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INTERPLAY BETWEEN *SENECIO JACOBAEA* AND PLANT, SOIL, AND ABOVEGROUND INSECT COMMUNITY COMPOSITION

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Abstract. To elucidate the factors that affect the performance of plants in their natural environment, it is essential to study interactions with other neighboring plants, as well as with above- and belowground higher trophic organisms. We used a long-term field experiment to study how local plant community diversity influenced colonization by the biennial composite *Senecio jacobaea* in its native range in The Netherlands in Europe. We tested the effect of sowing later-succession plant species (0, 4, or 15 species) on plant succession and *S. jacobaea* performance.

Over a period of eight years, the percent cover of *S. jacobaea* was relatively low in communities sown with 15 or 4 later-succession plant species compared to plots that were not sown, but that were colonized naturally. However, after four years of high abundance, the density of *S. jacobaea* in unsown plots started to decline, and the size of the individual plants was smaller than in the plots sown with 15 or 4 plant species. In the unsown plots, densities of aboveground leaf-mining, flower-feeding, and stem-boring insects on *S. jacobaea* plants were lower than on plants in sown plots, and there was a strong positive relationship between plant size and levels of herbivory.

In a greenhouse experiment, we grew *S. jacobaea* in sterilized soil inoculated with soil from the different sowing treatments of the field experiment. Biomass production was lower when *S. jacobaea* test plants were grown in soil from the unsown plots than in soil from the sown plots (4 or 15 species). Molecular analysis of the fungal and bacterial communities revealed that the composition of fungal communities in unsown plots differed significantly from those in sown plots, suggesting that soil fungi could have been involved in the relative growth reduction of *S. jacobaea* in the greenhouse bioassay. Our results show that, in its native habitat, the abundance of *S. jacobaea* depends on the initial composition of the plant community and that, on a scale of almost a decade, its interactions with plant and soil communities and aboveground invertebrates may influence the dynamics of this colonizing species.

Key words: biodiversity; biotic resistance; DGGE; grasslands; insect herbivory; plant–soil feedback; Senecio jacobaea.

Introduction

Plant growth, survival, reproduction, and subsequent population density are determined by competition with neighboring plants and interactions with organisms of higher trophic levels, both aboveground and belowground (Crawley 1983, Tilman 1997, Hambäck and Beckerman 2003, Bezemer et al. 2004, Wardle et al. 2004). However, why some plant communities are more prone to colonization by new plant species than others remains an intensely debated issue in ecology (e.g., Vitousek 1990, Crawley et al. 1999, Kennedy et al. 2002). Elton (1958) supposed that highly diverse plant communities are more competitive and thus more resistant to colonization than less diverse ones. Indeed, a number of studies on plant colonization in short-term experimental (grassland) systems show a negative relationship between species richness and colonization (e.g., Tilman 1997, Knops et al. 1999, Naeem et al. 2000, Kennedy et al. 2002, Fargione and Tilman 2005). The susceptibility of a community to plant colonization has been associated with available amounts of space, light, and nutrients (Naeem et al. 2000). Higher plant species richness frequently increases the use of these resources, and hence decreases the space for establishment by increasing the productivity of the community and decreasing root space (Tilman et al. 1996). Other studies suggest that plant species identity may be more important than species richness per se in determining whether or not a community can be colonized successfully (Crawley et al. 1999, Lepš et al. 2001). This involvement of both plant species richness and plant species identity may explain why patterns of colonization in self-assembled, natural systems do not always

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follow predictions of current competition models (Crawley et al. 1999, Levine and D'Antonio 1999, Meiners et al. 2004).

Pathogenic and herbivorous organisms, either above- or belowground, can also strongly influence the establishment and performance of a plant species within a community (Crawley 1983, Klironomos 2002, De Deyn et al. 2003). Soil pathogens, for example, can increase rapidly in the presence of their host, causing conditions that are unfavorable for subsequent growth or local recruitment of the same species or of other species (Van der Putten et al. 1993, Bever et al. 1997). Soil can also express biotic resistance to colonization by new plant species, for example due to the presence of pathogens (Beckstead and Parker 2003) or nonselective root feeders (Knevel et al. 2004). Several studies also show that aboveground herbivorous insects can reduce plant abundance (e.g., Louda and Potvin 1995, Maron and Vilà 2001). Although above- and belowground plant control factors are frequently studied in isolation, the impacts of plant neighbors and above- and belowground herbivores and pathogens on the colonization and performance of plant individuals are obviously not independent, but interact with each other (Hambäck and Beckerman 2003, Rudgers and Hoeksema 2003, Bezemer et al. 2004). The composition of neighboring plants, for example, can determine not only the number and diversity of aboveground herbivorous insects present in the community (Siemann et al. 1998, Knops et al. 1999, Koricheva et al. 2000), but also the composition of the belowground community (De Deyn et al. 2004). Moreover, the feeding damage of aboveground herbivores is frequently determined by the quality of the host plant, and this can be influenced by whether the plant also suffers from herbivory belowground, and vice versa (Bezemer et al. 2005, Bezemer and Van Dam 2005). To understand what determines the performance of a plant in its natural environment, it is thus essential to examine interactions with other neighboring plants, as well as with above- and belowground organisms of other trophic levels (Bezemer et al. 2004).

Here we report the results of an eight-year study on the population dynamics of ragwort (*Senecio jacobaea*) in its native range in Europe. The plant established in a long-term field experiment on ex-arable land (i.e., abandoned arable land) with plots that were either sown with 15 or 4 midsuccessional grassland species, or were not sown but were naturally colonized by plant species. Colonization was also allowed in sown plots, and further plant community development in all treatments was thus due to colonization and plant community assemblage processes. The sowing treatments resulted in long-term differences in plant communities. We analyze the dynamics of *S. jacobaea* and show the relationship between *S. jacobaea* performance, the surrounding plant community, and soil and aboveground insect communities.

Materials and Methods

Ragwort (*Senecio jacobaea)* plants usually spend their first year as rosettes, and they bolt and flower the following summer. Ragwort is a biennial but may become a short-lived perennial when damaged by cutting or defoliating. It usually reproduces by seed but can reproduce vegetatively via root and crown buds. Ragwort is a prominent invasive weed in Australia, New Zealand, and North America, and the relationship between *S. jacobaea* and its invertebrate herbivores is well studied. Most of these investigations have focused on the interaction between ragwort and the cinnabar moth *Tyria jacobeaea* (e.g., Crawley and Gillman 1989, Van der Meijden et al. 1991, McEvoy et al. 1993, McEvoy and Coombs 1999, Bonsall et al. 2003). In Europe, however, ragwort is attacked by a number of insects feeding on foliage, flowers, stems, and roots (Harrison and Thomas 1991, Kunin 1999). There are surprisingly few studies on soil-borne pathogens of *S. jacobaea* (but see Wardle et al. 1995, Hol and Van Veen 2002).

Site description and experimental design

In spring 1996, we set up an experimental field 100×50 m^2 on abandoned ex-arable land at Planken Wambuis, Ede, The Netherlands. Until then, the area had been cultivated with maize (*Zea mais*) in rotation with sugar beets (*Beta vulgaris*), potatoes (*Solanum tuberosum*), barley (*Hordeum vulgarum*), and occasionally rye grass (*Lolium perenne*). The surrounding area was 50 ha comprising heathland, mixed forest, and abandoned land. The soil was a sandy loam with the following particle size distribution: <2 μm, ~3%; 2–63 μm, ~17%; >63 μm, ~80%. At the start of the experiment the field was plowed and harrowed. The soil contained 4.5% organic matter, with a pH (H_2O) of 6.4. Abiotic soil conditions of the field have been described in detail elsewhere (Van der Putten et al. 2000, Bezemer et al. 2004). We initiated the experiment to determine the effects of sowing of later-succession plant species on the process of plant secondary succession and nature restoration with three sowing levels: 0 species (these plots were initially colonized by plants from the seed bank and from the surroundings), 4 species, and 15 species. The experimental treatments were installed using a randomized block design with five blocks. Each replicate plot measured 10×10 m^2, and each block contained one replicate of each sowing level. Within each block the plots were separated by lanes 2-m wide that were mown frequently.

All replicates with 15 species sown had the same combination of species (five grasses, *Festuca rubra* [Fr], *Phleum pratense* [Php], *Poa pratensis* [Pop], *Agrostis capillaris* [Ac], *Anthoxanthum odoratum* [Ao]; five legumes, *Lotus corniculatus* [Lc], *Trifolium pratense* [Tp], *Trifolium dubium* [Td], *Trifolium arvense* [Ta], *Vicia cracca* [Vc]; five other forbs, *Plantago lanceolata* [Pl], *Tanacetum vulgare* [Tv], *Hypericum perforatum*

[Hp], *Hypochaeris radicata* [Hr], *Linaria vulgaris* [Lv]). These species were not present when the site was abandoned. To prevent confusion between diversity of seed mixture and plant-specific traits (Huston 1997), each of the replicates with four species sown contained a different subset of the high-diversity mixture (two grasses, one legume, one other forb; block 1, Fr, Php, Lc, Pl; block 2, Fr, Pop, Vc, Hr; block 3, Pop, Ao, Tp, Tv; block 4, Ac, Ao, Td, Hp; block 5, Php, Ac, Ta, Lv). The high- and low-diversity mixtures consisted of the same number of seeds (grasses, 2500 seeds/m^2; legumes, 500 seeds/m^2; other forbs, 500 seeds/m^2). After sowing, plots were not weeded, so that the communities were the result of self-assemblage following initial sowing. Plants could colonize from the area surrounding the experimental field but also from other plots. Annually in September, all plots were mown, and the aboveground biomass was removed.

Vegetation dynamics

Annually (1996 to 2003), at peak standing biomass (late July to early August), plant species abundance in every experimental plot of 100 m^2 was recorded in 12 permanent quadrats of 1 m^2 each. Because cover was assessed for each species individually, total cover within a quadrat can exceed 100%, reflecting overlapping of the plant species. For each plot the results from the 12 permanent quadrats were used to calculate the mean number of plant species/m^2, the Shannon-Wiener index (H'), and evenness. In each permanent quadrat, we also assessed the total plant cover and percent cover of grasses, legumes, and other forbs. Every year in late August, we clipped aboveground biomass at 2 cm above the soil surface in 12 0.25×0.25 m^2 subplots adjacent to the permanent quadrats. Plant material was oven-dried at 70°C and weighed to calculate the mean aboveground biomass/m^2 for each plot.

Senecio jacobaea *performance*

In the abandoned area surrounding the field *S. jacobaea* established and became one of the dominant plant species in the vegetation (40%–50% abundance in 2002 and 2003; T. M. Bezemer, *personal observation*). The plant species was not sown but established naturally at the experimental site. Since this plant species is considered a "problem weed" in the Netherlands, and we observed that the rate of establishment and the percent cover varied between sowing treatments, we investigated the dynamics of this species in more detail within the setting of the sowing experiment. Therefore, the abundance of *S. jacobaea* was recorded annually as part of the plant species abundance measurements. For each replicate plot and each year, we also recorded the proportion of permanent quadrats in which *S. jacobaea* was present. Starting in 2000, aboveground biomass of *S. jacobaea* was separated from the total biomass and weighed, and for each plot we determined the proportion of subplots with *S. jacobaea* biomass.

In early July 2002, we randomly selected eight flowering plants in each plot, avoiding the permanent quadrats, and for each plant we measured height and the number of leaves and flower buds. We then clipped the plants at the soil surface and separated each plant into stem, leaf, and reproductive biomass. All material was oven-dried at 70°C and weighed.

Molecular analyses of the soil community

In mid-September 2002, we took soil samples from each plot following a stratified random-sampling pattern (24 cores per plot, 3 cm diameter, and 15 cm deep). Soil samples were homogenized and used for molecular analysis of the bacterial and fungal soil community. For DNA isolations we used 0.25 g wet soil per isolation and used the MO BIO soil DNA extraction kit (MO BIO Laboratories, Solana Beach, California, USA). Soil samples were washed twice in 120 mM K_2HPO_4, pH 8.0, prior to DNA extraction, in order to wash away extracellular DNA from the soil samples without the loss of intact cells. Extractions were performed as per the manufacturer's specifications except that vortex mixing was replaced with shaking 2×30 seconds at 5000 rounds/min in a minibead beater (BioSpec Products, Techno Lab, Alkmaar, The Netherlands), and final DNA elution was in 30 μL 10 mmol/L Tris, pH 8.0. The concentration of DNA extracts was determined by spectrophotometric measurements at 260, 280, and 300 nm. DNA quantity and quality were also inspected by 1% agarose gel electrophoresis with standard ethidium bromide staining and ultraviolet illumination (not shown).

For the polymerase chain reaction (PCR) denaturing gradient gel electrophoresis (DGGE) profiling strategy for bacteria, we used the primer pair 968f-GC/1401r (Heuer et al. 1997), and for fungal-specific PCR–DGGE the FR1-GC/FF390 primer pair (Vainio and Hantula 2000). All amplification reactions were performed in a volume of 25 μL and consisted of 15 nmol/L of each primer, approximately 50 ng of environmental template DNA, 1 U Expand High Fidelity DNA polymerase (Boehringer, Mannheim, Germany), and the manufacturer's recommended nucleotide concentrations and buffer conditions. All reactions were performed in a PTC200 thermal cycler (MJ Research, Waltham, Massachusetts, USA). DGGE utilized 6% acrylamide gels with a gradient of 45–65% denaturant for bacterial analyses and 40–55% denaturant for fungal analyses (100% denaturant = 7 mol/L urea with 40% formamide). DGGE was performed for 16 h at 75 V using the D-Gene system (Bio-Rad Laboratories, Hercules, California, USA). Approximately 1 μg of PCR product was loaded per well in a final volume of 20 μL. Gels were stained with ethidium bromide and washed twice for 15 min in deionized H_2O prior to UV transillumination and digital photography using the ImaGo system (B & L, Maarssen, The Netherlands). We analyzed DGGE banding profiles within the Imagemaster elite v4.20

(Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) for rolling ball ($r = 10$) background subtraction, normalization, and band detection. Matching of bands was performed in reference to a hypothetical composite lane containing bands at all positions found across the entire dataset. DGGE profile comparisons used a Pearson's similarity index, taking both band number and intensity into account, after signal normalization. For dendrogram construction we used unpaired group mean averages (UPGMA) using the ImageMaster 1D Database program, version 3.0 (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). We also carried out principal component analysis (PCA) with CANOCO 4.5 (Ter Braak and Šmilauer 2002) on DGGE banding data, including position and normalized intensity. Pseudoreplicates were performed for a number of samples to test the reproducibility of the PCR–DGGE methods across multiple DNA extractions and PCR amplifications. In all cases, patterns were nearly identical (Pearson's index > 0.95, not shown).

Nematodes

In early August 2002 we took soil cores (3 cm diameter, 15 cm depth) from the rhizosphere of 12 randomly selected flowering *S. jacobaea* plants in each plot. After the soil of each plot had been homogenized, 100 g were subsampled for nematode extraction using an Oostenbrink elutriator (Oostenbrink 1960). The total number of nematodes was counted, after which nematodes were heat killed and fixed in 4% formalin. A minimum of 150 nematodes were then identified per sample to genus or family level and allocated to feeding groups: plant parasites, bacterivores, fungivores, omnicarnivores. Numbers of nematodes were expressed per 100 g dry soil.

Soil bioassay

To determine the effect of soil (micro)organisms on *S. jacobaea* we carried out a greenhouse study. In the greenhouse, climatic conditions were set at 60% relative humidity, with light availability at 16L:8D, and temperature at 21° ± 1°C during the day and 16° ± 1°C at night. Natural daylight was supplemented by 400-W metal halide bulbs (one per 1.5 m^2). In mid-September 2002, we took two sets of soil samples from each plot (24 cores per set, 3 cm diameter, and 15 cm deep). One set of 24 cores was taken following a stratified random sampling pattern. The second set of 24 cores was taken from the rhizosphere of *S. jacobaea* plants. For each plot, the soil of each set was homogenized and sieved (<0.5 cm). We also collected a bulk sample of soil from outside the plots. This soil was also sieved (<0.5 cm) and sterilized by gamma irradiation by Isotron, Ede, The Netherlands, using an absorbed dose of minimally 25 kGy/s. This sterilized bulk soil was subdivided and gently homogenized with the soil from the plots (1:6 nonsterilized/bulk mass), so that the five replicates in the field remained to be replicates in the greenhouse experiment. We then filled 1-L pots with the different mixtures, and soil moisture content was set at 20% (v:v). The procedure enabled us to avoid confounding of results by nutrient availability differences on plant performance in sterilized vs. nonsterilized soil samples (Troelstra et al. 2001).

We then transplanted 1-wk-old *S. jacobaea* seedlings to each pot. Seedlings came from seeds collected from plants growing in the abandoned area surrounding the experimental field. The seeds were surface sterilized with 1% chlorix (household bleach) and germinated on glass beads. Pots were either planted with a single seedling or with three seedlings. Each soil origin within each plot was replicated three times resulting in a total of 180 pots (2 soil origins × 3 sowing levels × 5 blocks × 2 plant densities × 3 replicates). Plants were watered regularly, and soil moisture was kept at 20% throughout the experiment. Ten weeks after transplanting the seedlings we clipped aboveground biomass and washed the roots. Aboveground and root material was oven-dried at 70°C, and total dry mass per pot determined. The data from the three replicate soil samples per plot were averaged prior to analysis.

Aboveground insect herbivory on Senecio jacobaea

In early August 2002 we measured aboveground insect herbivory on *S. jacobaea* in each replicate plot at 16 predetermined positions, randomly distributed throughout the plot so as not to disturb the permanent quadrats. At each of the 16 positions, we clipped the nearest flowering *S. jacobaea* plant, and plants were kept individually in plastic bags. In the laboratory, for each plant we then recorded the presence of and damage by flower feeders, stem borers, and foliar feeders. To determine the amount of damage by flower-feeding insects, for 8 out of 16 plants, 25 randomly chosen flower buds per plant were individually examined. For stem borer densities, we recorded the number of "mines" and whether the larva was still present. For each plant we also recorded whether leaf miner damage occurred, and for damaged plants we recorded the number of mined leaves per plant. To determine whether plant quality differed between treatments, for a subset of three randomly chosen plants in each plot, all foliar, stem, and flower material was separated, oven-dried at 70°C, ground, and analyzed for total nitrogen content (Novozamsky et al. 1984).

Statistical analysis

Replicated measurements taken within one plot were averaged prior to statistical analyses to prevent pseudoreplication. All data (plot averages) were first checked for homogeneity of variance and normality, and log-transformed if necessary. Percentage data were arcsine transformed before analyses to achieve a normal distribution. We then analyzed data using sowing treatment (3 levels) as fixed and block (5 levels) as random effect. All data were analyzed using Statistica, version 6 (2004; StatSoft,

Tulsa, Oklahoma, USA). Measurements that were repeated during different years were analyzed using repeated-measures analysis of variance (RANOVA). This analysis takes into account the overall treatment effect, independent of time, as well as the within-treatment effect of whether treatments differ in their pattern of response over time. The three sowing levels were then compared using a Tukey test based on the overall effect (independent of time). Measurements taken once were analyzed using analysis of variance (ANOVA), and individual comparisons were based on a Tukey test. The plant–soil bioassay was analyzed using a full factorial three-way ANOVA with sowing treatment (3 levels), soil origin (2 levels), and plant density as main factors. The relationship between soil community composition in each plot and mean individual *S. jacobaea* aboveground biomass per plot was determined with linear regression analysis using the scores of the first two axes derived from principal component analysis carried out on fungal and bacterial DGGE profiles.

Results

Vegetation dynamics

Plots that were not sown had significantly more plant species/m^2 and higher H' diversity and evenness than sown plots (Fig. 1, Appendix A). On average species richness in plots originally sown with 15, 4, or 0 species was 12.2, 10.2, and 13.7, respectively. Plots sown with 15 species had, on average, significantly more species and a higher diversity (H') than plots sown with four species (Fig. 1). For all vegetation measurements the highest amount of variation between replicates was found, as expected, for plots sown with four species of which the individual replicates had been sown with different mixtures (Fig. 1). Aboveground biomass was highest in plots sown with 15 species (795 g/m^2), intermediate in plots sown with four species (610 g/m^2), and lowest in unsown plots (469 g/m^2) (Fig. 1D, Appendix A). The abundance of grasses, forbs, and legumes also differed significantly between sowing treatments (Appendix A). Abundance of grass was lowest in unsown plots (on average 20%) and did not differ significantly between plots sown with 4 (on average 43%) or 15 species (on average 39%) (Fig. 1E). Forb abundance was on average highest in unsown plots and lowest in plots sown with 15 species, but this difference diminished over time (Fig. 1F). Legume abundance was highest in plots sown with 15 species, in particular in 1998. Legume abundance was initially higher in plots sown with four species than in unsown plots, but after four years it did not differ anymore (Fig. 1G). The vegetation in unsown plots was less dense than in sown plots (either 4 or 15 species), as indicated by a lower total vegetation cover for unsown plots (Fig. 1H, Appendix A).

Senecio jacobaea *performance*

The abundance of *S. jacobaea* differed significantly between treatments (Appendix A). The invasion of the plots started in 1997, but percent cover of *S. jacobaea* increased more in unsown plots than in plots sown with 4 and 15 species (Fig. 2A). Mean annual abundance in plots sown with 15 and 4 species did not differ (Fig. 2A). In unsown plots mean abundance peaked at approximately 30% in 2000, whereas mean abundance did not exceed 10% in sown plots (either 4 or 15 species). From 2000 onward, mean abundance of *S. jacobaea* declined in unsown plots, and the pattern of abundance in plots sown with 15 and 4 species fluctuated and was, on average, 7%.

The decline of *S. jacobaea* abundance in unsown plots was even more evident when considering plant biomass. From 2000 onward, *S. jacobaea* aboveground biomass in unsown plots declined sharply, while it remained constant in plots sown with 15 and 4 species (Fig. 2B, Appendix A). In 2000, mean aboveground biomass/m^2 in unsown plots was 20 times higher than in plots sown with either 15 or 4 species, but in 2003 this difference was reduced to only three times more biomass in unsown plots than in sown plots (Fig. 2B).

The proportion of subplots with *S. jacobaea* biomass was far higher in unsown plots than in plots sown with 15 and 4 species, and this remained relatively constant over time (Fig. 2C). In 2002, individual *S. jacobaea* plants were significantly smaller and had significantly fewer leaves and flowers in unsown plots than in plots sown with 15 species. In plots sown with 15 species, *S. jacobaea* plants were largest, and the largest variation was found among individual replicates sown with 4 species (Table 1).

Molecular analyses of the soil community

There was a high overall level of similarity between bacterial PCR–DGGE banding patterns from different plots (Pearson's index = 0.74 ± 0.12 [mean ± SD]). No bands could be identified that were characteristic for sowing treatments, and dendrogram analysis showed no significant groupings of sample banding patterns (Appendix B). A similar result was obtained by principal component analysis (PCA), which showed no clustering of samples (Appendix B). Fungal PCR–DGGE revealed a lower diversity of bands and greater variability between samples compared to bacterial profiles (mean Pearson's index = 0.58 ± 0.18, not shown). Similar to what was observed for the bacterial PCR–DGGE analysis, no specific bands could be identified that corresponded to soil origin. Dendrogram analysis of fungal PCR–DGGE patterns did, however, reveal some clustering of patterns (Fig. 3A). Of particular interest, four of the five samples from unsown plots clustered together with reasonable bootstrap support. In a PCA the same four samples from unsown plots clustered apart from the intermixed samples of plots sown with 15 or 4 species (Fig. 3B). The last of the blocks from the unsown treatment (Block 2), which did not group with other samples of this treatment in the dendrogram analysis, occupied an intermediate position

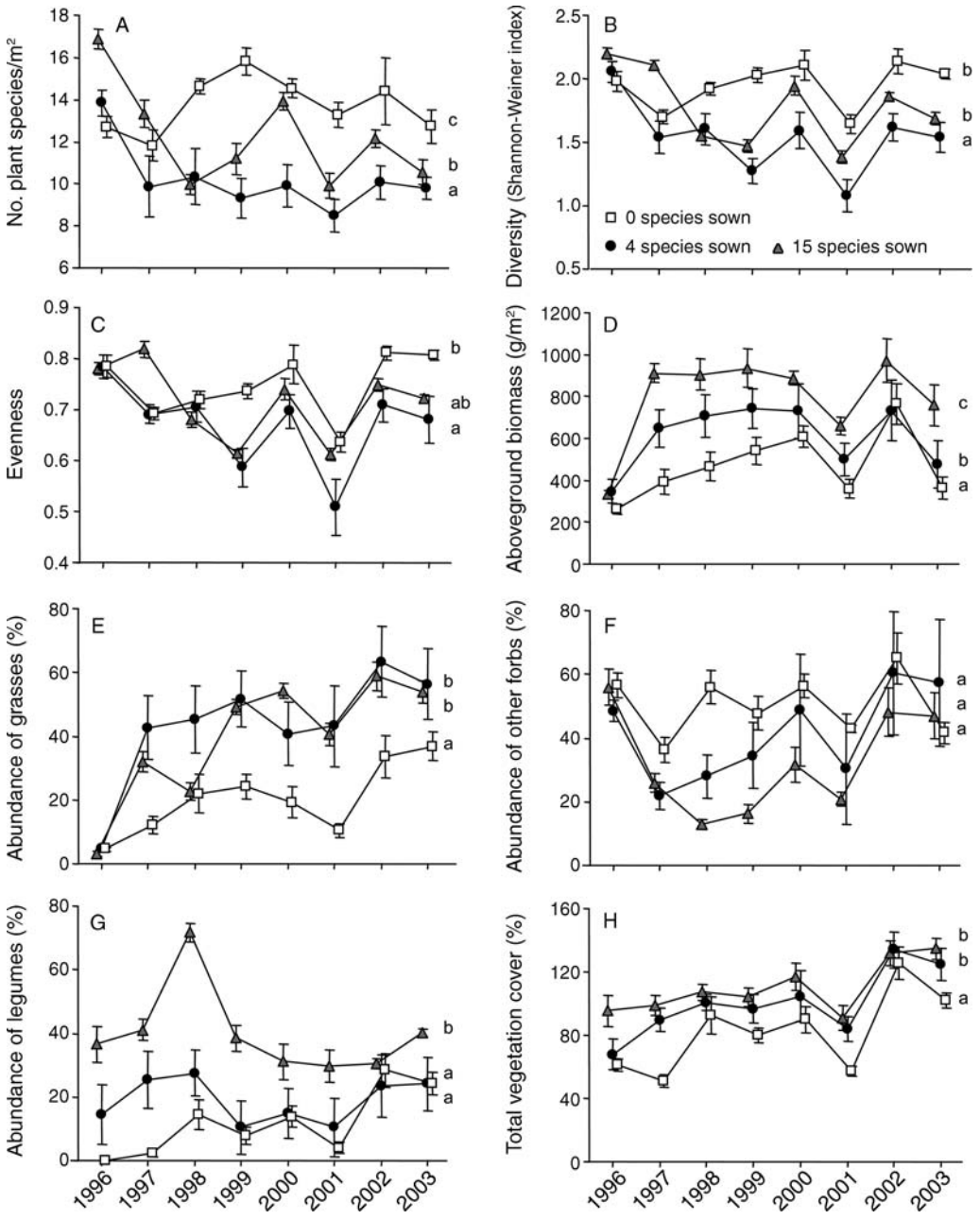

FIG. 1. Measures (mean ± SE, $n = 5$) of plant species/m^2, diversity, evenness, aboveground biomass, and relative abundance of legumes, other forbs, and total vegetation cover in plots with different sowing treatments for the years 1996–2003 (see panel B for key to symbols). Plots were not weeded but were mown annually, after which aboveground biomass was removed. Within each graph, different letters to the right of each line indicate significant differences ($P < 0.05$) based on a Tukey's hsd test following the overall effect analysis of the RANOVA (independent of time). Individual means have been slightly offset from the year to avoid overlapping error bars.

(Fig. 3B). However, analysis of similarity indices within treatments as compared to the entire dataset revealed no significant trends. There was a strong positive relationship between fungal DGGE clustering based on PCA and individual *S. jacobaea* aboveground biomass for the first PCA axis ($F_{1,13} = 18.86$, $P < 0.001$, $R^2 = 0.59$), indicating that *S. jacobaea* biomass is related to belowground fungal community composition. No significant relationships were found with respect to the bacterial DGGE analysis.

Nematodes

Significantly more omnicarnivorous nematodes were found in plots sown with 15 species than in plots sown

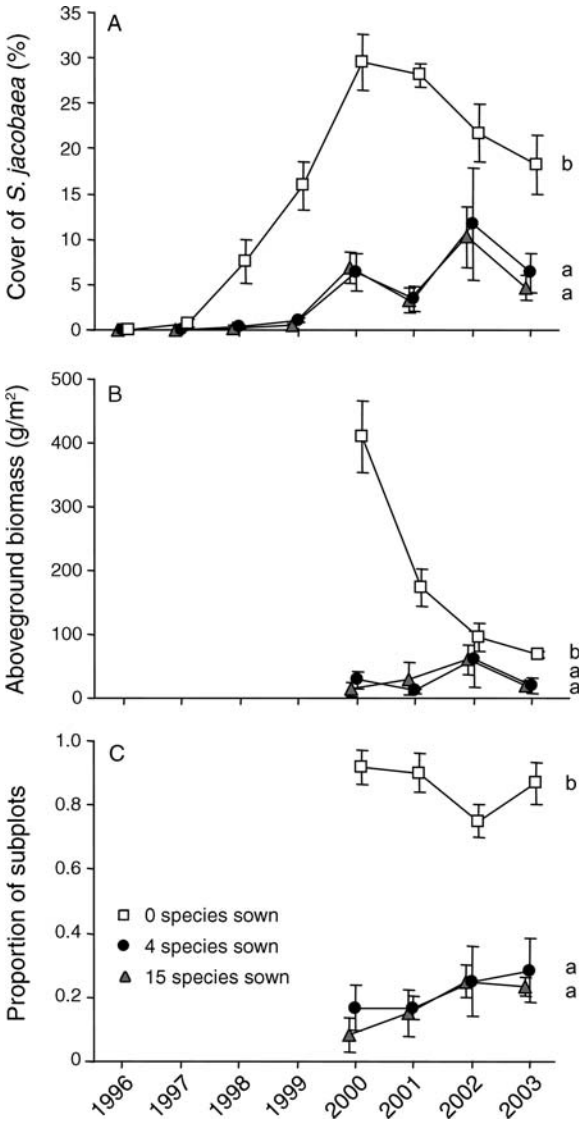

FIG. 2. Mean (±SE; $n = 5$) percent cover and aboveground biomass of *Senecio jacobaea*, and proportion of subplots (12 per plot) with *S. jacobaea* biomass. Letters to the right of each line indicate significant differences, as in Fig. 1.

with 4 species or unsown plots ($F_{2,8} = 5.66$, $P = 0.03$). A similar but nonsignificant trend was found for plant-feeding nematodes ($F_{2,8} = 3.34$, $P = 0.09$). *Paratylenchus* sp. was the most dominant plant-feeding nematode, and this taxon was, on average, twice as abundant in plots sown with 15 species as in plots sown with 4 species or unsown plots. The abundance of bacterial- and fungal-feeding nematodes did not differ between treatments.

Soil bioassay

In the greenhouse experiment, plants growing in soil collected from unsown plots had significantly less biomass than plants growing in soil collected from plots sown with 15 or 4 species (Fig. 4), resulting in a significant sowing treatment effect ($F_{2,44} = 30.17$, $P < 0.001$). Total biomass per pot was significantly larger when three plants were growing together than for one plant in a pot ($F_{1,44} = 22.17$, $P < 0.001$), but this difference was not large, and there was no interaction between plant density in the pots and soil origin ($F_{2,44} = 2.55$, $P = 0.09$; Appendix C). There was no significant difference in biomass between randomly collected soil samples from the experimental plots and soil samples collected from the rhizosphere of *S. jacobaea* in the experimental plots ($F_{1,44} = 0.04$, $P = 0.83$; Fig. 4, Appendix C).

Aboveground insect herbivory

Two species of flower feeders were found: *Pegohylemyia seneciella* (Diptera: Anthomyiidae) and larvae of an unidentified lepidopteran. In August 2002, flower feeders were present in the majority of *S. jacobaea* plants in all treatments, but the proportion of plants with flower feeders tended to be higher in sown plots (15 and 4 species) than in unsown plots (Fig. 5A); however, this was not significant ($F_{2,8} = 3.26$, $P = 0.09$; Appendix D). Leaves were mined by *Lyriomya synchenesiae* (Diptera: Agromyzidae), and significantly fewer plants had leaf miner damage in unsown plots than in sown plots ($F_{2,8} = 6.65$, $P = 0.02$). Stem borer larvae were not identified because adults failed to emerge, but significantly fewer plants with stem borers were found in unsown plots than in sown plots ($F_{2,8} = 12.11$, $P = 0.004$; Appendix D, Fig. 5A). For damaged plants, leaf miner and stem borer

TABLE 1. Plant characteristics (mean ± SE) for individual *Senecio jacobaea* plants growing in plots with different sowing treatments ($n = 5$), and results of ANOVA.

Measurement	No. species sown			Sowing treatment		Block	
	15	4	0	$F_{2,8}$	P	$F_{4,8}$	P
Plant height (cm)	$76.7^{b} \pm 3.6$	$68.1^{b} \pm 7.9$	$61.3^{a} \pm 3.9$	4.40	0.05	4.25	0.04
No. leaves per plant	$21.6^{b} \pm 0.6$	$17.2^{b} \pm 1.8$	$14.2^{a} \pm 0.5$	11.67	0.004	1.26	0.36
No. flowers per plant	$80.1^{b} \pm 7.3$	$58.6^{b} \pm 13.1$	$33.5^{a} \pm 3.3$	9.52	0.007	2.13	0.17
Leaf biomass (g/plant)	$1.57^{b} \pm 0.13$	$1.20^{b} \pm 0.35$	$0.47^{a} \pm 0.07$	14.26	0.002	2.19	0.16
Reproductive biomass (g/plant)	$0.75^{b} \pm 0.02$	$0.61^{b} \pm 0.14$	$0.33^{a} \pm 0.05$	13.58	0.002	2.64	0.11
Stem biomass (g/plant)	$3.61^{b} \pm 0.35$	$2.66^{b} \pm 0.77$	$1.28^{a} \pm 0.21$	18.34	0.001	3.87	0.05
Aboveground biomass (g/plant)	$5.93^{b} \pm 0.46$	$4.47^{b} \pm 1.23$	$2.08^{a} \pm 0.34$	17.55	0.001	3.25	0.07

Note: Within rows, means followed by different letters are significantly different ($P < 0.05$) based on a Tukey's hsd for Sowing treatment.

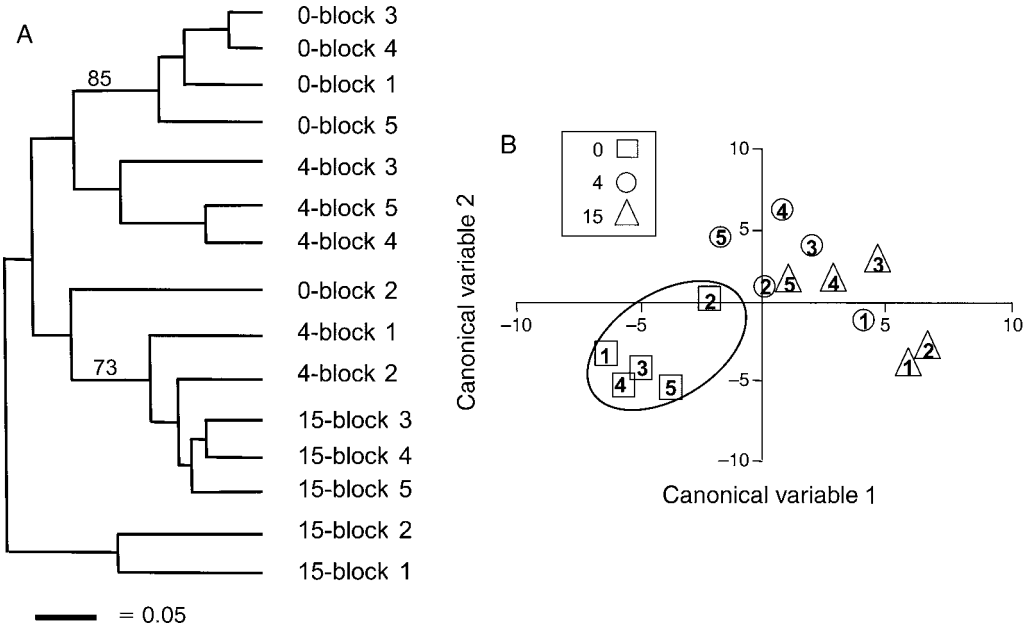

FIG. 3. (A) Dendrogram based upon unpaired group mean averages (UPGMA) analysis of fungal polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) patterns recovered from plots with different sowing treatments. Bootstrap analyses (100 replicates) are only shown for clusters with >70% support that contain more than two samples. (B) Principal component analysis of fungal PCR–DGGE banding patterns. The numbers indicate field blocks.

densities did not differ between sowing levels (Fig. 5B), but for plants with flower feeders, the proportion of damaged flowers was significantly lower in unsown plots than in sown plots (either 15 or 4 species) ($F_{2,8} = 22.02$, $P < 0.001$; Appendix D, Fig. 5B). Nitrogen levels in flowers were significantly higher in plants in plots sown with 15 species than in unsown plots. However, nitrogen levels in stem and leaf material did not differ significantly between sowing treatments (Appendix E). There was a strong positive relationship between average plant height per plot and the percentage of plants with herbivory (flower feeders, $F_{1,13} = 9.79$, $P = 0.007$, $R^2 = 0.43$; leaf miners, $F_{1,13} = 12.10$, $P = 0.004$, $R^2 = 0.48$; stem borers, $F_{1,13} = 7.13$, $P = 0.02$, $R^2 = 0.35$), while the relationship between plant nitrogen and herbivory was only significant for flower feeders ($F_{1,13} = 6.59$, $P = 0.02$,

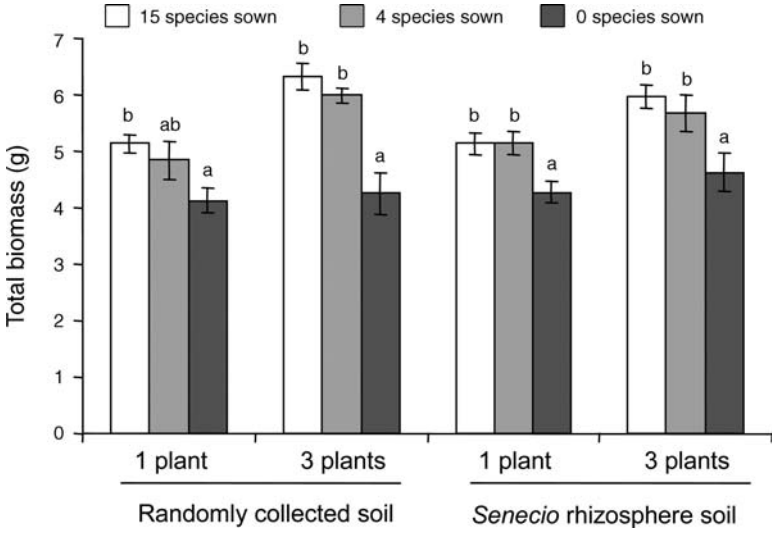

FIG. 4. Mean (±SE; $n = 5$) total biomass of one or three *S. jacobaea* plants grown in sterilized soil mixed (6:1) with soil from plots sown with 15, 4, or 0 species. Within each plot, soil samples were either taken randomly or from the rhizosphere of *S. jacobaea* plants. Within each soil-origin/plant-density combination, bars with different letters are significantly different ($P < 0.05$) based on a Tukey's hsd test.

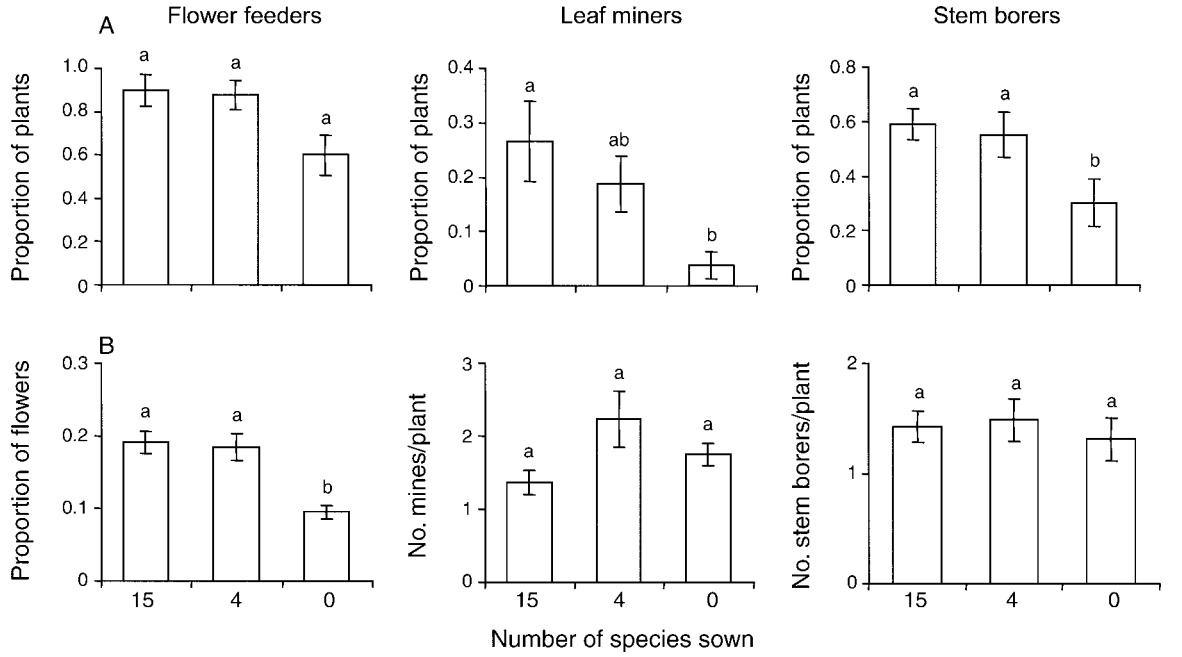

FIG. 5. Mean herbivory (±SE; $n = 5$) on *S. jacobaea* plants growing in plots with different sowing treatments (15, 4, or 0 species). For each type of herbivore, histograms show (A) the proportion of plants in each plot with herbivory, independent of the amount of herbivory per plant, and (B) the level of herbivory for damaged plants. Herbivory for flower feeders was calculated as the proportion of flowers with presence of flower feeders based on 25 flowers per plant. Within each panel, bars with different letters are significantly different ($P < 0.05$) based on a Tukey's hsd test.

$R^2 = 0.34$). When plant size (aboveground biomass) was included as a covariable in the ANOVA, the effect of sowing treatment was no longer significant (flower feeders, $F_{2,7} = 3.75$, $P = 0.08$; leaf miners, $F_{2,7} = 0.36$, $P = 0.71$; stem borers, $F_{2,7} = 0.78$, $P = 0.49$).

DISCUSSION

Sowing midsuccessional plant species on ex-arable land initially inhibits the establishment of early successional plant species (Van der Putten et al. 2000). Our study shows that this inhibition can continue for at least eight years. Although weed suppression by sown species is a well-known phenomenon (e.g., Turnbull et al. 2000, Mouquet et al. 2004, Gross et al. 2005), we are not aware of any other study showing that the effects of sowing remain for such a long period. Moreover, we posit that besides direct competitive interactions between plants, the interactions with aboveground and belowground biota may also be able to influence the temporal patterns of abundance of colonizing plants. Our results thus support the hypothesis that the initial assemblage of a plant community is crucial in determining the composition of a plant community in the longer term, but they also point at more complex trophic interactions driving the observed patterns in plant community development. The importance of the initial plant assemblage was originally proposed by Gleason (1917), who argued that functional characteristics of plants within communities, rather than diversity per se, are most important in driving plant community composition, influencing community assemblage, and by association, invasibility. Although most plant succession and plant community assemblage studies have paid relatively little attention to interactions between plants and aboveground and belowground biota, we show for *S. jacobaea* that the long-term development of plants within the vegetation can be profoundly influenced by such interactions and that these interactions have strong temporal dynamics.

The diversity of the seed mixture that was sown also influenced longer-term community characteristics such as species richness, productivity, and legume abundance. Interestingly, abundance of *S. jacobaea* in plots that were initially sown with 15 or 4 species did not differ, and was far lower than in unsown plots in spite of the high plant species diversity of the latter. This is in contrast with a large number of studies that have shown, both in microcosms and in the field, that plant diversity increases resistance to colonization (Knops et al. 1999, Naeem et al. 2000, Van Ruijven et al. 2003; but see Levine and D'Antonio 1999). As mentioned by Meiners et al. (2004), many of these earlier studies were performed over a short duration of time and used assembled, unnatural communities from which all species that were not sown or planted were weeded out continuously (e.g., Tilman et al. 1996, Van Ruijven et al. 2003). It is likely that in our self-assembled communities, vegetation characteristics such as openness and grass

abundance have influenced the ability of *S. jacobaea* to successfully invade certain plots but not others (see also Crawley et al. 1999, Van der Putten et al. 2000). Like many other "fugitive" and ephemeral plant species, *S. jacobaea*, requires gaps in the vegetation for successful germination and establishment (Harper and Wood 1957), and the relative species-diverse unsown plant communities had lower biomass and higher vegetation openness than plant communities in sown plots. Probably high species diversity will only suppress *S. jacobaea* when accompanied by high productivity.

While competition may have influenced initial establishment of *S. jacobaea*, the "boom and bust" development of its abundance over eight years in the nonsown plots could not be easily explained from differences in vegetation openness. Our study suggests that soil organisms, in particular soil fungi, determined the success of *S. jacobaea*. As far as we are aware, this study is the first to show that the performance of *S. jacobaea* in its native habitat may be negatively influenced by soil organisms. In our study, soil collected from different plots was inoculated into sterile bulk soil, which excludes the possibility that abiotic effects (notably nutrient supply) were responsible for the observed effects (Troelstra et al. 2001). Since *S. jacobaea* plant biomass increased dramatically over the first four years of the experiment in unsown plots but declined thereafter, this species may be influenced by a negative plant–soil feedback ultimately influenced by initial plant sowing. Negative feedbacks between plant and soil communities develop when soil pathogens increase rapidly in the presence of their host, ultimately causing conditions that are unfavorable for subsequent growth or local recruitment (Bever et al. 1997). The strong initial increase of *S. jacobaea* biomass in unsown plots may have amplified the density of a selection of pathogenic soil organisms. If this were the case, however, we would have expected a greater effect on *S. jacobaea* growing in inoculums from rhizosphere samples than in inoculum from randomly collected soil, and we did not observe this. Alternatively, it is possible that soil pathogens have become widely spread, or that once developed they were quite persistent. The persistence of soil pathogens in the absence of their host plant has been proposed to influence the pattern of succession or oscillations in plant abundance (Van der Putten 2003).

Recently, there has been a growing awareness that soil pathogens can influence spatiotemporal patterns in natural vegetation (Van der Putten 2003), and that invasive plant species in particular may benefit in the introduced area from escape from pathogens that control the plant in its native area but that are not present in the introduced area (Klironomos 2002, Reinhart et al. 2003). Our results allow us to speculate that *S. jacobaea* in its invasive range, where it performs well (e.g., North America, Australia), may have escaped from its native soil pathogens. However, subsequent studies are needed to test this hypothesis.

Insect damage was lower on (smaller) plants growing in unsown plots. This suggests the presence of an indirect aboveground–belowground interaction (Bezemer and Van Dam 2005). Plant openness in unsown plots has led to high abundance of *S. jacobaea*, which may have triggered the density-dependent development of soil pathogens that subsequently reduced plant size, finally leading to reduced aboveground insect damage. Whether or not this is true remains to be studied by experimentally manipulating *S. jacobaea* densities in sowing plots. Nevertheless, it appears that aboveground insect herbivores preferentially attacked bigger, and presumably healthier, plants. *S. jacobaea* dominated the area surrounding the experimental site, and host plants were thus abundantly available. When plant size was included in the analyses as a covariable, the difference in herbivory between sowing treatments was no longer significant. This also suggests that plant size was responsible for the differences in herbivory and not another factor that differed between sowing treatments, such as the availability of alternative food sources (e.g., nectar) for adult herbivores or predators (Siemann et al. 1998). While the overall response to the sowing treatments was similar for the three insect-feeding guilds, there were also differences between guilds. Our data suggest that plant size appears to be the major factor determining host suitability for leaf miners and stem borers, but that in addition to plant size, flower quality appears to be also important for flower feeders, indicated by the positive relationship between flower nitrogen level and flower damage. We do not know whether larger flowers within a plant were preferentially attacked, but other studies indicate that this is typically the case (e.g., Strauss 1997, Leege and Wolfe 2002).

While the most straightforward explanation for reduced herbivory on *S. jacobaea* in unsown plots appears to be reduced plant quantity, we cannot exclude the possibility that plant quality also differed among treatments. Plant nitrogen levels did not differ greatly, and there was no significant relationship between nitrogen levels and herbivory, but it is possible that there were differences in aboveground plant secondary chemistry. *S. jacobaea* produces alkaloids that are known to act as feeding deterrents for many insect herbivores (McEvoy et al. 1993), and root damage in *S. jacobaea* has been shown to result in increases in foliar alkaloid content (Hol et al. 2004).

In conclusion, few studies have examined the interactions between plant competition, soil organisms, and aboveground insects for a plant species in its natural environment. We show that for *S. jacobaea*, abundance appears to be initially controlled by characteristics of the vegetation, but that the pattern of abundance may also be influenced by the soil community. Molecular analysis of the soil community suggests that, in particular, the fungal community might be involved in this effect. The

aboveground invertebrate community appears to be associated with differences in plant size rather than plant abundance, as small plants had less damage by aboveground invertebrate herbivores than large plants. We suggest that these above- and belowground loops demand more consideration when attempting to explain the dynamics of plant communities.

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Literature Cited

Beckstead, J., and I. M. Parker. 2003. Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? Ecology **84**:2824–2831.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561–573.

Bezemer, T. M., G. B. De Deyn, T. M. Bossinga, N. M. van Dam, J. A. Harvey, and W. H. van der Putten. 2005. Soil community composition drives aboveground plant-herbivore-parasitoid interactions. Ecology Letters **8**:652–661.

Bezemer, T. M., O. Graça, P. Rousseau, and W. H. van der Putten. 2004. Above- and below-ground trophic interactions on creeping thistle (*Cirsium arvense*) in high and low diversity plant communities: potential for biotic resistance? Plant Biology **6**:231–238.

Bezemer, T. M., and N. M. Van Dam. 2005. Linking aboveground and belowground multitrophic interactions via induced plant defenses. Trends in Ecology and Evolution **20**:617–624.

Bonsall, M. B., E. Van der Meijden, and M. J. Crawley. 2003. Contrasting dynamics in the same plant-herbivore interaction. Proceedings of the National Academy of Sciences of the United States of America **100**:14932–14936.

Crawley, M. J. 1983. Herbivory: the dynamics of animal-plant interactions. Blackwell, Oxford, UK.

Crawley, M. J., S. L. Brown, M. S. Heard, and G. R. Edwards. 1999. Invasion-resistance in experimental grassland communities: species richness or species identity? Ecology Letters **2**: 140–148.

Crawley, M. J., and M. P. Gillman. 1989. Population dynamics of cinnabar moth and ragwort in grassland. Journal of Animal Ecology **58**:1035–1050.

De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos **106**:576–586.

De Deyn, G. B., C. E. Raaijmakers, R. H. Zoomer, M. P. Berg, P. C. De Ruiter, H. A. Verhoef, T. M. Bezemer, and W. H. van der Putten. 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature **422**:711–713.

Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen, London, UK.

Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementary effects. Ecology Letters **8**:604–611.

Gleason, H. A. 1917. The structure and development of the plant association. Bulletin of the Torrey Botanical Club **53**:7–26.

Gross, K. L., G. G. Mittelbach, and H. L. Reynolds. 2005. Grassland invasibility and diversity: responses to nutrients, seed input, and disturbance. Ecology **86**:476–486.

Hambäck, P. A., and A. P. Beckerman. 2003. Herbivory and plant resource competition: a review of two interacting interactions. Oikos **101**:26–37.

Harper, J. L., and W. A. Wood. 1957. *Senecio jacobaea* L in biological flora of the British Isles. Journal of Ecology **45**: 617–637.

Harrison, S., and C. D. Thomas. 1991. Patchiness and spatial pattern in the insect community on ragwort *Senecio jacobaea*. Oikos **62**:5–12.

Heuer, H., M. Krsek, P. Baker, K. Smalla, and E. M. H. Wellington. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Applied and Environmental Microbiology **63**:3233–3241.

Hol, W. H. G., M. Macel, J. A. van Veen, and E. van der Meijden. 2004. Root damage and aboveground herbivory change concentration and composition of pyrrolizidine alkaloids of *Senecio jacobaea*. Basic and Applied Ecology **5**: 253–260.

Hol, W. H. G., and J. A. Van Veen. 2002. Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. Journal of Chemical Ecology **28**:1763–1772.

Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia **110**:449–460.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. Nature **417**:636–638.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature **417**:67–70.

Knevel, I. C., T. Lans, F. B. J. Menting, U. M. Hertling, and W. H. van der Putten. 2004. The role of the enemy release hypothesis and the biotic resistance hypothesis in the establishment of the alien *Ammophila arenaria* in South Africa. Oecologia **141**:502–510.

Knops, J. M. H., D. Tilman, N. M. Haddad, S. Naeem, C. E. Mitchell, J. Haarstad, M. E. Ritchie, K. M. Howe, P. B. Reich, E. Siemann, and J. Groth. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters **2**:286–293.

Koricheva, J., C. P. H. Mulder, B. Schmid, J. Joshi, and K. Huss-Danell. 2000. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. Oecologia **125**:271–282.

Kunin, W. E. 1999. Patterns of herbivore incidence on experimental arrays and field populations of ragwort, *Senecio jacobaea*. Oikos **84**:515–525.

Leege, L. M., and L. M. Wolfe. 2002. Do floral herbivores respond to variation in flower characteristics in *Gelsemium sempervirens* (Loganiaceae), a distylous vine? American Botanist **89**:1270–1274.

Lepš, J., et al. 2001. Separating the chance effect from other diversity effects in the functioning of plant communities. Oikos **92**:123–134.

Levine, J. M., and C. M. D'Antonio. 1999. Elton revisited: a review of evidence linking diversity and invasibility. Oikos **87**:15–26.

Louda, S. M., and M. A. Potvin. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. Ecology **76**:229–245.

Maron, J. L., and M. Vilà. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypothesis. Oikos **95**:361–373.

McEvoy, P. B., and E. M. Coombs. 1999. Biological control plant invaders: regional patterns, field experiments, and structured population models. Ecological Applications **9**: 387–401.

McEvoy, P. B., N. T. Rudd, C. S. Cox, and M. Huso. 1993. Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. Ecological Monographs **63**: 55–75.

Meiners, S. J., M. L. Cadenasso, and S. T. A. Pickett. 2004. Beyond biodiversity: individualistic controls of invasion in a self-assembled community. Ecology Letters **7**:121–126.

Mouquet, N., P. Leadley, J. Meriguet, and M. Loreau. 2004. Immigration and local competition in herbaceous plant communities: a three-year seed-sowing experiment. Oikos **104**:77–90.

Naeem, S., J. M. H. Knops, D. Tilman, K. M. Howe, T. Kennedy, and S. Gale. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. Oikos **91**:97–108.

Novozamsky, I., V. J. G. Houba, E. Temminghof, and J. J. van der Lee. 1984. Determination of 'total' N and 'total' P in a single soil digest. Netherlands Journal of Agricultural Science **32**:322–324.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85–102 *in* J. N. Sasser and W. R. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill, North Carolina, USA.

Reinhart, K. O., A. Packer, W. H. van der Putten, and K. Clay. 2003. Plant-soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. Ecology Letters **6**:1046–1050.

Rudgers, J. A., and J. D. Hoeksema. 2003. Inter-annual variation in above- and belowground herbivory on a native, annual legume. Plant Ecology **169**:105–120.

Siemann, E., D. Tilman, J. Haarstad J, and M. Ritchie. 1998. Experimental tests of the dependence of arthropod diversity on plant diversity. American Naturalist **152**:738–750.

Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. Ecology **78**:1640–1645.

Ter Braak, C. J. F., and P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination. Version 4.5. Microcomputer Power, Ithaca, New York, USA.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology **78**:81–92.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718–720.

Troelstra, S. R., R. Wagenaar, W. Smant, and B. A. M. Peters. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. New Phytologist **150**:697–706.

Turnbull, L. A., M. J. Crawley, and M. Rees. 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos **88**:225–238.

Vainio, E. J., and J. Hantula. 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycological Research **104**: 927–936.

Van der Meijden, E., C. A. M. van Wijk, and R. E. Kooi. 1991. Population dynamics of the cinnabar moth (*Tyria jacobaea*): oscillations due to food limitation and local extinction risks. Netherlands Journal of Zoology **41**:158–173.

Van der Putten, W. H. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. Ecology **84**: 2269–2280.

Van der Putten, W. H., et al. 2000. Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. Oecologia **124**:91–99.

Van der Putten, W. H., C. van Dijk, and B. A. M. Peters. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature **362**:53–56.

Van Ruijven, J., G. B. De Deyn, and F. Berendse. 2003. Diversity reduces invasibility in experimental plant communities: the role of plant species. Ecology Letters **6**:910–918.

Vitousek, P. M. 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. Oikos **57**:7–13.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**: 1629–1633.

Wardle, D. A., K. S. Nicholson, and A. Rahman. 1995. Ecological effects of the invasive weed species *Senecio jacobaea* L. (ragwort) in New Zealand pasture. Agriculture, Ecosystems and Environment **56**:19–28.

APPENDIX A

Results of repeated-measures ANOVAs for vegetation properties and performance of *Senecio jacobaea* (*Ecological Archives* E087-125-A1).

APPENDIX B

Dendrogram and canonical variance analysis of bacterial PCR–DGGE patterns (*Ecological Archives* E087-125-A2).

APPENDIX C

Results of an ANOVA test for the bioassay experiment (*Ecological Archives* E087-125-A3).

APPENDIX D

Results of an ANOVA test for herbivore occurrence and density on *Senecio jacobaea* (*Ecological Archives* E087-125-A4).

APPENDIX E

Nitrogen contents of flower, leaf, and stem tissues of *Senecio jacobaea* (*Ecological Archives* E087-125-A5).