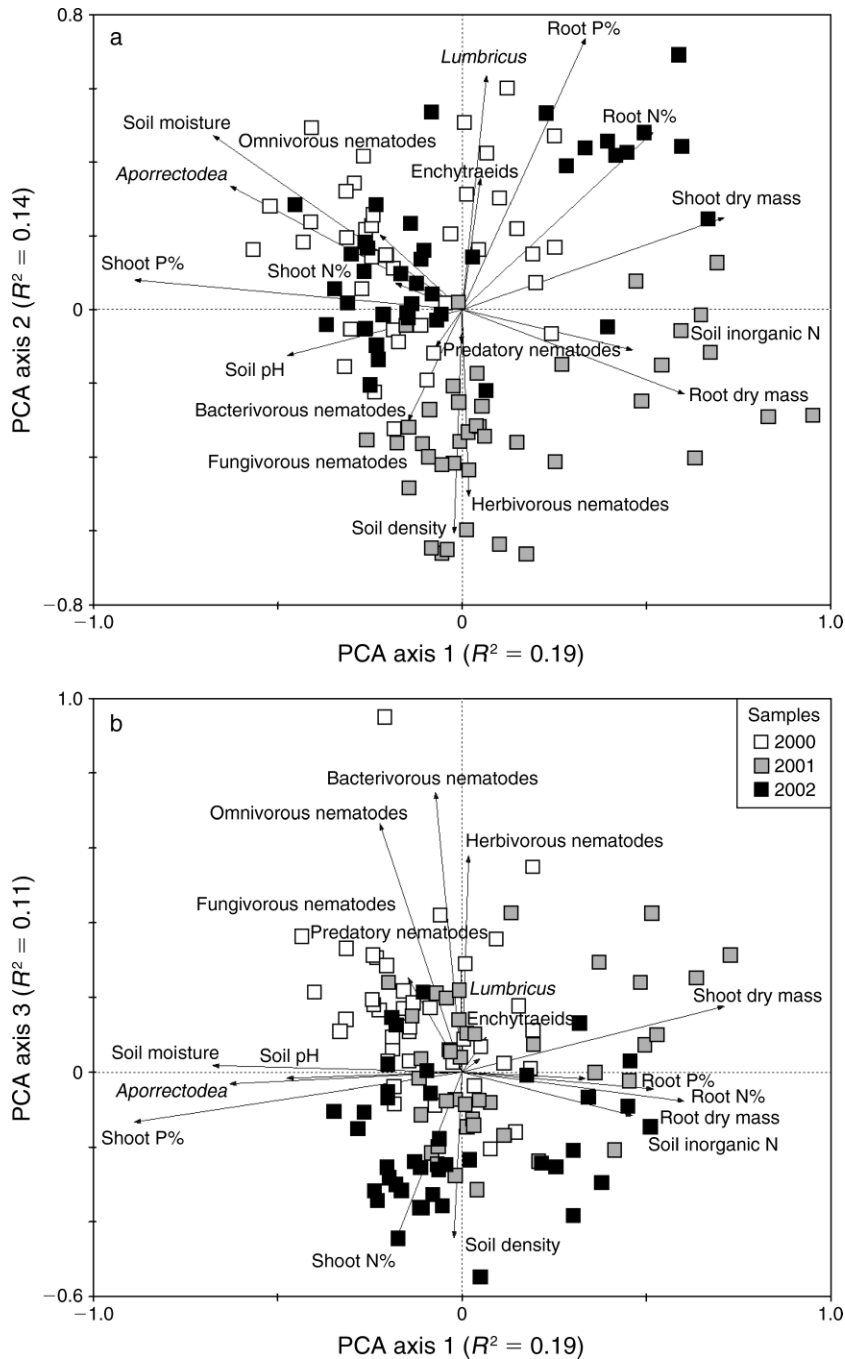
FIG. 2. PCA graph of field data (except for shoot production) in (a) axis 1 × axis 2 and (b) axis 1 × axis 3 ordination planes with the three experimental years (2000–2002) as an overlay. Interpretation of the graph is as in Fig. 1.

Among meso- and macrofauna, grazing decreased the biomass of enchytraeids and *Lumbricus* earthworms on average by 55% and 42%, respectively, but increased the biomass of *Aporrectodea* earthworms by 69% (Fig. 7f–h, Table 4). The effects of mowing on earthworms were parallel to those of grazing, i.e., a 44% decrease in *Lumbricus* biomass and a 48% increase in *Aporrectodea* biomass, while other animal groups were not affected

(Fig. 7a–h, Table 4). Adding dung and urine in M plots had no effect on soil animals (Fig. 7a–h, Table 4), whereas the presence of cows, when compared to MDU plots, increased the abundance of fungivorous nematodes 3.5-fold (Fig. 7b, Table 4) and decreased the biomass of enchytraeids by 56% (Fig. 7f, Table 4).

Abiotic soil attributes.—Soil density and pH were on average higher in G than in C plots, and relative to C
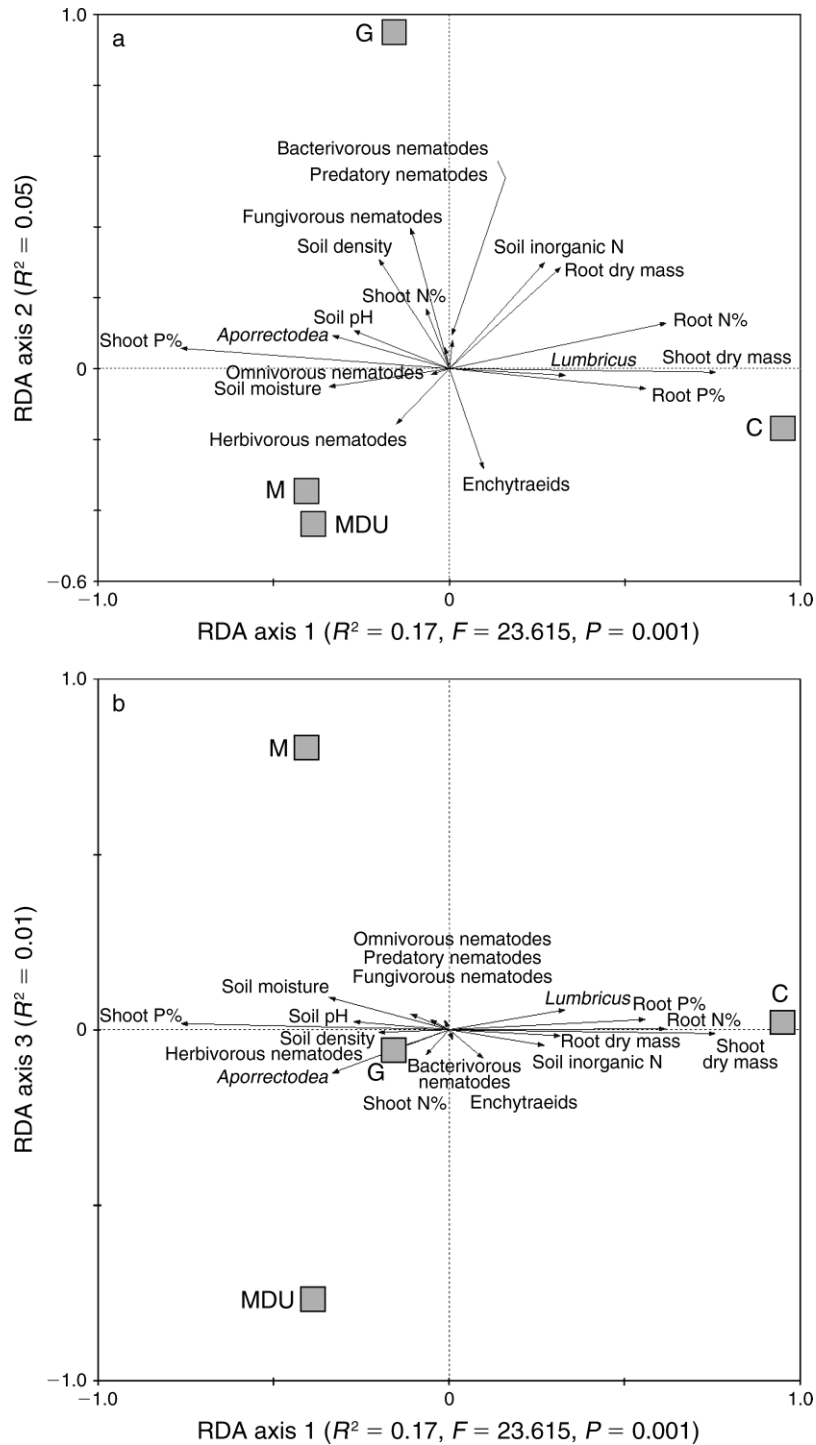
Fig. 3. Partial redundancy analysis (RDA) graph of field data (except for shoot production) in (a) axis 1 × axis 2 and (b) axis 1 × axis 3 ordination planes constrained by the four pasture treatments (C = control, M = mowing, MDU = mowing combined with addition of dung and urine, G = cow grazing) after controlling for the effect of treatment blocks and years. Interpretation of the graph is as in Fig. 1.

TABLE 2.   Standardized Mantel statistics (r_M) for four groups of field variables from simple and partial Mantel tests.

	Soil†			Plants‡			Nematodes§		
	r_M	P	Controlled group	r_M	P	Controlled group	r_M	P	Controlled group
Plants‡	0.233	0.001							
	0.231	0.001	nematodes						
	0.233	0.001	annelids						
Nematodes§	0.055	0.165		0.051	0.186				
	0.061	0.123	annelids	0.039	0.249	soil			
	0.044	0.220	plants	0.052	0.176	annelids			
Annelids‖	0.090	0.068		0.019	0.337		−0.061	0.113	
	0.088	0.087	plants	−0.002	0.505	soil	−0.062	0.107	plants
	0.094	0.056	nematodes	0.022	0.329	nematodes	−0.066	0.086	soil

† Consists of soil pH, soil moisture, soil density, and soil inorganic N.
‡ Consists of shoot and root dry mass and shoot and root N and P concentrations.
§ Consists of bacterivorous, fungivorous, herbivorous, omnivorous, and predatory nematodes.
‖ Consists of enchytraeids and *Aporrectodea* and *Lumbricus* earthworms.

plots grazing increased soil moisture in 2001, but had no effect on soil inorganic-N concentration (Fig. 8, Table 4). The effects of mowing followed those of grazing in soil moisture (a positive effect in 2001–2002), but differed in other variables; i.e., mowing had no effect on soil density and pH and decreased the concentration of inorganic N in soil on average by 67% (Fig. 8, Table 4). Adding dung and urine in mowed plots had no effect on soil attributes, whereas the presence of cows, relative to MDU plots, increased average soil inorganic-N concentration 2.6-fold and soil density by 7% (Fig. 8, Table 4).

The feedback test

Phleum pratense seedlings growing in the soil collected from G plots produced on average 95% and 57% more shoot and root mass, respectively, and had on average 80% and 37% higher shoot and root N content, respectively, than seedlings growing in the soil collected from C plots (Fig. 9). None of these contrasts was, however, statistically significant in univariate *t* tests due to the great variation generated by grazing on plant growth and N uptake. The PCA and RDA graphs (Figs. 10 and 11) show a similar strong, positive effect of the soil collected from G plots on plant performance, with high loadings of plant dry mass and N content on PCA axis 1 (Table 5) and highly significant grazing effect in the partial RDA when the block effect was controlled for (Fig. 11). In contrast to the PCA (Fig. 10), the RDA indicates that shoot biomass allocation and shoot N allocation are also positively associated with the soil collected from G plots (Fig. 11).

The soil feedback created by mowing was in complete contrast with the one created by grazing: seedlings growing in the soil collected from M plots produced 28% and 25% less shoot (*t*-test, $P = 0.001$) and root ($P = 0.024$) mass, respectively, and had 18% and 22% lower shoot ($P = 0.004$) and root ($P = 0.021$) N content, respectively, than seedlings growing in the soil collected from C plots (Fig. 9). Adding dung and urine to mowed field plots created no feedback on *P. pratense* seedlings, whereas the presence of cows (when compared to MDU) induced a strong feedback (Figs. 9 and 11). The average shoot and root mass, as well as the average shoot and root N content were considerably higher in seedlings that grew in the soil collected from G plots than in seedlings that grew in the soil collected from MDU plots, but again, due to the great variation generated by grazing the effect was statistically significant in the case of root mass only (*t* test, $P = 0.042$) (Fig. 9).

In univariate *t* tests, none of the field treatments had effects on *P. pratense* biomass allocation, N allocation, litter-N uptake, or root-colonization rate of AM fungi (Fig. 9). However, there was a negative correlation between root mass and AM colonization rate ($r = -0.34$, $P = 0.033$, $n = 40$ replicates) and a positive correlation between AM colonization rate and plant litter-N uptake ($r = 0.34$, $P = 0.030$, $n = 40$ replicates). These correlations are corroborated by the RDA graph, which shows that while both AM colonization rate and plant litter-N uptake are negatively associated, root mass is positively associated with the soil collected from G plots (Fig. 11).

DISCUSSION

The aim of our study was to reveal the relative significance of the three mechanisms—defoliation, dung and urine return, and physical presence of animals—in the effects of grazing on plants, belowground organisms, and soil-nutrient availability. As we expected, grazing affected almost all plant and most of the belowground parameters, and the capability of the three different grazing mechanisms to explain these effects differed a lot. Ten out of 14 recorded grazing effects were at least partly explained by defoliation (all plant parameters, both earthworm groups and soil water content), three effects were explained by the physical presence of cows (fungal-feeding nematodes, enchytraeids, and soil density) and only one was partly explained by dung and urine return (shoot N concentration).

We predicted that the effects of grazing on plants would be explained by both defoliation and dung and urine return as these mechanisms should directly affect
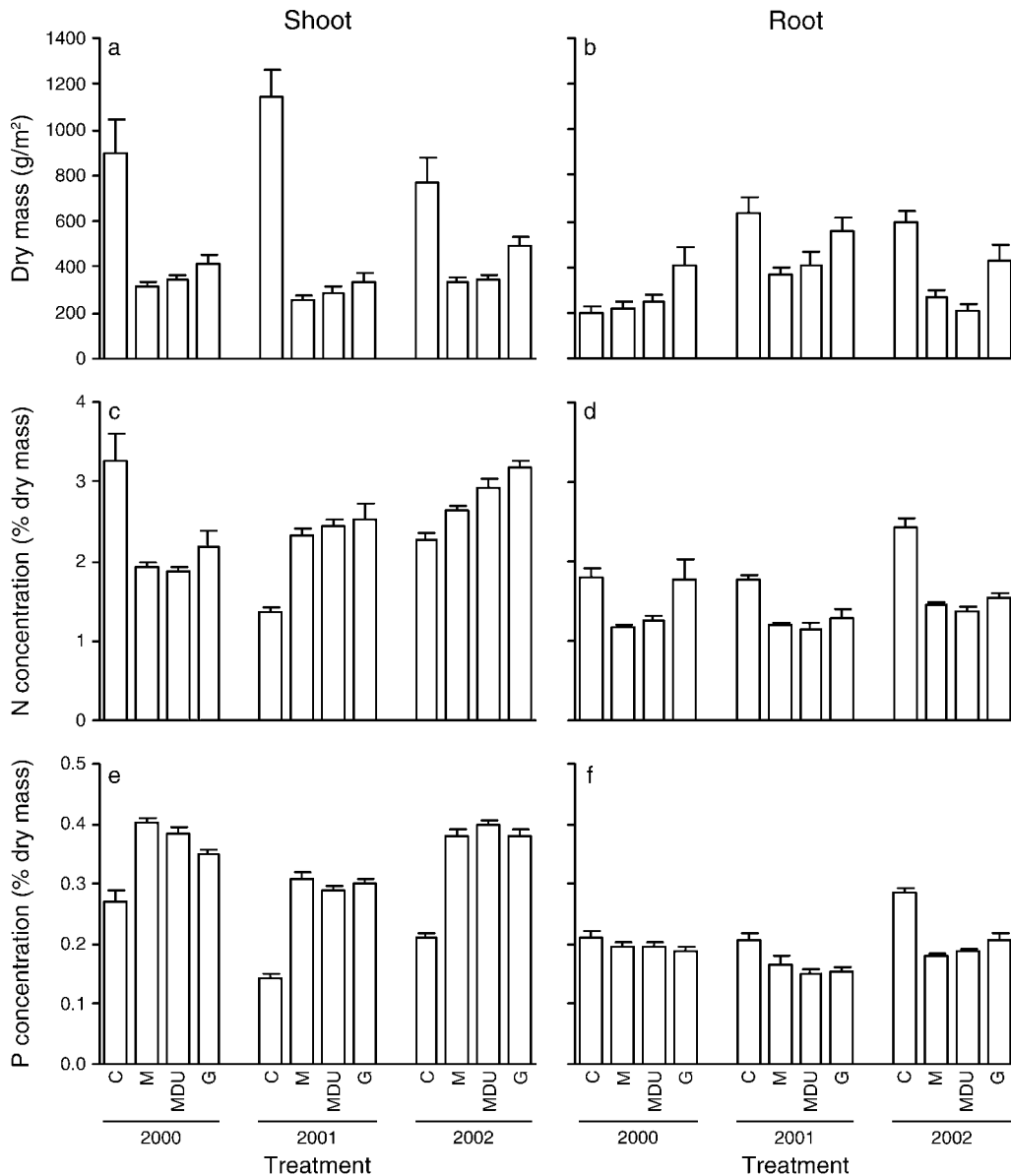
FIG. 4. Plant shoot and root attributes (includes all aboveground and belowground plant material; data are means + SE, $n = 10$ replicate observations) in *Phleum pratense–Festuca pratensis* grassland in relation to pasture treatments during three growing seasons: total harvested shoot biomass and root biomass (from upper 10-cm soil layer), and their N and P concentrations. Treatment abbreviations are as in Fig. 1.

plant performance. Our results show that defoliation has a major role in explaining grazing effects on plants, but, in contrast to what we assumed, this is not the case for dung and urine return. We further predicted that grazing effects on soil microfauna will mostly be explained by defoliation and dung and urine return (because these mechanisms should explain grazing effects on plants, and microfauna are closely associated with plants roots) and those on meso-and macrofauna by the physical presence of cows (due to soil compaction). However, this prediction is not corroborated by our data. While the grazing effects on enchytraeids were explained by cow

presence, thus supporting our prediction, the effects on earthworms were explained by defoliation and those on fungal-feeding nematodes by the presence of cows. The different mechanisms also counteracted each other in a few response variables. For instance, the mean concentration of soil inorganic N was not affected by grazing because defoliation decreased, and cow presence simultaneously increased, the concentration. Finally, it is remarkable that although the field measurements in 2001 differed significantly from those in 2000 and 2002—due to the 2001 harvest being preceded by a long dry period—we did not find evidence that grazing effects

TABLE 3. Results of repeated-measures ANOVA of the effects of year and pasture treatments (C, control; M, mowing; MDU, mowing combined with addition of dung and urine; and G, cow grazing) on plant shoots and roots in *Phleum pratense–Festuca pratensis* grassland during the growing seasons of 2000–2002, together with significant ($P < 0.05$) treatment contrasts from *t* tests.

| | | Within-subject effects | | | | Between-subject effects | |
| | | Year | | Year × Treatment | | Treatment | |
Dependent variable	ε	$F_{2,72}$	P	$F_{6,72}$	P	$F_{3,36}$	P
Shoots							
Shoot production†	0.86	6.40	0.005	0.58	0.651	1.66	0.209
Shoot mass	0.88	1.04	0.350	3.29	0.009	84.49	<0.001
N concentration	0.89	25.13	<0.001	21.39	<0.001	5.94	<0.002
P concentration	0.95	130.44	<0.001	6.79	<0.001	110.61	<0.001
Roots							
Dry mass	0.84	21.62	<0.001	4.20	0.002	13.13	<0.001
N concentration	0.90	16.10	<0.001	2.00	0.086	24.31	<0.001
P concentration	0.95	31.81	<0.001	6.86	<0.001	25.89	<0.001

Notes: "Shoots" includes all aboveground plant material, and "roots" includes all belowground plant material. Degrees of freedom of within-subject F statistics were corrected using Greenhouse-Geisser ε. Contrasts (G vs. C, M vs. C, MDU vs. M, and G vs. MDU) were tested using entire data sets when repeated-measures ANOVA revealed no significant Year × Treatment effect, whereas when such effect was found, contrasts were tested separately for each year.

† Shoot production comprises both mowed and harvested shoot mass, is not available from treatment G, and has degrees of freedom 2, 54 for Year; 4, 54 for Year × Treatment; and 2, 27 for Treatment.

or the mechanisms explaining these effects had been different in 2001 in comparison to those in 2000 and 2002. This shows that although plant growth and abundance of soil organisms vary with years, due to varying environmental conditions, grazing effects remain predictable. On the whole, our results indicate that understanding the effects of defoliation gives satisfactory predictions for the effects of grazing on plant growth and plant community structure, whereas when predicting the effects on soil fauna and soil N availability, this is not the case.

Grazing and plant parameters in the field

Herbivores are known to affect plant community structure in grasslands by altering the colonization and extinction dynamics of plant species (Olff and Ritchie 1998). In our pasture, grazing had a major effect on plant community structure by restraining the emergence of *Elymus repens* among the vegetation. *E. repens* is a very viable weed in light sandy soils like the one we had, but its abundance diminishes dramatically during the pasture rotation when the old vegetation is killed using the glyphosate herbicide and the new pasture established through seeding of the desired grasses. However, some *E. repens* rhizomes can survive the herbicide application, and when the vegetation is not mowed or grazed, *E. repens* rapidly outcompetes the other grasses and colonizes the area. Of the different mechanisms of grazing, defoliation was clearly responsible for restraining *E. repens* colonization since dung and urine addition (when compared to the mowed plots) and presence of cows (when compared to the mowed plots with dung and urine addition) encouraged *E. repens* growth. The effect of grazing on plant community composition was thus mediated by two simultaneous but contrasting mechanisms; while defoliation reduced the ability of *E.*

repens to outcompete the other grasses in the pasture, fertilization provided by the excreta encouraged the colonization. These results support earlier findings that *E. repens* shoot mass decreases with increasing grazing and mowing pressure (Le Roux et al. 2003) and that N fertilization increases the proportion of *E. repens* in plant communities (Käding et al. 2003). The reason why cow presence increased *E. repens* proportion among the vegetation in our study is probably explained by defoliation being less efficient in the grazed than in the mowed plots.

Standing shoot mass was significantly lower in the grazed than control plots at every harvest, reflecting the steady consumption of grass by the cows. We did not measure the effect of grazing on aboveground NPP (comprising both grazed and harvested shoot mass), but found that aboveground NPP was not affected by mowing. This indicates that plants were fully able to compensate for the lost shoot tissue. In a compilation of studies, the average response of aboveground NPP to grazing was negative (Milchunas and Lauenroth 1993), but grazing can also increase aboveground NPP (McNaughton 1976, Pandey and Singh 1992, Frank et al. 2002). In our study the consumption of herbage by cows was controlled to optimize herbage production, which probably explains why mowing, which imitated the schedule and pressure of grazing, did not have negative effects on aboveground plant production.

Two earlier reviews suggest that while defoliation mainly decreases root mass (Ferraro and Oesterheld 2002), animal grazing more often increases than decreases root mass (Milchunas and Lauenroth 1993). It has been proposed that this discrepancy results from defoliation studies being mostly carried out using pot plants, while grazing studies have plants growing in the field (McNaughton et al. 1998). Our findings do not

TABLE 3. Extended.

| | Statistically significant contrasts | | |
Over all years	2000	2001	2002
none			
	G < C, M < C	G < C, M < C	G < C, M < C, G > MDU
	G < C, M < C	G > C, M > C	G > C, M > C, MDU > M
	G > C, M > C	G > C, M > C	G > C, M > C
	G > C	M < C, G > MDU	G < C, M < C, G > MDU
G < C, M < C, G > MDU	G < C	G < C, M < C	G < C, M < C

purely follow either of these patterns since the effect of grazing on root mass turned from positive to negative during the experiment. We suggest that this is a consequence of two mechanisms acting simultaneously. First, soil compaction due to trampling is known to lead to increased root biomass in upper soil layers (Bouwman and Arts 2000). We measured root mass from the upper 10-cm layer of soil, so soil compaction is likely to explain why at the first sampling, when the mowing treatment still had no effect on root mass, root mass was already higher in the grazed than control plots. Second, as *E. repens* obviously colonized the control plots gradually, contribution of heavy *E. repens* rhizomes to root mass increased with time in the control plots. This can explain why root mass did not differ between the control and mowed plots at the first harvest (*E. repens* coverage was still low), but was later higher in the control than mowed plots. However, as it is likely that grazing and mowing also affected root growth in our experiment (Ferraro and Oesterheld 2002), it is not possible to fully figure out how much the negative long-term effect of grazing and mowing on root mass was mediated by plant community change and how much by root-growth change. Nevertheless, our results show that defoliation and physical presence of cows were responsible for the observed effects on root mass, while dung and urine return had no role.

During the last two years of the study, plants that were grazed had higher shoot N and P concentrations than did control plants. These effects were largely
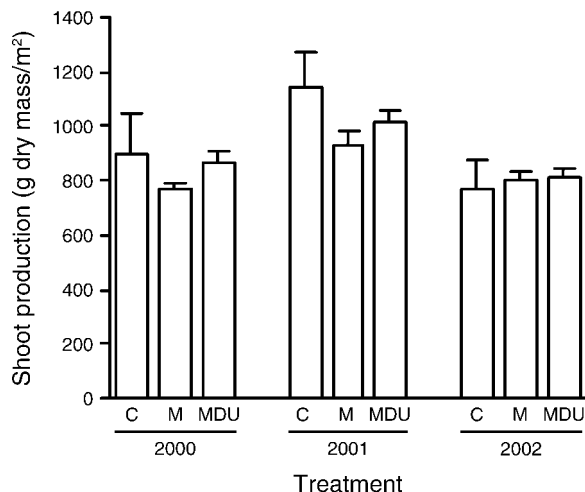

FIG. 5. Plant shoot production by the fourth grazing rotation (the sum of harvested and mowed shoot mass, not available from grazed plots; data are means + SE, *n* = 10 replicate observations) in *Phleum pratense–Festuca pratensis* grassland in relation to pasture treatments during three growing seasons. Treatment abbreviations are as in Fig. 1.
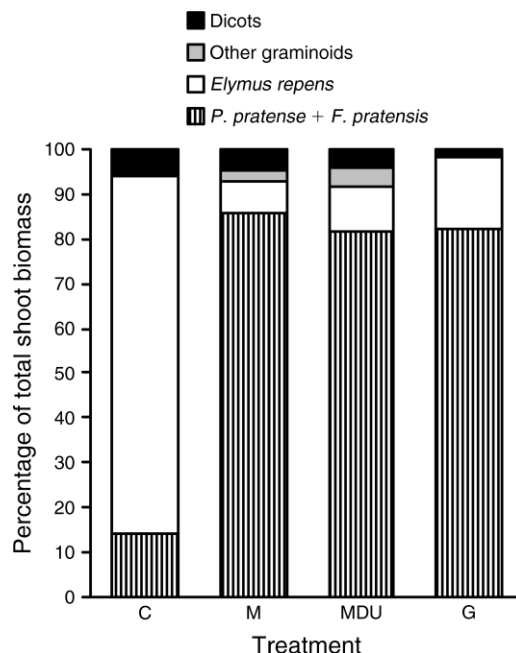

FIG. 6. Percentages contributed by plant taxa to total plant shoot mass (data are means, *n* = 10 replicate observations) in *Phleum pratense–Festuca pratensis* grassland in relation to pasture treatments in 2002. Treatment abbreviations are as in Fig. 1.
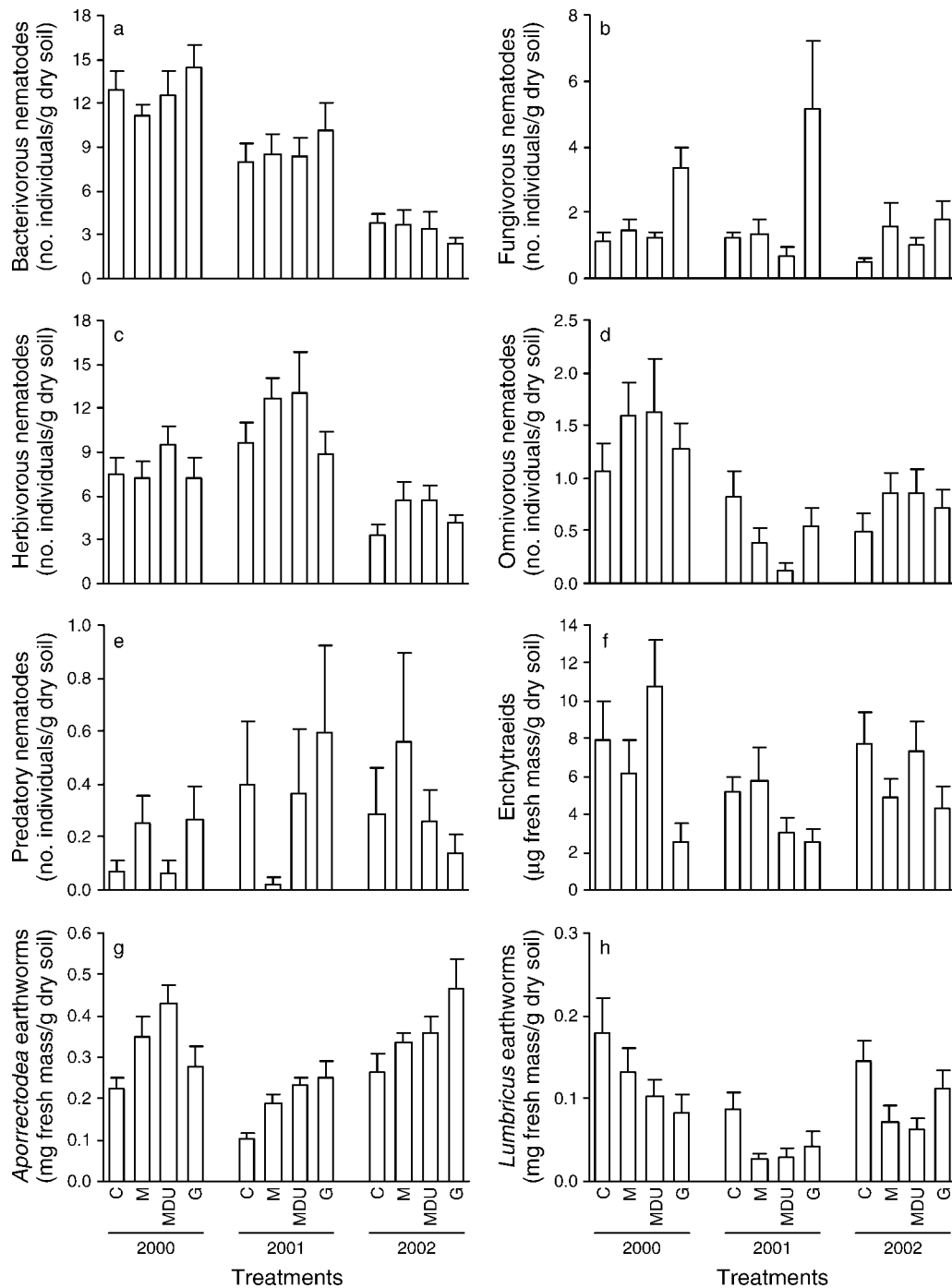
FIG. 7. The abundance of soil fauna (data are means + SE, $n = 10$ replicate observations) in *Phleum pratense–Festuca pratensis* grassland in relation to pasture treatments during three growing seasons: bacterivorous, fungivorous, herbivorous, omnivorous, and predatory nematodes; enchytraeids; and *Aporrectodea* and *Lumbricus* earthworms. Treatment abbreviations are as in Fig. 1.

explained by defoliation as the effects of mowing followed closely those of grazing. In grasses, defoliation and animal grazing typically increase shoot N concentrations (Wilsey et al. 1997, Green and Detling 2000). Elevated shoot N concentrations are often due to regrowing shoot tissues having higher nutrient concen-

trations than the more mature grazed tissues, but can also be due to improved nitrogen availability in the plant rhizosphere after defoliation (Holland and Detling 1990, Hamilton and Frank 2001) or due to higher relative allocation of nutrients to regrowing shoots (Ruess 1988, Louahlia et al. 2000). In our study, grazing and mowing

TABLE 4. Results of repeated-measures ANOVA of the effects of year and pasture treatments [control (C), mowing (M), mowing combined with addition of dung and urine (MDU), and cow grazing (G)] on soil animal abundances and abiotic soil attributes in *Phleum pratense–Festuca pratensis* grassland during the growing seasons of 2000–2002, together with significant ($P < 0.05$) treatment contrasts from t tests.

		Within-subject effects				Between-subject effects					
		Year		Year × Treatment		Treatment		Statistically significant contrasts			
Response variable	ε	$F_{2,72}$	P	$F_{6,72}$	P	$F_{3,36}$	P	Over all years	2000	2001	2002
Abundance of nematode trophic groups											
Bacterivores	0.99	65.37	<0.001	0.88	0.515	0.49	0.693	none			
Fungivores	0.94	4.02	0.025	1.83	0.111	8.04	<0.001	G > C, G > MDU			
Herbivores	0.93	28.32	<0.001	0.74	0.614	2.82	0.053	G < MDU			
Omnivores	0.97	17.44	<0.001	1.97	0.083	0.49	0.692	none			
Biomass of enchytraeids and earthworms											
Enchytraeids	0.87	1.98	0.152	0.38	0.871	2.77	0.056	G < C, G < MDU			
Aporrectodea earthworms	0.96	20.00	<0.001	1.91	0.095	10.00	<0.001	G > C, M > C			
Lumbricus earthworms	0.99	15.71	<0.001	0.83	0.549	5.83	0.002	G < C, M < C			
Abiotic soil attributes											
Water content	0.91	120.45	<0.001	4.20	0.002	6.33	0.001		none	G > C, M > C	M > C
Density	0.92	13.71	<0.001	1.18	0.331	4.95	0.006	G > C, G > MDU			
pH	0.96	1.23	0.299	1.39	0.232	1.91	0.146	G > C			
Inorganic-N concentration	0.83	3.45	0.046	1.65	0.161	7.26	0.001	M < C, G > MDU			

Notes: Degrees of freedom of within-subject F statistics were corrected using Greenhouse-Geisser ε. Contrasts (G vs. C, M vs. C, MDU vs. M, and G vs. MDU) were tested using entire data sets when repeated-measures ANOVA revealed no significant Year × Treatment effect, whereas when such effect was found, contrasts were tested separately for each year.

lowered root N and P concentrations, which indicates that elevated shoot nutrient concentrations were not due to elevated N and P availability in soil, but instead due to higher relative allocation of nutrients to regrowing shoots. This is supported by our finding that grazing and defoliation did not increase concentrations of inorganic N in soil. Instead, grazing had no effect and mowing decreased N availability by 67%. These results agree with a recent greenhouse study, in which the elevated shoot N concentrations of defoliated *Phleum pratense* plants were found to be purely explained by higher relative N allocation to shoots in these plants (Ilmarinen et al. 2007). In contrast to later harvests, shoot N concentrations were decreased by grazing and mowing at the first harvest of our study. The reason for this is not clear, but could be related to pasture establishment (tillage, fertilization) producing early shoot growth of particularly high N concentration, which after three grazing rotations was still detectable in shoot N concentration in the control, but not in the other plots.

Aboveground grazing and defoliation have been suggested to enhance the quality of roots in terms of increasing root N concentration and decreasing root C-to-N ratio (Seastedt 1985, Seastedt et al. 1988), and there are observations from greenhouse (Hokka et al. 2004) and grazing (Johnson and Matchett 2001) studies that support this idea. However, there is also recent

evidence that defoliation can increase root C-to-N ratio in field grass swards (Bazot et al. 2005), and our results support this: both grazing and mowing reduced N concentration and raised the C to N ratio of below-ground plant parts (C:N 19.8, 24.4, and 29.8 for C [control], G [grazed by cows], and M [mowed] plots, respectively). Changes in root growth and quality have further been suggested to affect the numbers of root feeders, with both positive (Seastedt et al. 1988) and negative (Todd 1996) effects being found, but in our case root feeders did not respond to changes in root quantity or quality.

In contrast to what we expected, returning dung and urine to mowed plots had few effects on plant growth and plant nutrient concentrations, the only effect being the increased shoot N concentration in 2002. This lack of dung and urine effects is well exemplified in the redundancy analysis (RDA) of the field data, which shows that none of the plant attributes was positively associated with the MDU (mowed, with dung and urine added) treatment (see Fig. 3). This is surprising considering that the amount of nutrients in dung and urine more than doubled the total amount of nutrients added to MDU plots (215 and 10 kg·ha^{-1}·yr^{-1} N and P, respectively, added to each plot through NPK fertilization; 244 and 24 kg·ha^{-1}·yr^{-1} N and P, respectively, added to MDU plots through dung and urine fertiliza-
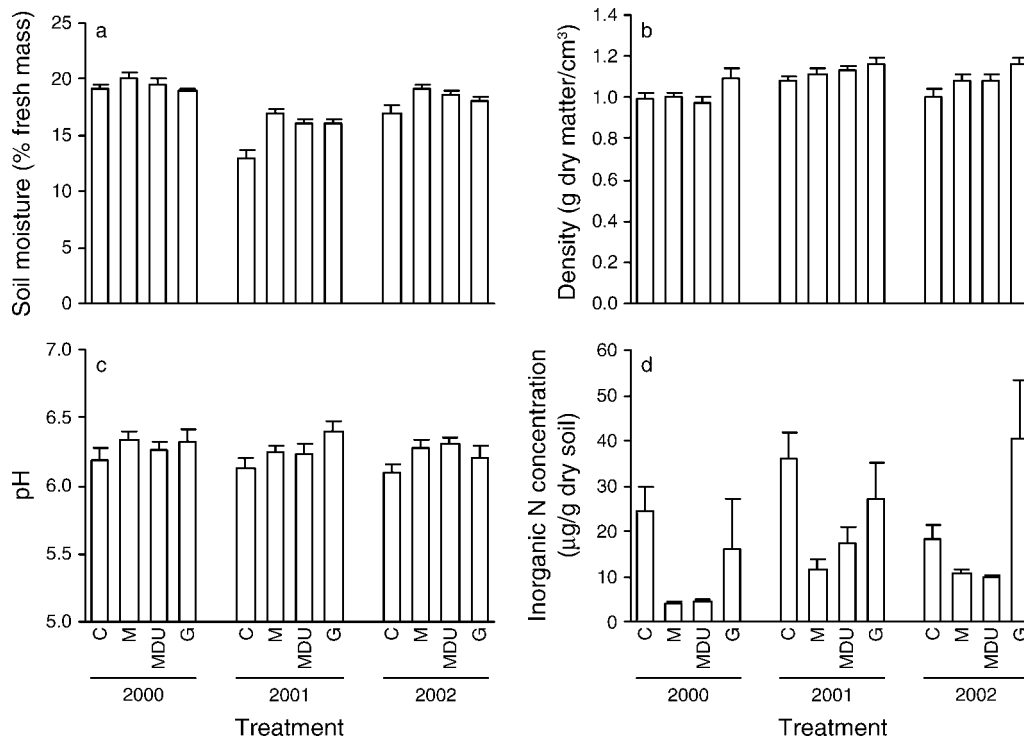
FIG. 8. Abiotic soil attributes (data are means + SE, $n = 10$ replicate observations) in *Phleum pratense–Festuca pratensis* grassland in relation to pasture treatments during three growing seasons: soil moisture, density, pH (H_2O), and inorganic-N (combined concentration of NH_4-N, NO_2-N, and NO_3-N). Treatment abbreviations are as in Fig. 1.

tion). It has earlier been shown that N and P leaching from mowed and grazed pastures similar to ours is low (Saarijärvi et al. 2004), making leaking an unlikely explanation. One potential explanation is that the regular NPK fertilization of the pasture already provided ample nutrition for plant growth. However, in the feedback trial, biomass production and total N uptake of *P. pratense* seedlings differed substantially between soils collected from different field treatments, which indicates that the amount of nutrients in pasture soil was not in excess of plant requirements. Another potential explanation is that nutrients in dung and urine were mostly utilized by soil microbes. Soil microbes are efficient competitors for nutrients (Kaye and Hart 1997) and they can successfully acquire the majority of available N in grassland ecosystems (Bardgett et al. 2003). Cow dung also contains ~40% C of the dry mass (Bol et al. 2000), which can stimulate microbial growth and lead to increased microbial immobilization of nutrients, as shown in a laboratory experiment with sheep-dung addition (Bardgett et al. 1998a). It is therefore possible that nutrients available in the dung and urine mixture were effectively assimilated by decomposers with few nutrients becoming available for plant uptake, until at the last harvest when shoot N concentrations were found to be higher in the MDU than M plots. However, this idea is not supported by our measurements of soil organisms. We did not measure the

abundance of soil microbes, but the animal part of the decomposer community, i.e., microbial-feeding nematodes and enchytraeids and earthworms, did not appear to benefit from the dung and urine addition. Finally, it is possible that the effects of dung and urine return were weak because a substantial part of total N disappeared as volatilized ammonia (NH_3) before reaching the soil. Around 60% of the total N of cow slurry is ammonia, and when slurry is evenly spread on ley in summer conditions, >50% of the ammonia can volatilize during the few days following application (Mattila and Joki-Tokola 2003). This suggests that, besides removing the patchiness of excreta return, spreading dung and urine mixture evenly on field plots can also increase the loss of N through ammonia volatilization in comparison to the situation in the presence of cows.

Grazing and soil parameters in the field

Some recent greenhouse and field studies suggest that soil microfauna, such as nematodes, can readily respond to plant defoliation (Mikola et al. 2001, 2005b, Hokka et al. 2004, Bazot et al. 2005). However, few effects of grazing on nematode diversity and abundance have been found in different grassland ecosystems, ranging from cattle pastures (McSorley and Frederick 2000, McSorley and Tanner 2007) to semi-natural (Zolda 2006) and natural grasslands (Merrill et al. 1994) and having grazing periods of even more than 50 years (Wall-

Freckman and Huang 1998). Also, when effects are found, they seem to depend on the grassland type and the time of sampling (Wang et al. 2006). Our findings that bacterial-feeding, root-feeding and omnivorous nematodes did not respond to grazing at any study year support these earlier observations in grazed grasslands. However, we found a very significant positive influence of grazing on the abundance of fungal-feeding nematodes. This effect was clearly explained by the physical presence of cows, since mowing and application of dung and urine mixture had no effect on fungal feeders. Of the factors that are related to the presence of cows, patchy nutrient return appears to be the most plausible explanation for the increased fungivore numbers. This is because the high mean abundance of fungal feeders in G plots stems from a few high values, with most values remaining at the level of other treatment plots (for instance in 2001, G plots produced four high values—4, 10, 11 and 20 fungal feeders per gram of soil—with the other six values ranging from 0.4 to 2 fungivores/g soil). It is further likely that the high numbers of fungal feeders originate from plots where cowpats were deposited since applications of urine or manure slurry, which contains urine, have earlier been found to have stronger positive influence on bacteria and bacterial feeders than on fungi and fungal grazers (Opperman et al. 1989, Griffiths et al. 1998, Williams et al. 2000, Bittman et al. 2005). That even application of urine and dung mixture in MDU plots did not have a similar positive influence on fungal feeders indicates that the spatial distribution of resource return has a significant role in determining the effects on soil organisms. This idea is further supported by soil inorganic-N concentrations, which were not higher in MDU than in M plots (despite the addition of nutrients to MDU plots) but were significantly higher in G than in MDU plots (despite the similar amount of nutrients returning to both treatments). As in the case of fungal-feeding nematodes, these results derive from an uneven distribution of nutrients in the grazed area (the differences between the minimum and maximum values of inorganic-N concentrations were 3, 35, and 5 μg/g dry soil for MDU plots and 116, 63, and 120 μg/g dry soil for G plots in years 2000, 2001, and 2002, respectively). It is likely that soil microbes and plants are able to assimilate all returning nutrients when they are evenly distributed over the soil area (along with part of the total N disappearing through ammonia volatilization), which causes no difference between M and MDU plots in soil mineral N concentrations. In contrast, when nutrient return is concentrated in dung and urine patches, the high concentration of nutrients exceeds the immediate need of microbes and plants, and nutrients remain available in the soil for longer. The results of plant biomass and plant N content obtained in the feedback trial corroborate this idea: both of these plant attributes show the same pattern of means and variance as do soil N concentration at the last harvest. These results
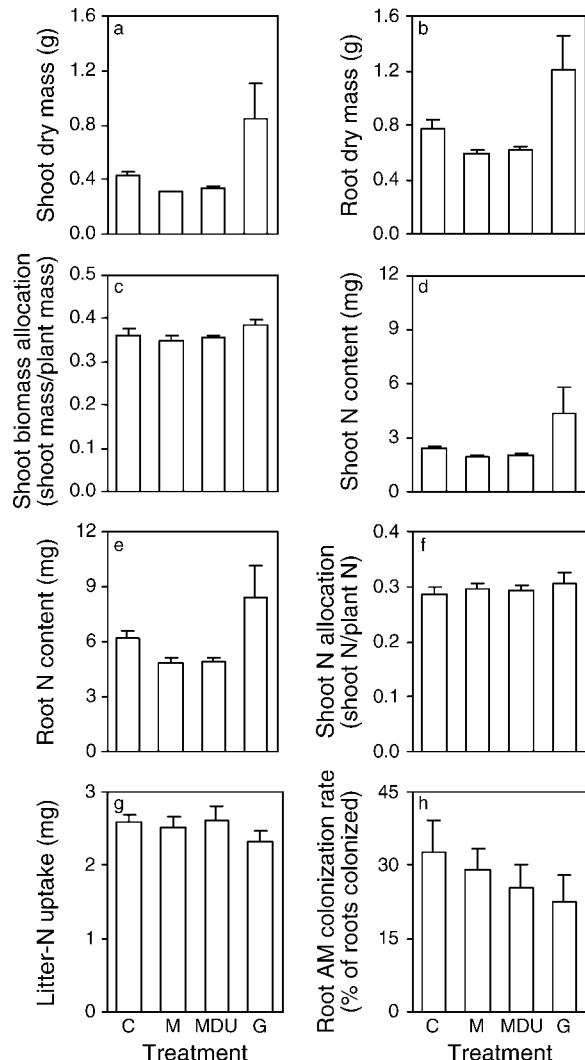

FIG. 9. Attributes of *Phleum pratense* seedlings (data are means + SE, *n* = 10 replicate observations) growing in soils collected from field plots subjected to different pasture treatments: dry mass of shoots and roots; relative biomass allocation to shoots; N content of shoots and roots; relative N allocation to shoots; amount of N taken from the added litter; and root colonization rate of arbuscular mycorrhizal fungi. Soil treatments are: C (control), M (mowed), MDU (mowed and dung and urine added), and G (grazed by cows) plots.

support earlier observations that concentrations of mineral N are spatially highly variable in grazed grasslands (Bogaert et al. 2000).

Earlier studies of the effects of grazing on soil meso- and macrofauna show that earthworm biomass, and especially that of *Lumbricus terrestris*, increases with increasing cattle stocking rate (Muldowney et al. 2003), while numbers of enchytraeids decrease with increasing sheep stockings (King and Hutchinson 1976). Our findings agree with these in that grazing had a negative effect on enchytraeids, but, in contrast to earlier findings, *Lumbricus* earthworms were lower in grazed
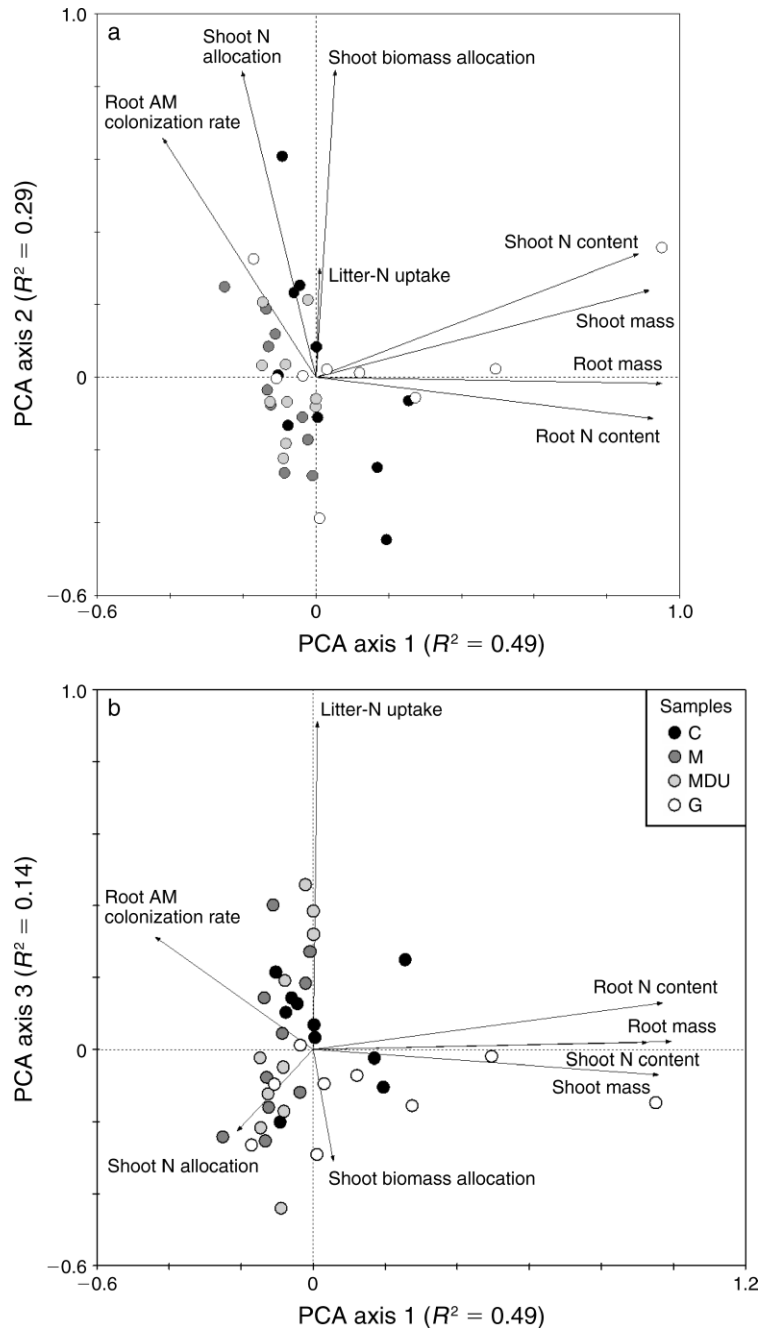
FIG. 10. PCA graph of feedback data in (a) axis 1 × axis 2 and (b) axis 1 × axis 3 ordination planes with the soil origin as an overlay; soils were collected from C (control), M (mowed), MDU (mowed and dung and urine added), and G (grazed by cows) plots. Interpretation of the graph is as in Fig. 1.

than control plots and only *Aporrectodea* earthworms were positively affected by grazing. Our results further indicate that the mechanisms responsible for these effects differed among faunal groups: while the effects of grazing on enchytraeids were explained by the physical presence of cows, which was our original prediction, the effects on both groups of earthworms were explained by defoliation. The difference in the

response of the two earthworm genera can be related to their living habits. Species of *Lumbricus* are typical anecic earthworms that build vertical burrows and feed on plant litter available on the soil surface. It is therefore likely that the lower abundance of *Lumbricus* in grazed and mowed plots was due to there being less plant shoot litter entering the soil surface in these than in control plots. Species of *Aporrectodea* are classified as endogeic
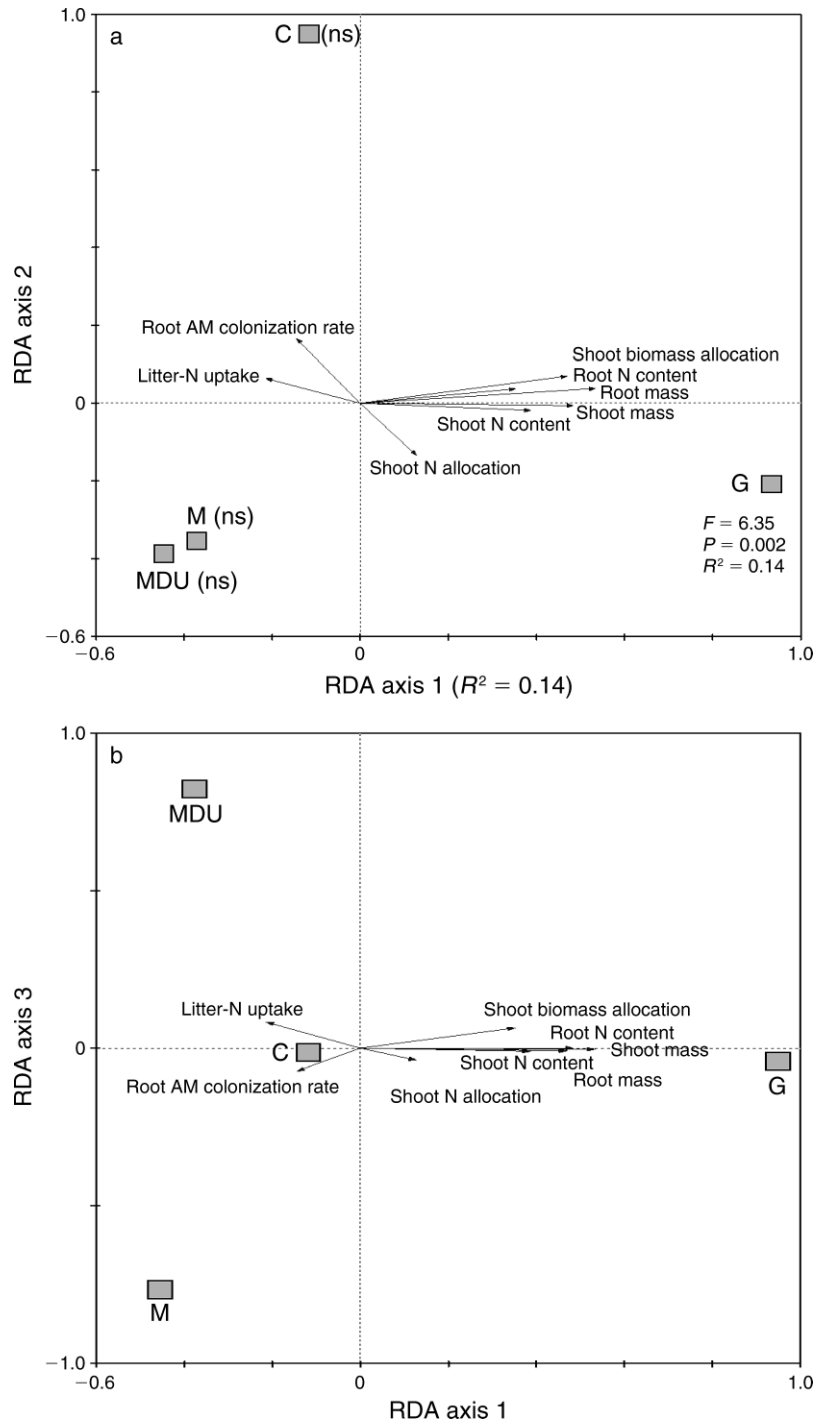
FIG. 11. Partial RDA graph of feedback data in (a) axis 1 × axis 2 and (b) axis 1 × axis 3 ordination planes constrained by the soil origin (C, M, MDU, and G as is previous figures) after controlling for the effect of blocks and years. "ns" means treatment effect on dependent variables is not statistically significant. Interpretation of the graph is as in Fig. 1.

species that build lateral burrows in the soil and feed on soil organic matter. It is known that defoliation can increase exudation of organic compounds from plant roots to the rhizosphere (Holland et al. 1996, Paterson and Sim 1999) and increase root mortality and turnover (Frank et al. 2002), which can lead to increased availability of resources for animals that feed on soil organic matter. Our finding that grazing effects on earthworms were not explained by trampling and soil compaction, but instead by defoliation of plants

TABLE 5. Variable scores from a PCA of the feedback trial data.

Variable	PCA axis			
	1	2	3	4
Shoot dry mass	**0.9591**	0.2504	−0.0700	−0.0024
Root dry mass	**0.9936**	−0.0181	0.0216	0.0756
Shoot biomass allocation	0.0558	**0.8834**	−0.3108	−0.0546
Shoot N content	**0.9272**	0.3544	0.0188	−0.0076
Root N content	**0.9697**	−0.1190	0.1292	0.1302
Shoot N allocation	−0.2116	**0.8806**	−0.2273	−0.2085
Root AM colonization rate	−0.4393	**0.6871**	0.3133	0.4836
Litter-N uptake	0.0108	0.3138	**0.9118**	−0.2566
Eigenvalue	0.4935	0.2911	0.1375	0.0458

Notes: The scores are directly proportional to the correlations between the response variables and the PCA axes. Score values $\geq |0.5|$ are in boldface type and indicate a large contribution of the variable to the properties of the respective PCA axis.

affecting earthworm resources, gets support from a recent study by Curry et al. (2008). They found that earthworm populations were not adversely affected by increasing trampling at higher cow-stocking rates when this was accompanied by increasing fertilization and plant production. In contrast to earthworms, enchytraeids were clearly negatively affected by the presence of cows in our study, and unlike in the case of fungal-feeding nematodes, there is no evidence of patchiness creating this response. This leaves trampling and soil compaction as most likely reasons for the reduced enchytraeid biomass in the grazed plots.

Of the measured soil parameters, grazing increased soil density, pH, and moisture content (in 2001 only) but had no effect on soil inorganic-N concentrations. Defoliation explained the positive effect of grazing on soil moisture content, probably because defoliation reduced plant transpiration. Increasing soil density is in turn explained by the physical presence of animals, which agrees with earlier findings that animal trampling decreases the amount of soil pores and increases soil bulk density (King and Hutchinson 1976, Greenwood and McKenzie 2001). The neutral effect of grazing on soil N concentration is in turn a sum of two simultaneous mechanisms compensating for each other's influence. While defoliation reduced the availability of N in soil, probably as a consequence of plants remaining in active vegetative-growth stage when defoliated, the presence of cows, when compared to the MDU plots, increased N availability. As discussed above, the cow effect derives from an uneven return of nutrients into the grazed area, which both reduces ammonia volatilization and makes soil microbes and plants unable to exhaust nutrients from concentrated patches. In earlier investigations, grazing has usually been found to increase net N mineralization (Holland and Detling 1990, McNaughton et al. 1997, Frank and Groffman 1998, Frank et al. 2000), and it has been shown that this can happen

without consequences on the amount of extractable N (McNaughton et al. 1997). Our results agree with the neutral effect of grazing on extractable mineral N and show that this can be a result of two contrasting mechanisms—defoliation and patchy excreta return—taking place simultaneously. The positive effect of grazing on pH could not be explained by any particular mechanism in our study, which agrees with earlier studies that have not found a consistent effect of grazing on soil pH (Milchunas and Lauenroth 1993).

Grazing and soil feedback

The laboratory test revealed a clear soil feedback on plant growth and plant N uptake: plants that grew in the soil collected from G plots produced more biomass and acquired more N than plants growing in the soils collected from other field plots (although this was not statistically significant). Also, plants growing in C plots produced more biomass and took up more N from the soil than plants growing in M and MDU plots. Both of these effects were most probably mediated by soil inorganic-N concentrations since soil inorganic N concentration in the 2002 harvest correlated strongly with shoot mass ($r = 0.97$, $P < 0.001$, $n = 40$ replicates), root mass ($r = 0.96$, $P < 0.001$), shoot N content ($r = 0.90$, $P < 0.001$) and root N content ($r = 0.94$, $P < 0.001$) of *P. pratense* seedlings measured in the feedback study. None of the other soil variables (including root-feeding nematodes and *Lumbricus* earthworms that had a treatment pattern similar to the plant parameters at the last harvest) correlated with these plant parameters. This indicates that the soil feedback on plant growth was not mediated by grazing-induced changes in soil biota, but simply by mineral-N availability, which in turn was controlled by plant N demand in mowed plots and patchy return of dung and urine in grazed plots (as discussed above). It has been shown that plants growing on urine patches postpone their senescence in comparison to plants growing off patches (Day and Detling 1990), which agrees with our finding that nutrients remain available for longer in grazed grassland because they are patchily distributed and cannot be quickly exhausted. In our feedback test, plants acquired on average 8% of the available litter-N, but this amount was not affected by the history of the soil or the availability of mineral N in soil. The fact that litter-N uptake, which is a decomposer-mediated process, was not affected by the soil history supports our conclusion that the soil feedback was not mediated by soil biota. It also agrees with an earlier finding that elevated levels of mineral N in urine patches do not affect N mineralization rate in the soil (Augustine and Frank 2001).

When considering the role of soil animals in the soil feedback, it is important to bear in mind that the soil was sieved before the feedback test. This was done to avoid a false soil feedback on plant growth through decaying roots in the soil. The risk of a false feedback is particularly high when the field treatments cause

changes in root quantity and quality (as do grazing and defoliation in our study), because the amount and quality of roots remaining in the feedback soil is likely to partly determine the availability of nutrients for the seedlings later planted into the soil. On the other hand, the disturbance on soil organisms and soil structure that is created by soil sieving has a potential to cover differences caused by the field treatments and thus preclude soil feedbacks. That we found a soil feedback that appeared to be directly mediated by inorganic-N availability, rather than soil animal abundances, could thus be argued to be due to the effects mediated by soil organisms being destroyed during soil preparation. However, our conclusions of N availability mediating the soil feedback on plant growth are also supported by the field data. The Mantel tests show that while soil parameters were clearly linked with plant parameters in the field, nematode and annelid abundances were not (Table 2), indicating a minor role for animals in plant performance in the field, too. Therefore, although the soil preparation for the feedback test may to some extent diminish the role of soil organisms in the soil feedback, it is likely that the organisms would not dominate the feedback over the inorganic-N availability in non-sieved soil either. The weak connection between plant attributes and soil-animal abundances found in our arable soil contrasts the common view of a significant role of soil animals in plant growth and nutrient uptake (cf. Mikola et al. 2002), but agrees well with recent studies suggesting that the link between soil decomposer abundances and plant nutrient uptake may not be straightforward or easy to predict (Saj et al. 2007, Sørensen et al. 2008).

In the feedback test, we also measured the arbuscular mycorrhizal (AM) colonization rate of *P. pratense* seedlings to test whether the field treatments had affected the ability of plants to acquire mycorrhizal symbionts from the soil. We did not find clear evidence for this since root AM colonization rates did not differ between seedlings growing in the soils of different history despite the RDA graph suggesting a negative association between root AM colonization rate and the soils collected from G plots. However, we found a significant negative correlation between root mass and AM colonization rate and a positive correlation between AM colonization rate and plant litter-N uptake, which were also supported by the RDA graph. These finding indicate that those plants that grew in better N conditions (i.e., in the soil collected from G plots), allocated fewer resources to their mycorrhizal symbionts, with a consequence of less N captured from the soil organic matter (despite these plants having more root mass). Although these effects were weak in our study, they reveal an interesting new perspective on how grazers can indirectly affect plant nutrition. It seems that return of inorganic N into pasture soil in concentrated patches can, on average, divert plants from allocating resources to structures that help in capturing N from organic sources.

To sum up, our results indicate that many, but not all, grazing effects can be explained by plant defoliation. This is especially true for plant attributes, ranging from nutrient allocation within a plant to resource competition between plant species, but also for some soil animal groups. Dung and urine appear to have major effects on soil animals and soil N availability when they return to soil in concentrated patches, but not when applied evenly over the soil surface. The soil feedback created by grazing seems to be mediated by soil mineral-N availability, rather than soil biota, and also in this case the patchy return of N to soil in grazed systems has a paramount role. Altogether, these results suggest that changes in plant ecophysiology caused by the defoliation and the great spatial variation of soil nutrients created by the grazing animals are the two key mechanisms through which large herbivores can control grassland ecosystems.

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APPENDIX A

A table showing precipitation, temperature, and duration of the growing season at the experimental area, 2000–2002, and corresponding long-term means (1961–1990) (*Ecological Archives* M079-008-A1).

APPENDIX B

A figure showing plant-available soil moisture at 20-cm depth during growing seasons 2000–2002 (*Ecological Archives* M079-008-A2).

APPENDIX C

Schematic depiction of the arrangement of treatment plots and replicate blocks in the pasture (*Ecological Archives* M079-008-A3).