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**Correspondence** and requests for materials should be addressed to B.R. (e-mail: barbara@seismo.berkeley.edu).

## Soil invertebrate fauna enhances grassland succession and diversity

Gerlinde B. De Deyn\*, Ciska E. Raaijmakers\*, H. Rik Zoomer†, Matty P. Berg‡, Peter C. de Ruiter‡, Herman A. Verhoef‡, T. Martijn Bezemer\* & Wim H. van der Putten\*

\* Department of Multitrophic Interactions, Centre for Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 40, 6666 ZG, Heteren, The Netherlands

† Department of Animal Ecology, Institute of Ecological Science, Vrije Universiteit De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

‡ Department of Environmental Sciences, Copernicus Research Institute for Sustainable Development and Innovation, Utrecht University, P.O. Box 80115, 3508 TC Utrecht, The Netherlands

One of the most important areas in ecology is to elucidate the factors that drive succession in ecosystems and thus influence the diversity of species in natural vegetation. Significant mechanisms in this process are known to be resource limitation<sup>1–3</sup> and the effects of aboveground vertebrate herbivores<sup>4,5</sup>. More recently, symbiotic and pathogenic soil microbes have been shown to exert a profound effect on the composition of vegetation<sup>6–9</sup> and changes therein<sup>10,11</sup>. However, the influence of invertebrate soil fauna on succession has so far received little attention<sup>12,13</sup>. Here we report that invertebrate soil fauna might enhance both secondary succession and local plant species diversity. Soil fauna from a series of secondary grassland succession stages selectively suppress early successional dominant<sup>14</sup> plant species, thereby enhancing the relative abundance of subordinate<sup>14</sup> species and also that of species from later succession stages. Soil fauna from the mid-succession stage had the strongest effect. Our results clearly show that soil fauna strongly affects the composition of natural vegetation and we suggest that this knowledge might improve the restoration and conservation of plant species diversity.

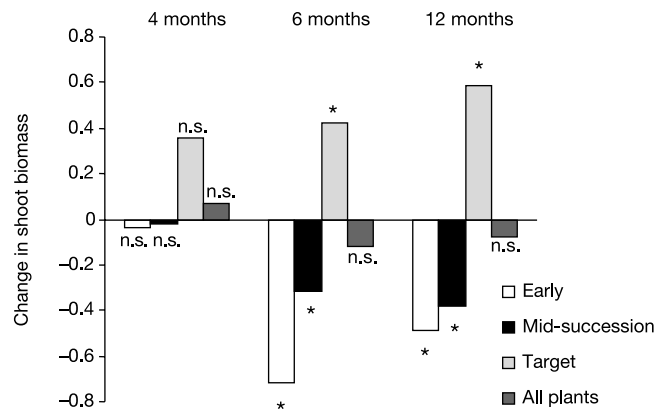
The succession of species in natural communities is one of the most fundamental concepts in ecology<sup>15</sup>. Successional patterns in vegetation can be reasonably well predicted owing to their correlation with changes in resource availability, whereas the competitive abilities of plants for these resources determines which species might become dominant<sup>2</sup>. However, biotic interactions above and below ground can greatly change the outcome of these competitive interactions. Aboveground vertebrate herbivores can indirectly benefit subdominant plant species through selective feeding on dominants<sup>4,5</sup>. Below the soil surface, root symbionts can enhance plant species diversity by improving the nutrient uptake and growth

of subdominants<sup>6</sup>, and root pathogens can do so by suppressing dominant host plant species<sup>7</sup>. Selective improvement or suppression of plant species can eventually lead to cyclic<sup>8,9,16</sup> or unidirectional successional<sup>11</sup> shifts in local species composition.

Although soil microorganisms seem to have a profound effect on vegetation succession and plant species diversity, the role of invertebrate soil fauna has not yet been resolved. In early secondary succession grasslands, the application of soil insecticides reduces the abundance of root-feeding insects, resulting in the promotion of early successional forbs<sup>12</sup>, suggesting that root-feeding insects might enhance the process of succession<sup>13</sup>. Similar effects of root-feeding insects were confirmed in microcosm<sup>17</sup> and mesocosm<sup>18</sup> studies. In mid-succession extensively grazed grasslands, cyclic replacement of grass and sedge species were correlated primarily with the abundance of plant-feeding nematodes<sup>19</sup>. However, soil organisms with a larger body size seem to overrule the effects of smaller soil organisms<sup>18</sup>, casting doubt on the results of studies that focus on the role of specific components of invertebrate soil fauna.

To clarify the specific contribution of the invertebrate soil fauna (hereafter called 'soil fauna') to secondary vegetation succession and to plant species diversity, we conducted an experiment in which natural soil fauna communities (Table 1) were applied to combinations of plant species from production, restoration and conservation grasslands. We constructed experimental grassland communities in which plant species from a production grassland (early succession stage), from a grassland subjected to nature restoration management during the past 20 years (mid-succession) and from a species-rich natural grassland community (the target of secondary grassland succession) were grown in mixed stands. All plant communities were established in sterilized soil and were inoculated with soil fauna from one of the three successional stages. This made it possible to determine the role of the soil fauna from a range of secondary succession grasslands on the outcome of interactions between the plant species from their own and from the other two successional stages. The soil fauna added represented the densities and composition of the field communities and included microfauna (nematodes), mesofauna (micro-arthropods) and macrofauna (beetle larvae).

Our results show that the addition of all three successional communities of soil fauna resulted in significant shifts in the



**Figure 1** Change in the shoot biomass of early and mid-succession plant species and plants from the target grassland community in the presence of soil fauna relative to the control (no soil fauna added) calculated as  $(B_1 - B_c)/B_c$ , where  $B_1$  and  $B_c$  are the mean shoot biomasses with and without soil fauna added, respectively. The experimental grassland communities were grown in microcosms for 1 year. Addition of the soil fauna impaired the early and mid-succession plants and stimulated the development of plants from the target grassland community. Data presented are averages over all treatments with different origins of soil fauna. Significance: asterisk,  $P < 0.05$ ; n.s., not significant.

Table 1 Numbers and biomass of soil fauna in the inoculum from the grasslands

Organism group	Individuals per treatment (inoculum origin)			Biomass ( $\mu\text{g}$ dry wt) per individual <sup>27</sup>
	Early	Mid-succession	Target	
Total nematodes	29,430 $\pm$ 9,124	28,118 $\pm$ 6,135	12,460 $\pm$ 3,466	0.33
Root-feeding nematodes	7,235 $\pm$ 3,002	9,130 $\pm$ 2,662	3,500 $\pm$ 631	0.33
Elateridae larvae	0	11 $\pm$ 7	5 $\pm$ 3	9,000
Collembola	590 $\pm$ 231	413 $\pm$ 139	713 $\pm$ 117	7.62
Mites	196 $\pm$ 59	1,698 $\pm$ 325	2,638 $\pm$ 400	1.66

Data are means  $\pm$  s.e.m. ( $n = 4$ ). Nematodes were extracted from each grassland by decantation of 100 soil cores, each 2.5 cm in diameter and 10 cm deep. Collembola, mites and Elateridae larvae were isolated from soil cores collected from the top 5-cm soil layer of each grassland; the area extracted was equal to that of the inoculated microcosms. The soil cores were extracted with Tullgren funnels<sup>26</sup> (30  $\times$  30 cm<sup>2</sup>, 30 °C top, 12 °C at the bottom). The micro-arthropods were collected from the funnels and inoculated every second day for 3 weeks.

plant communities towards the dominance of the plant species from the late succession (target) community ( $P < 0.01$ ), whereas in the control soils the plant communities ultimately became dominated by mid-succession plant species. To identify the mechanisms underlying the enhancement of succession by the soil fauna, we examined the root and shoot biomass distributions of the various plant species in relation to the various soil communities. The soil fauna decreased the shoot biomass of the early succession plant species after 6 months ( $P_6 = 0.006$ ) and 12 months ( $P_{12} = 0.025$ ), as well as plant species from the mid-succession stage ( $P_6 = 0.032$ ,  $P_{12} = 0.015$ ), whereas the shoot biomass of the target plant species was increased ( $P_6 = 0.047$ ,  $P_{12} = 0.021$ ; Fig. 1). The soil fauna did not affect the total shoot biomass after 6 or 12 months but decreased the total root biomass at the end of the experiment by about one-half ( $P = 0.005$ ). These effects on the roots indicate that the differences are due mainly to root herbivory and that the effects increase with time. The initial biomass of the root-feeding soil fauna was negatively correlated with both final root biomass and final shoot biomass ( $R^2 = 0.24$ ,  $P < 0.01$ ;  $R^2 = 0.18$ ,  $P < 0.05$ , respectively). There was no significant correlation between collembola, or mites, and root biomass. Fungal feeders in these soil taxa might have been feeding on fungal hyphae that could develop from propagules that recolonized the microcosms throughout the course of the experiment.

Besides favouring plant species from the target site relative to plant species from early and mid-succession grasslands, addition of the soil fauna also enhanced plant species diversity. In the presence of soil fauna, the proportion of the dominant plant species in the total biomass decreased, resulting in enhanced plant evenness ( $P = 0.023$ ; Fig. 2). Plant evenness, based on shoot biomass, was positively correlated with the initial biomass of the root-feeding soil fauna ( $R^2 = 0.25$ ,  $P < 0.01$ ), and negatively with the root biomass at the end of the experiment ( $R^2 = 0.13$ ,  $P < 0.05$ ). Although it was not possible to determine the root masses of individual plant species, our results suggest that the invertebrate root herbivores were selectively feeding on roots of dominant plants. Plant evenness was enhanced primarily by the soil fauna from the mid-succession

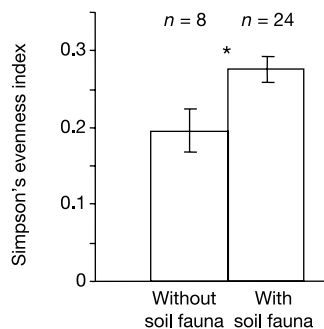


Figure 2 Simpson's evenness index (mean  $\pm$  s.e.m.) of microcosms with experimental grassland communities without and with soil fauna. The soil fauna enhanced the evenness of the plant community (ANOVA;  $P < 0.05$ ).

grassland ( $P < 0.05$ ). In particular, the mid-succession soil fauna most selectively decreased roots and shoots of the mid-succession grasses (Fig. 3), which became the dominant plant species in controls lacking soil fauna (data for individual plant species are not shown).

Root-feeding soil fauna of the early succession communities was made up predominantly of root-feeding nematodes, whereas the faunal communities from the mid-succession and target sites consisted mainly of root-feeding nematodes and Elateridae (click beetle) larvae. Plant evenness was correlated best with initial root-feeding nematode biomass ( $R^2 = 0.42$ ,  $P = 0.0001$  for the root-feeding nematodes alone, and  $R^2 = 0.25$ ,  $P < 0.01$  for nematodes and Elateridae larvae together). The selective effects of soil fauna on the plant communities might therefore be enhanced by differences in community composition, because the nematode taxa present in the mid-succession grassland were more similar to that from the early grassland than to that from the target grassland (Sørensen's similarity index  $I_S$ ;  $P < 0.001$ ).

In summary, our results show that the soil fauna communities from early succession, mid-succession and target stages of secondary grassland profoundly enhance vegetation succession and the homogeneity of the plant community by reducing the biomass of the dominant plant species. The strongest effect was found with the soil fauna from the mid-succession grassland, while plant species from this succession stage were affected most. Reduction of the root mass of the dominant plants by the soil fauna provided an indirect advantage for the subdominant plant species, which were only marginally suppressed in the presence of soil fauna. This was confirmed by the negative correlation between the initial biomass

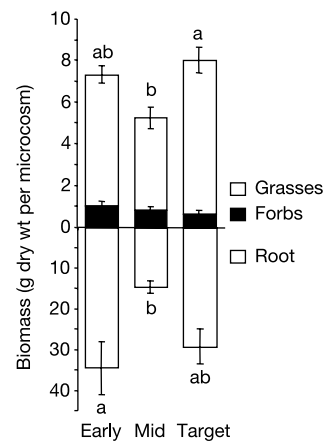


Figure 3 Effect of soil fauna from early, mid-succession and target grassland communities on total root and shoot biomasses of grasses and forbs (mean  $\pm$  s.e.m.;  $n = 8$ ). The plant communities in the microcosms were established from grassland plants from early and mid-secondary succession stages and species from the target (a semi-natural grassland). The soil fauna from the mid-succession grassland reduced the biomass of grass shoots and that of the total amount of roots (a and b denote significant differences in grass shoot or root biomass, Tukey;  $P < 0.05$ ).

of the root feeders and the biomass of plant shoots and roots at the termination of the experiment.

The effects of soil fauna on succession are analogous to the feeding of aboveground vertebrate herbivores<sup>5,20</sup> but differ markedly from the effects of arbuscular mycorrhizal fungi, which directly improve growth conditions of the subordinate plants<sup>6</sup>. The contribution of soil fauna and of mycorrhizal fungi to plant community processes in grasslands seems to be additive rather than counter-acting<sup>18</sup>. The net effect of the soil community on early and mid-succession and on plant species diversity seems to be due to a selective suppression of the dominant plant species by soil fauna. This might be attributed to higher root quality and accessibility (more suitable nutrient composition or less chemical or mechanical defence) or to lower tolerance to herbivory of the early succession plant species. By contrast, in late succession stages, where nutrients become limited, the growth conditions of the subordinate plant species might be selectively improved by mycorrhizal fungi<sup>10</sup>. However, their effect depends on the grassland community studied, because the competitive outcome is driven by the differential host plant responses of dominant and subordinate plant species to colonization by mycorrhizal fungi<sup>21</sup>. Our results show that there is a strong linkage between succession in vegetation and in soil community composition, and that the patterns observed above ground are the result of a continuous feedback process<sup>22</sup> between plants and soil organisms. Restoration and conservation of biodiversity in natural vegetation should therefore not be focused exclusively on reducing soil fertility or on introducing aboveground vertebrate grazers. A better understanding of the dynamic contribution of soil organisms to plant community processes is also important. Including the role of soil fauna might profoundly enhance the predictability of succession and might therefore affect the success of programmes aimed at the restoration and conservation of biodiversity. □

## Methods

### Microcosm design maintenance and collection

We established 32 microcosms of plant species mixtures from (1) recently abandoned production grassland (early); (2) grassland under restoration for 20 years (mid-succession); and (3) species-rich natural grassland, the biodiversity restoration and conservation target (target). Experimental units consisted of microcosms (17 cm long, 17 cm wide and 22 cm deep) filled with  $\gamma$ -irradiated (25 kGy) sterilized soil from the mid-succession grassland, planted with 24 seedlings of *Poa trivialis* L., *Lolium perenne* L., *Stellaria media* L., *Rumex obtusifolius* L. (early species), *Agrostis capillaris* L., *Festuca rubra* L., *Plantago lanceolata* L., *Prunella vulgaris* L. (mid-succession species), *Anthoxanthum odoratum* L., *Festuca ovina* L. and *Campanula rotundifolia* L. (target species). In addition, owing to poor germination of *Succisa pratensis* L., half of the replicates received two *Centaurea jacea* L. seedlings, and the other half received one *Centaurea jacea* L. and one *Succisa pratensis* L. seedling (target species). The seeds were provided by a small specialized company and obtained from the field. One week after germination the seedlings were planted in a grid of 6 × 4 positions. Each of the eight replicates was assigned a different plant configuration to avoid positioning effects. The plants were watered daily; once a week the initial soil moisture level was measured by weighing and readjusted to 20%.

After 6 weeks the soil fauna was added (Table 1). To prevent the organisms from spreading from one microcosm to the other, a rim of Fluon was applied to the inner and outer edges of the microcosms and trolleys. Three trolleys were loaded with each microcosm with the same origin of soil fauna (early, mid-succession or target) or without inoculum (control). The trolleys with microcosms were maintained in a greenhouse in a random arrangement. There was minimally 16 h of light (240 W m<sup>-2</sup>) and a light/dark temperature regime of 21/16 °C. Every 2 weeks the positions of the trolleys were changed at random to minimize position effects.

After 4 and 6 months, shoots were clipped at 4 cm above the soil surface and the dry weights of all individual plant species were determined after 2 days at 75 °C. After 12 months, shoots of each plant species were clipped at ground level and total root biomass was collected, dried and weighed. Plant evenness was calculated as Simpson's evenness index<sup>23</sup> ( $SIEI = (1/\sum p_i^2)(1/S)$ , where  $p_i$  is the proportional contribution of the  $i$ th species to the canopy and  $S$  is the number of species present). To determine the numbers and identity of the nematodes in the microcosms, six soil cores 2.5 cm in diameter and 20 cm deep (the entire depth of the microcosm) were collected and extracted with Oostenbrink elutriators<sup>24</sup>. We analysed four replicates for nematodes; micro-arthropods were collected from the remaining four replicates. All nematodes present in 10% of the total extracted soil volume were counted and identified to genus level. The genera were distributed into feeding groups as described in ref. 25. The similarity in taxon composition was calculated as the Sørensen similarity index  $I_S = 2J/(A + B)$ , with  $J$  the number of taxa present in both

samples,  $A$  the number of taxa in sample  $A$  and  $B$  the number of taxa in sample  $B$ . At final collection micro-arthropods were isolated from soil cylinders (10 cm in diameter, 5 cm deep) with Tullgren funnels<sup>26</sup> (10 cm in diameter by 6 cm deep, 30 °C top, 5 °C at the bottom) and determined to species level (collembola) or higher taxonomic levels (mites). The collembola and mites were considered to be microbivores; the Elateridae larvae were considered to be root feeders.

### Statistics

The data were analysed with Statistica version 10.0. All plant data were analysed by one-way analysis of variance (ANOVA), using soil fauna inoculum or soil fauna origin as factors. Normality was checked for by the Kolmogorov-Smirnov (Lilliefors) test, homoscedasticity with the Levene test. The statistical difference between groups was determined by Tukey's multiple-range test, with  $P < 0.05$  indicating significance. If normality was not achieved, data were transformed before statistical analysis. The total shoot biomass and the shoot biomasses of early and mid-succession plant species were square-root transformed, whereas root biomasses were natural-logarithm transformed before analysis. Plant biomass per harvest was analysed as total biomass and biomass per plant successional origin and plant type (for example grasses versus forbs).

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**Correspondence** and requests for materials should be addressed to G.D.D. (e-mail: g.dedejn@nioo.knaw.nl).