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SHORT COMMUNICATION

Dissimilar response of plant and soil biota communities to long-term nutrient addition in grasslands

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Abstract The long-term effect of fertilizers on plant diversity and productivity is well known, but long-term effects on soil biota communities have received relatively little attention. Here, we used an exceptional long-lasting (>40 years) grassland fertilization experiment to investigate the long-term effect of Ca, N, PK, and NPK addition on the productivity and diversity of both vegetation and soil biota. Whereas plant diversity increased by liming and decreased by N and NPK, the diversity of nematodes, collembolans, mites, and enchytraeids increased by N, PK, or NPK. Fertilization with NPK and PK increased plant biomass and biomass of enchytraeids and collembolans. Biomass of nematodes and earthworms increased by liming. Our results suggest that soil diversity might be driven by plant productivity rather than by plant diversity. This may imply that the selection of measures for restoring or conserving plant diversity may decrease soil biota diversity. This needs to be tested in future experiments.

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Introduction

For many decades, ecologists have been interested in factors determining the diversity and productivity of ecosystems. Until recently, the focus in terrestrial ecosystems was mainly on aboveground species, even though the corresponding belowground communities support a much larger biodiversity and play a major role in ecosystem processes. The recent increase in appreciation for soil communities is due to studies that suggest that soil organisms may influence the vegetation, making the interaction between above- and belowground communities itself an important regulating factor for ecosystem productivity and diversity (Van der Heijden et al. 1998; De Deyn et al. 2003).

Soil fertility is a well-known determinant of grassland productivity and diversity (Wardle 2002). Many studies show that fertilization decreases plant-species diversity and increases productivity (e.g., Harpole and Tilman 2007; Pywell et al. 2007). However, these studies are often dealing with short-term nutrient additions (e.g., Foster and Gross 1998; Stevens et al. 2006; Clark et al. 2007).

So far, the relation between nutrients and belowground communities has received relatively little attention. It is to be expected that nutrients affect soil biota productivity via increased plant biomass production, which in turn enhances microbial growth, thereby increasing resources for soil fauna (Cole et al. 2005). Yet, energy fluxes through the food web may drastically change as a result of fertilization. The effect of nutrients on plant diversity might affect soil biota diversity as well. It has been suggested that heterogeneous vegetation offers diverse resources and habitats, thereby promoting resource and habitat-partitioning among soil biota (Hooper et al. 2000).

In 1958, a grassland field experiment was set up to study the long-term effect of fertilizers on plant productivity and diversity (Elberse et al. 1983). This field experiment has only duplicates per treatment, but the duration of the experiment is exceptional and of great value to answer long-term research questions. It is offering the opportunity to support or reject the following hypotheses: (1) nutrient addition will increase plant productivity, and this will in turn increase the biomass of soil biota, as more resources will become available to microbial decomposers and, subsequently, to higher trophic levels in the soil food web and (2) nutrient addition will decrease plant diversity, and this will in turn decrease the diversity of soil biota, as a result of a less diverse mixture of resources and physical habitats available to soil organisms.

Material and methods

The Ossekampen Grassland Experiment was established in 1958 on a species-rich, old pasture on a heavy river-clay soil near Wageningen, the Netherlands (51°58′ 15″ N; 5°38′ 18″ E), as described by Elberse et al. (1983). From 1959 onwards, yearly fertilizer treatments were N, PK, NPK, and lime (Ca) (Table 1). The fertilizers used were superphosphate, potassium sulfate, ammonium nitrate, and lime marl. All plots (16×2.5 m) were replicated twice (five treatments including the control, resulting in ten plots) and were mown twice a year (July and October).

The vegetation composition was described by the 25 cm^2 frequency and order method (De Vries 1937). In May 2001, 50 samples of 25 cm^2 were taken on each plot along a diagonal and two parallel lines on either side of the diagonal. A strip of 1.25 m over the length of the plot was harvested to estimate aboveground plant biomass production.

In March 2000, 50 soil samples (Ø 2.3 cm, 5 cm depth), were taken in each plot along a diagonal transect. The organic matter content was determined as weight loss of dry soil after ignition at 550°C for 16 h. Total C and N were analyzed by combustion of air-dried soil at 1,050°C. Total P was determined as described in Van der Wal et al (2007).

In May 2001, about 150 soil cores (2.3 cm Ø, 10 cm depth) were randomly taken in each plot. Per plot, soil cores were mixed, homogenized, and sieved before nematode analyses. Additionally, in each plot, duplicates were taken for enumeration of microarthropods (Ø 6 cm, 7.5 cm depth) and enchytraeids (6 cm Ø, 15 cm depth). For earthworms, duplicates of 20×20 cm, 15 cm depth, were collected in each plot. Soil was stored at field moisture content at 4°C before analysis. Nematodes, microarthropods, and enchytraeids were extracted and determined as described in Mulder et al. (2008). Briefly, free-living nematodes were extracted from 100 g soil with the elutriator, and total numbers were counted. Nematodes were fixed in formaldehyde 4%, and from each sample, at least 150 randomly selected nematodes were identified to genus and, whenever possible, to species. For the sampling of microarthropods (collembolans and mites), intact soil cores were placed in Tullgren-Berlese funnels for 5 days. The temperature in the upper part of the funnel was set at 30°C and kept at 5°C in the lower part. All animals escaping from the heat were collected at the lower end of the funnels in 70% ethanol and observed at a magnification of ×200–1,000 with a compound light microscope. Microarthropods were identified at the species level. Enchytraeids were extracted using modified Baermann funnels with increasing light and heat each 30 min after the start of the extraction and a total extraction time of 3 h. The total

Table 1 Fertilizer treatments of the Ossekampen plots from 1959 (N from 1966) until 2008

Treatment	Start	N application (kg ha^{-1} year^{-1})	P application (kg ha^{-1} year^{-1})	K application (kg ha^{-1} year^{-1})	Ca application (kg ha^{-1} year^{-1})
Control	1959	0	0	0	0
N	1966	160	0	0	0
PK	1959	0	52 (1959–1980) 17 (1981–1985) 22 (1986-2008)	332 (1959–1980) 108 (1981–1985) 165 (1986-2008)	0
NPK	1959	160	52 (1959-1980) 31 (1981-1985) 33 (1986-2008)	332 (1959-1980) 291 (1981-1985) 311 (1986-2008)	0
Ca	1959	0	0	0	710 (1959-1980) 355 (1981-2008)

number of enchytraeids per sample was counted, and species were identified to the highest taxonomic level possible.

Plant diversity and soil faunal diversity (nematodes, microarthropods, and enchytraeids) were calculated as the Shannon index $H' = -\Sigma(p_i \times \ln p_i)$, where p_i represents the relative abundance of species i, and the Shannon's equitability (evenness) $E_H = H'/\ln S$, where S represents the total number of species present in the community. To be able to assess the effect of nutrients on plant and soil communities, different fertilization treatments were lumped based on two analyses. The first one was a principal component analysis (PCA) using CANOCO 4.5 (Ter Braak and Smilauer 2002). Plant diversity, aboveground plant production, and soil biota (as diversity and biomass) were entered in the analysis. Based on clustering of samples, treatments were grouped. The second analysis was based on the soil macronutrients. Nutrient ratios have been suggested to reflect the interaction between the environment and the occurring (micro)organisms (Schade et al. 2005; Cleveland and Liptzin 2007). The N/P ratio showed clear differences between treatments (Table 2, one-way ANOVA $p<0.001$). Based on both the N/P ratio and the PCA, treatments were grouped in enrichment group 1 (untreated control and Ca), group 2 (PK and NPK), and group 3 (N). The effect of nutrients on communities of plants and soil biota were then analyzed using Redundancy Analysis (RDA; Ter Braak and Smilauer 2002). The significance of the first canonical axis was investigated by Monte Carlo tests, using three enrichment groups as factors.

Results

Soil organic matter content was not significantly different between treatments (range from 21.1–22.2%), except for limed plots (18.5%). Soil pH was higher in the control (5.3) and especially in the limed plots (6.7) compared to the N, PK, and NPK treatments (4.8, 5.0 and 4.9, t test, $p=0.07$).

Principal component analysis (major PCA-axes explain 64% of the variance) showed that the Ca and control treatments clustered together, as well as the PK and NPK treatments (Fig. 1). Redundancy analysis (major RDA-axes explain 56% of the variance) showed a remarkable relation between nutrients, vegetation, and soil biota along the first ordination axis ($p=0.006$). The diversity and evenness of plant species were positively correlated with the Ca treatment and control, whereas aboveground plant biomass production correlated with PK and NPK fertilization (Fig. 1). Like plant biomass, biomass of enchytraeids and collembolans was positively correlated with PK and NPK fertilization. Biomass of nematodes and earthworms was, however, correlated with the Ca treatment and the control. As opposed to the results for plant species, diversity and evenness of soil biota groups were positively correlated with either PK and NPK or N fertilization.

Table 2 Average molar ratio of soil C/N and N/P measured in 2000

Ratio	Treatment				
	Control	Ca	PK	NPK	N
C/N	14.2 a	13.5 a	14.9 a	15.0 a	13.5 a
N/P	10.3 a	10.6 a	5.6 b	5.9 b	11.9 c

Letters denote significant differences between treatments at $p<0.05$ (t test)

Discussion

This field experiment enabled us to study the long-term effect of nutrients on the diversity and productivity of grassland plants and soil biota. Although only duplicate datasets were available for each treatment, the similarity in responses of the two replicates indicated that trends in above- and belowground communities showed consistent patterns. Biomass and diversity responses of plants to fertilization are consistent with other long-term fertilization grassland field experiments (Silvertown et al. 2006; Honsova et al. 2007). The diversity and evenness of plant species was highest in the Ca treatment. This can be explained by the presence of a larger pool of vascular plant species suited for high pH soils in Continental Europe than the pool of plant species for acidic soils (Ellenberg et al. 1992).

The PCA analysis showed that biomass of enchytraeids, collembolans, and mites were increased in soils with relatively high N content. We may, therefore, conclude that the mesofauna prefers high quality resources, i.e., food sources with high nutrient content. A comparable trend is shown by Bardgett and Cook (1998) and Cole et al. (2005) as they reported increased densities of collembolans and mites after application of NPK and N in combination with Ca to grasslands. Another study of Loranger-Merciris et al. (2007) showed that high content of N in leaves is attractive for soil fauna, resulting in an abundant macrofauna community. In general, nutrient supply (N, PK, and NPK) increased soil biota diversity and evenness. In contrast, diversity and evenness of plant species was highest in the control and Ca treatment. Therefore, we conclude that under these circumstances, nutrient supply has an opposite effect on the diversities of plants and soil biota. This is unexpected, as we hypothesized that nutrient addition would decrease plant diversity, followed by a decrease in soil biota diversity as a less diverse mixture of resources will be present belowground.

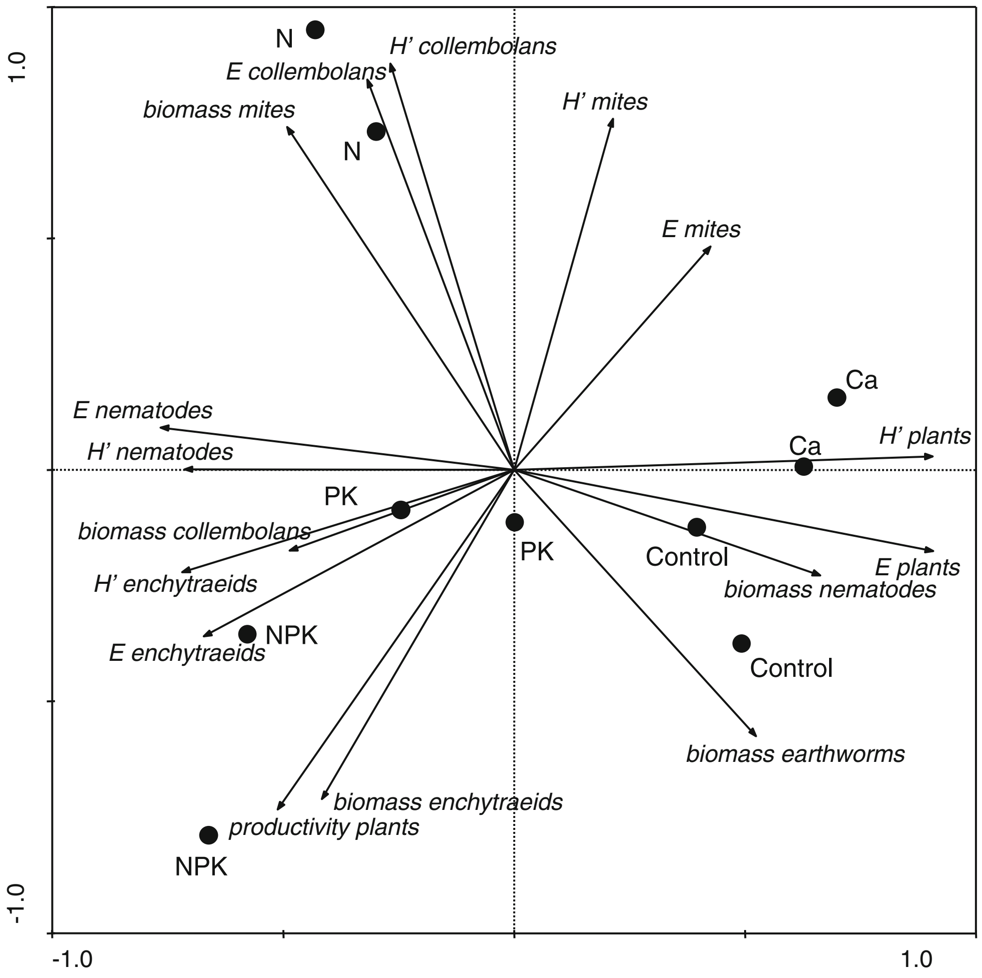

Fig. 1 PCA plot of plant and soil communities at the Ossekampen Grassland Experiment. Diversity (*H'*), evenness (*E*), and productivity (*biomass*) of plants and of soil biota are entered in the analysis as species (*arrows*). *Dots* are representing field samples with corresponding treatments (*Control*, *Ca*, *N*, *PK*, *NPK*)

Soil faunal diversity seems, thus, not to be driven by greater plant diversity. It has been suggested that fungal-feeding soil animals are general fungal feeders, and this may also account for soil biota feeding on dead organic matter (Maraun et al. 2003; Ayres et al. 2006; Mulder et al. 2008). The fact that soil biota diversity increases after nutrient supply may be due to an overall increase in quality and quantity of organic matter in these treatments, resulting in an increase in food sources in more soil pores compared to the control. Furthermore, it has been suggested that productive ecosystems will increase higher trophic levels as sufficient C will become available for them (bottom–up effect) (Moore and De Ruiter 2000). A top–down effect may also occur; nitrogen supply increases predatory mites, and this may contribute to an increase in diversity of soil biota as a result of feeding of top predators on dominant prey species (Dyer and Letourneau 2003). Another possibility is that not plant diversity as a whole, but particular plant species characteristics played a role in structuring the soil biota. For example, NPK and N strongly increased the frequency of grasses and decreased the frequency of forbs. The percentage of legumes increased in both the Ca and PK treatments. A change in functional groups of plants, in this case an increase in grasses, may have structured the soil biota rather than a change in plant species diversity (Porazinska et al. 2003).

Plant productivity increased simultaneously with NPK additions and biomass of enchytraeids and collembolans. This could confirm our first hypothesis that an increase in resources (organic matter) will increase the biomass of soil biota. However, this relation was not found for all groups of soil biota; biomass of nematodes and earthworms did not follow this pattern. Hence, it appears that the interaction between productivity of above- and belowground communities is also influenced by other variables, such as soil pH, resource quality, or even the interaction between plant and soil biota communities itself.

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