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Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags

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We examined the relationship between plant species diversity, productivity and the development of the soil community during early secondary succession on former arable land across Europe. We tested the hypothesis that increasing the initial plant species diversity enhances the biomass production and consequently stimulates soil microbial biomass and abundance of soil invertebrates. We performed five identical field experiments on abandoned arable land in five European countries (CZ, NL, SE, SP and UK) which allowed us to test our hypothesis in a range of climate, soil and other environmental factors that varied between the experimental sites. The initial plant diversity was altered by sowing seed mixtures of mid-successional grassland species with two or five grass species, one or five legumes and one or five forbs. The results of low and high sown diversity treatments were compared with plots that were naturally colonized by species present in the seed bank. In three out of the five field sites, there was no correlation between plant species number and plant biomass production, one site had a positive and the other a negative relation. Treatments with a high diversity seed mixture had a higher biomass than the naturally colonized plots. However, there was no significant difference between high and low sown diversity plots at four out of five sites. The three-year study did not give any evidence of a general bottom-up effect from increased plant biomass on biomass of bacteria, saprophytic fungi or abundance of microarthropods. The biomass of arbuscular mycorrhizal was negatively related to plant biomass. The abundance of nematodes increased after abandonment and was related to plant biomass at four sites. Our results support the hypothesis that plant species diversity may have idiosyncratic effects on soil communities, even though studies on a longer term could reveal time lags in the response to changes in composition and biomass production of plant communities.

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It has been suggested that changes in plant species diversity affect several ecosystem processes, such as primary productivity, nutrient retention, and vegetation dynamics (Naeem et al. 1996, Tilman et al. 1996, Hector et al. 1999, Schläpfer and Schmid 1999, Tilman 1999, Chapin et al. 2000, Loreau et al. 2001). A positive impact of species diversity on plant productivity has been explained by the complementarity of resource use among plant species or their functional groups (Diaz and Cabido 2001, Dukes 2001, Loreau and Hector 2001, Tilman et al. 2001). Alternatively, it has been argued that an increase in plant diversity will increase the probability of including highly productive species into the plant community (Huston 1997, Lepš et al. 2001).

While the diversity-productivity debate has been focused on plants mainly, little attention has been given to possible consequences of plant species diversity for soil communities under field conditions (Wardle et al. 1999a, Wardle 2002). While the effects of plant diversity on conversion and retention of energy and nutrients in soil is due to soil organisms mainly (Wardle et al. 1997, Hooper et al. 2000, Knops et al. 2001), the response of the different trophic levels in the soil community to plant diversity and productivity is rather inconclusive. The microbial community shows either a positive relation (Wardle and Nicholson 1996, Wardle and Lavelle 1997, Bardgett and Shine 1999, Bardgett et al. 1999, Wardle et al. 1999b, Broughton and Gross 2000, Donnison et al. 2000) or no response (Wardle et al. 1997, 1999a) to diversity and productivity of the plant community.

It is an even more complex task to predict the effects of the plant community on higher trophic levels of a soil community, as interactions between trophic levels can be controlled not only by bottom-up but also by top-down interactions (Schaefer 1993, De Ruiter et al. 1995, Mikola and Setälä 1998b). Most studies on the relationship between plant diversity and soil animal abundances seem rather inconclusive or with no direct correlations (Yeates 1987, Wardle et al. 1999a). Although, it has sometimes been suggested that higher trophic levels are more strongly affected by plant diversity changes than lower trophic levels such as microorganisms (Spehn et al. 2000, Mikola et al. 2001).

In the debate of threats to biological diversity and ecosystem functioning, land use change has been identified as one of the most immediate causes (Vitousek et al. 1997, Sala et al. 2000). Diversity losses in plant communities can limit plant recruitment and decrease plant productivity, which will pose transient effects on the ecosystem functioning (Symstad and Tilman 2001). There is a growing awareness of how agricultural practices decrease diversity, not only of plants but also of soil microorganisms (Brussard et al. 1996, Helgason et al. 1998, Read 1998). Today, substantial effort is made to restore diversity of former arable land. Indeed, management modifying the initial plant community increases the rate of transfer towards more natural grasslands or forest communities (Hansson and Fogelfors 1998, Van der Putten et al. 2000). Different management regimes of grasslands are known to alter the biomass of soil fauna and to change the composition of microbial communities (Bardgett et al. 1996, 2001, Bardgett and Cook 1998, Donnison et al. 2000, Hedlund 2002). However, effects of restoring diversity of plant communities on the composition of soil communities have received far less attention.

In the European CLUE project (Changing land usage: enhancement of biodiversity and ecosystem development) we investigated how the rate and direction of secondary succession on former arable land may be affected by changing the diversity and species composition of the initial plant community. We examined these effects in identical field experiments carried out in five different European countries, covering a range of site-specific and climate conditions (Van der Putten et al. 2000). The plant communities were experimentally changed by sowing high (15 species) and low (4 species) diversity seed mixtures of mid-successional grassland communities. In each country plant species were used that occurred naturally in mid-successional target communities. For the low diversity mixture replicates we used different subsets of the total species pool to avoid samping errors (Huston 1997). This multi-site approach differs from studies by Tilman et al. (1996) and Hector et al. (1999), as the soil was not sterilised or removed prior to sowing, and it was not weeded in order to control plant diversity throughout the experiment.

We tested the hypothesis that an increase in the initial plant species diversity at the start of secondary succession enhances the amount of biomass produced and consequently stimulates the soil microbial biomass and the abundance of soil invertebrates.

Material and methods

Field experiment

In five European countries, an identical field experiment was carried out on abandoned arable land with sown low and high plant diversity treatments and natural colonization. The field sites were installed in Sweden (SE), United Kingdom (UK), the Netherlands (NL), Spain (SP), and Czech Republic (CZ). The basic characteristics of the different experimental locations are given in Table 1. Experimental plots were installed in April–May 1996 on agricultural land on which agricultural practices had ceased at the end of 1995. The experiment was organised according to a block design with five replicate blocks. The three treatments, NC-natural colonization, LD-low diversity (4 species sown) and HD-high diversity (15 species sown), were ran-

Table 1. Characteristics of experimental sites in five European countries.

Country Abbreviation	Czech Republic CZ	The Netherlands NL	Sweden SE	Spain SP	United Kingdom UK
Site	Benesov	Mossel	Trolleholm	Muñovela	Bradenham
Co-ordinates	49.20N 15.00E	52.04N 13.15E	55.45N 13.15E	40.54N 5.45W	51.40N 0.48W
Altitude (m a.s.l.)	659	30	85	840	140
Mean temperature (°C)	6.4	9.4	7.5	10.8	9.6
Warmest month	July (16.4)	July (22.1)	July (17.1)	August (29.9)	July (16.5)
Coldest month	January (−2.7)	January (4.3)	January (−0.9)	January (0.8)	January (3.6)
Average rainfall (mm $year^{-1}$)	680	840	700	500	750
Wettest month (mm yr^{-1})	July (78)	August (130)	July (66)	November (99)	October (65)
Driest month (mm yr^{-1})	February (36)	February (75)	February (33)	June (17)	February (41)
Last crop (1995)	*Hordeum vulgare*	*Zea mays*	*Lolium perenne*	*Medicago sativa*	*Hordeum vulgare*
Other crops	*Solanum tuberosum* *Pisum sativum*	*Solanum tuberosum* *Lolium perenne*	*Triticum aestivum* *Brassica napus*	*Triticum sativum* *Hordeum vulgare*	*Triticum aestivum* *Brassica napus* Set-aside
Surrounding vegetation	Arable land and recently established species-poor grassland	Heath, mixed forest and abandoned arable land	Deciduous forest and cultivated field	Dehesa-like woodland and cultivated field	Chalk grassland and deciduous forest
Soil type and texture	Brown soil, Loam	Brown soil, Sandy loam	Brown soil, Clayey till	Chromic luvisol, Loamy clay	Brown rendzina, Loam
PH KCl	5.0	5.8	5.8	7.3	7.6
% $CaCO_3$	0.11	0.15	0.11	0.07	73.8
% Organic matter	4.9	4.5	5.8	2.1	5.9
EC (μS m^{-1})	146.0	7.3	7.1	194.0	18.0
P-Olsen	24.3	110.0	32.0	24.6	22.8
Grain size distribution (μm)					
<2	14.0	3.4	15.6	20.2	15.0
>63 >2	29.0	13.9	45.0	18.5	44.7
>63	60.0	79.7	34.2	61.3	13.0

domly allocated to the plots in each block. Each block consisted of three treatment plots of 10×10 m and three plots of 2×2 m with identical seed mixtures.

Fifteen plant species (five grasses, five legume and five other forb species) were sown as the high diversity treatment. To avoid confounding the species identity effect with the diversity effect (Huston 1997) each low diversity treatment was sown with a different sub-set of the 15 species used in the high diversity treatment. The low diversity subsets contained a random selection of two grasses, one legume and one forb species. Each forb and legume species was sown in a low diversity plot of one block, while each grass species was sown in two blocks. Naturally colonized plots were treated similarly to the others but not sown. The low and high diversity mixtures consisted of the same total amounts of seed (grasses: 2500 seeds m^{-2}, legumes and forbs: 500 seeds m^{-2}). Since the five study sites differed in climate, soil type, and cropping history, it was not possible to work with the same pool of plant species. Therefore, the plant mixtures used at each site consisted of species typical of mid-successional stages of grassland succession to be expected at each site following land abandonment (Table 2). For further details see Van der Putten et al. (2000)

Vegetation analyses

Every year at peak standing biomass (May/June in Spain and July/August in the other countries), the numbers of plant species in each 10×10 m plot were determined in 12 $1m^2$ sub-plots. Plant above-ground biomass was clipped at 5 cm above the soil surface and harvested in a 25×25 cm area adjacent to each $1m^2$ plot. Additionally in CZ, UK and SP, the standing biomass was sorted into grasses, legumes, and other forbs. All samples were dried to constant weight at 80°C and weighed. Clipping was done in different sub-plots each year.

In 1996 and 1997 root biomass was determined by collecting soil cores of 5 cm diameter and 15 cm depth. In each treatment replicate of 10×10 m, four of the 25×25 cm sub-plots were randomly chosen for this purpose. The cores were collected from the centres of the sub-plots. The soil cores were collected immediately

Table 2. Plant species used for the high diversity sown treatments.

Group	Czech Republic	the Netherlands	Sweden	Spain	United Kingdom
Grasses	*Festuca rubra*	*Festuca rubra*	*Festuca rubra*	*Festuca rubra*	*Festuca rubra*
	Phleum pratense	*Phleum pratense*	*Phleum pratense*	*Phleum pratense*	*Phleum pratense*
	Cynosurus cristatus	*Poa pratensis*	*Cynosurus cristatus*	*Poa pratensis*	*Cynosurus cristatus*
	Holcus lanatus	*Agrostis capillaris*	*Agrostis capillaris*	*Poa trivialis*	*Holcus lanatus*
	Triseum flavescens	*Anthoxanthum odoratum*	*Anthoxanthum odoratum*	*Bromus inermis*	*Trisetum flavescens*
Other forbs	*Plantago lanceolata*	*Plantago lanceolata*	*Plantago lanceolata*	*Plantago lanceolata*	*Plantago lanceolata*
	Galium verum	*Tanacetum vulgare*	*Galium verum*	*Galium verum*	*Galium verum*
	Prunella vulgaris	*Linaria vulgaris*	*Prunella vulgaris*	*Sanguisorba minor*	*Sanguisorba minor*
	Centaurea jacea	*Hypochaeris radicata*	*Campanula rotundifolia*	*Achillea millefolium*	*Centaurea nigra*
	Lychnis floscuculi	*Hypericum perforatum*	*Leontodon hispidus*	*Matricaria chamomilla*	*Leontodon hispidus*
Legumes	*Lotus corniculatus*	*Lotus corniculatus*	*Lotus corniculatus*	*Lotus corniculatus*	*Lotus corniculatus*
	Trifolium pratense	*Trifolium pratense*	*Trifolium pratense*	*Trifolium pratense*	*Trifolium pratense*
	Medicago lupulina	*Trifolium arvense*	*Medicago lupulina*	*Medicago lupulina*	*Medicago lupulina*
	Trifolium dubium	*Trifolium dubium*	*Anthyllis vulneraria*	*Trifolium subterraneum*	*Trifolium dubium*
	Lathyrus pratensis	*Vicia cracca*	*Trifolium repens*	*Trifolium fragiferum*	*Anthyllis vulneraria*

after the clipping of the above-ground biomass and stored in a cold room at 5°C until further processing. The roots were separated from the soil by washing with tap water, dried at 45°C for 48 h and weighed.

Microbial biomass

Fatty acid analyses were made on soil samples from HD and NC plots of the Swedish site. From each experimental plot, 3 soil samples of about 5 g of soil were taken. Lipids were extracted according to the method developed by Frostegård et al. (1993). The resulting fatty acid methyl esters were separated on a Hewlett Packard 6890 gas chromatograph. Among 22 identified PLFA, the following were chosen to represent bacterial biomass: i15:0, a15:0, i16:0, 16:1w9, i17:0, a17:0, cy17:0, 18:1w7, cy19:0, according to Frostegård and Bååth (1996). Seven NLFA were identified and 16:1w5 was chosen as an AM fungal marker (Olsson et al. 1995, Hedlund 2002).

Microbial biomass in the Swedish and Spanish sites was estimated using substrate induced respiration (SIR). Six soil samples were taken from each replicate plot and 6 g from each sample was put in sterile glass vials (20 ml) for measuring respiration, as CO_2 production. 15 mg glucose was added to each vial to increase the respiration of microorganisms in soil that is proportional to the microbial biomass (Anderson and Domsch 1978). The vials were gently shaken to mix glucose with soil and then purged with air for 30 s and sealed with an autoclaved rubber septum. The vials were incubated at $+20$°C for 2 h and then analysed for CO_2 with a gas chromatograph according to Hedlund and Augustsson (1995). Soil microbial biomass from the Dutch site was measured with the fumigation technique (Maly et al. 2000).

Microarthropods

Five soil samples (3 cm diameter, 5 cm depth) from the 2×2 m experimental plots were taken from the CLUE field sites in the Netherlands, Spain and Sweden in June 1996 and 1997. The samples were extracted for three days in a high-gradient extractor with 50% ethylene glycol as preservative. Numbers of collembolans and mites were determined.

Earthworms

The earthworm faunas from the experimental sites in the Netherlands, Sweden, United Kingdom and Czech Republic were assessed in July or August 1998 at the peak time of vegetation biomass. The earthworms were extracted from four squares (25×25 cm) in every 10×10 m plot. The vegetation was cleared and five l of a household detergent solution (20 ml detergent l^{-1}) were applied on each square (East and Knight 1998). All earthworms that appeared in the squares within 30 min were collected and stored in 70% ethanol. The numbers of worms and the fresh weight were determined after drying the worms for one minute on filter paper.

Nematodes

In August 1996, 1997 and 1998 soil samples were taken from the top 10 cm of the soil. Twenty four cores from

each 10×10 m plot were mixed and a sub-sample of 100 g was used to extract nematodes (Korthals et al. 2001) by a modified Oosterbrink elutriator (Oosterbrink 1960). The nematodes were heat killed and fixed in 4% formaldehyde, identified and divided into feeding groups according to Yeates et al. (1993).

Statistical analyses

The effects of the treatment on above-ground and below-ground plant biomass were compared using analyses of variance (ANOVA) according to a randomised block design, with country, treatments and years as fixed factors. The relation between plant species number and biomass production was determined by a regression analysis of plant biomass (log values) and the number of plant species of each plot. When there were significant interaction effects the data was analysed by using a posteriori multicomparison (Student-Newman-Keuls test) with the error term of the ANOVA used for standard error (Underwood 1997). Data on plant biomass of each plot were compared to microbial biomass, nematode numbers, number of soil invertebrates with a regression analyses. However, in the cases of microarthropods and microbial biomass, the plant biomass was estimated from the 10×10 m plots and the soil organisms from the corresponding 2×2 m plots.

Results

Plant species and biomass production

The responses of plant species diversity and biomass production to the treatments differed among countries and years, so that no clear effects of the treatments could be detected in a 3-way ANOVA (Table 3). However, from a multicomparison between means of each factor we detected that at all field sites, the sowing of plant species initially increased the plant species diversity in sown treatments the first year (SNK-test: Q-value > 5.3, all countries, $P < 0.001$; Fig. 1). In the following two years the number of plant species developed into different patterns depending on country. In 1998 the plant species number was lower in sown HD plots in the Netherlands ($Q = 5.9$, $P < 0.001$) and Sweden ($Q = 6.5$, $P < 0.001$), showing no responses to treatments in the UK and Spain, while the site in the Czech

Table 3. Results of 3-way ANOVA of plant biomass data from all 5 field sites.

Factor analysed	Source of variation	df	F-value	P
above-ground biomass	Country	4	55.10	<0.001
	Year	2	140.00	<0.001
	Treatment	2	38.08	<0.001
	Block	1	0.04	ns
	Country × Treatment	8	13.16	<0.001
	Treatment × Year	4	8.51	<0.001
	Country × Year	8	4.73	<0.001
	Country × Treatment × Year	16	0.85	ns
species diversity	Country	4	78.95	<0.001
	Year	2	280.87	<0.001
	Treatment	2	88.22	<0.001
	Block	1	0.69	ns
	Country × Treatment	8	52.65	<0.001
	Treatment × Year	4	36.32	<0.001
	Country × Year	8	19.37	<0.001
	Country × Treatment × Year	16	2.18	<0.01
root biomass	Country	4	16.18	<0.001
	Year	1	55.11	<0.001
	Treatment	2	8.97	<0.001
	Block	1	2.30	ns
	Country × Treatment	8	2.74	<0.01
	Treatment × Year	2	8.81	<0.001
	Country × Year	4	2.88	<0.05
	Country × Treatment × Year	8	3.23	<0.01
above- and below-ground biomass	Country	4	105.90	<0.001
	Year	1	521.97	<0.001
	Treatment	2	39.12	<0.001
	Block	1	0.16	ns
	Country × Treatment	8	4.48	<0.001
	Treatment × Year	2	27.67	<0.001
	Country × Year	4	32.92	<0.001
	Country × Treatment × Year	8	2.36	<0.05

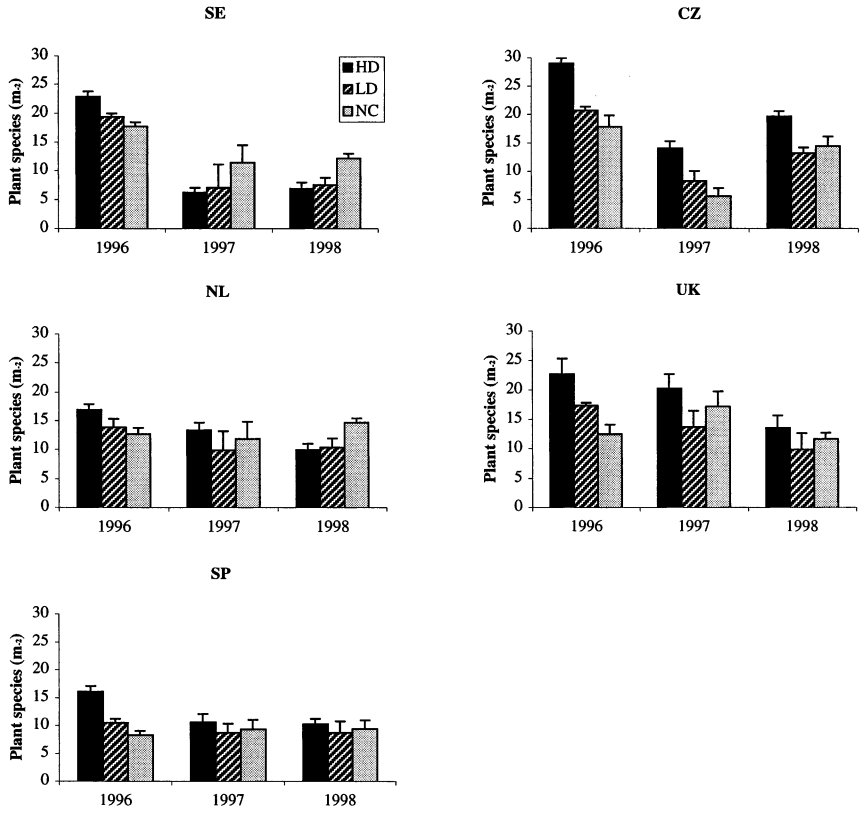


Fig. 1. Plant species number (per m^2) at the five field sites (Sweden – SE, Czech Republic – CZ, The Netherlands – NL, United Kingdom – UK and Spain – SP) of the treatments natural colonization (NC), low diversity sown (LD) and high diversity sown (HD). Error bars indicate standard deviation of mean values.

Republic ($Q = 6.5$, $P < 0.001$) had a remaining higher level of plant species number in sown HD plots compared to that of naturally colonized plots.

Sowing seed mixtures increased plant biomass in HD plots compared to that of the naturally colonized plots over all sites in 1997 and 1998 except for the Spanish site, where sowing did not result in higher plant biomass ($Q > 3.359$, all comparisons $P < 0.05$; Fig. 2, Table 3). During the three years of the experiment it was only in the Netherlands ($Q = 4.707$, $P < 0.01$) that plots with a high diversity seed mixture reached a higher biomass than those sown with a low diversity seed mixture.

During the experimental period the proportion of functional groups of plants varied mainly according to time. The proportion of grasses increased in the Czech Republic and Spain in all plots irrespective of treatment ($Q > 5.120$, $p < 0.001$; Fig. 2, Table 4). At the same time (1996–1998) the proportion of forbs decreased in both countries ($Q > 4.745$, $P < 0.001$). In the UK the proportion of grasses decreased ($Q = 4.336$, $P < 0.001$) and legumes increased ($Q = 5.005$, $P < 0.001$) but only in HD plots.

Over all sites the root biomass was not significantly affected by the sowing treatments as the biomass varied between countries and years (Table 3). However, in the Czech Republic the HD sown plots had a higher below-ground plant biomass than naturally colonized plots ($Q = 4.687$, $P < 0.001$; Fig. 2). The allocation between above- and below-ground plant biomass, estimated as a root to shoot ratio, was not affected by the sowing in any country (Table 3).

During the first year of the experiment, none of the sites showed a relation between plant biomass and plant species number (linear regression analyses of log biomass and species number values; Fig. 3). In the second year (1997) one site (CZ) had a positive relation (regression, $r^2 = 0.34$, $P < 0.05$) and one (SE) a negative relation ($r^2 = 0.66$, $P < 0.001$) between plant species number and biomass. In 1998 the Swedish site ($r^2 = 0.67$, $P < 0.001$) and the Dutch site ($r^2 = 0.42$, $P < 0.01$) had negative relationships between biomass production and plant species number. Summing up above- and below-ground plant biomass to determine total plant biomass resulted, in a comparison over all sites, in random patterns between plant species diversity and biomass production (results not shown).

Plant biomass in relation to the biomass or abundance of soil organisms

The overall microbial biomass measured as SIR (SE, SP) and with the fumigation technique (NL) did not show any reponses to the treatments in the three coun-

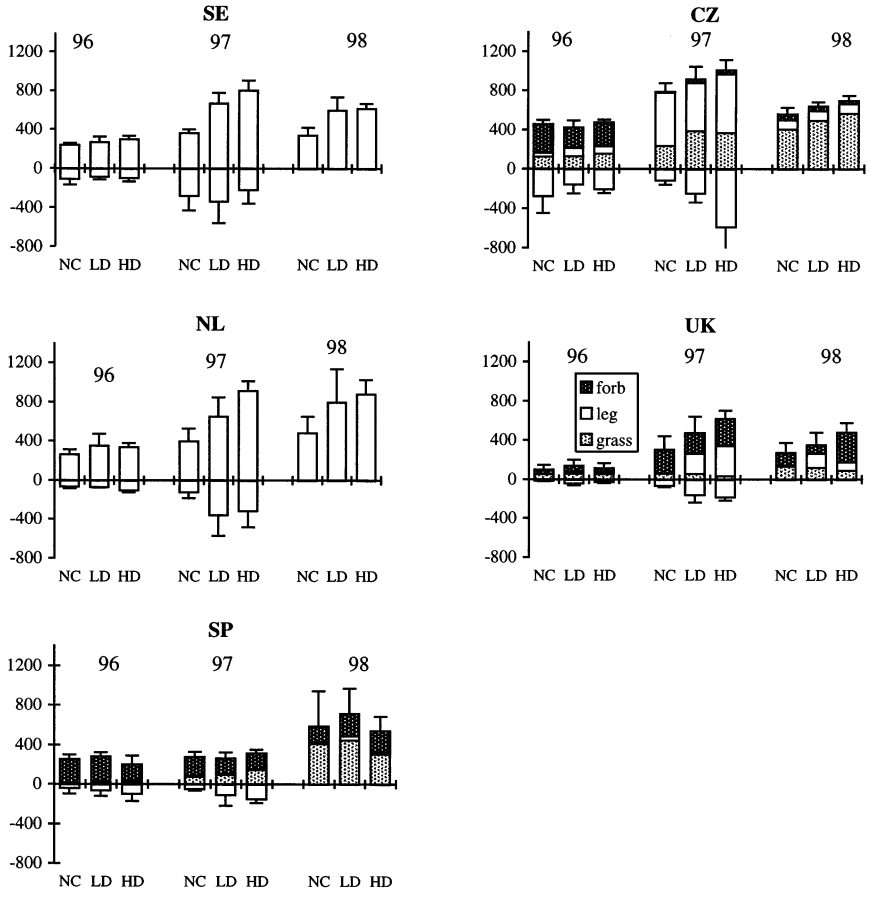


Fig. 2. Above-ground (positive values on y axis) and below-ground (negative values on y axis) biomass (g m^{-2}) at the five field sites for three years studies (1996, 1997 and 1998): Codes as in Fig. 1. In UK, CZ and SP the above-ground biomass was separated into functional groups (grasses, legumes and other forbs, see legend in Fig. 1).

Table 4. Proportions of each functional group of plant above-ground biomass tested in a 3-way ANOVA

Functional group	Factor	df	F-value	P
grasses	Country	2	29.33	<0.001
	Year	2	41.23	<0.002
	Treatment	2	0.36	ns
	Block	1	0.87	ns
	Country × Treatment	4	13.16	ns
	Treatment × Year	4	8.51	ns
	Country × Year	4	4.73	<0.001
	Country × Treatment × Year	8	0.85	ns
legumes	Country	2	46.08	<0.001
	Year	2	62.94	<0.001
	Treatment	2	1.95	ns
	Block	1	0.01	ns
	Country × Treatment	4	0.89	ns
	Treatment × Year	4	0.77	ns
	Country × Year	4	36.98	<0.001
	Country × Treatment × Year	8	0.96	ns
forbs	Country	2	254.72	<0.001
	Year	2	5.09	<0.01
	Treatment	2	1.13	ns
	Block	1	1.90	ns
	Country × Treatment	4	3.39	<0.5
	Treatment × Year	4	2.48	<0.05
	Country × Year	4	10.91	<0.001
	Country × Treatment × Year	8	2.21	<0.05

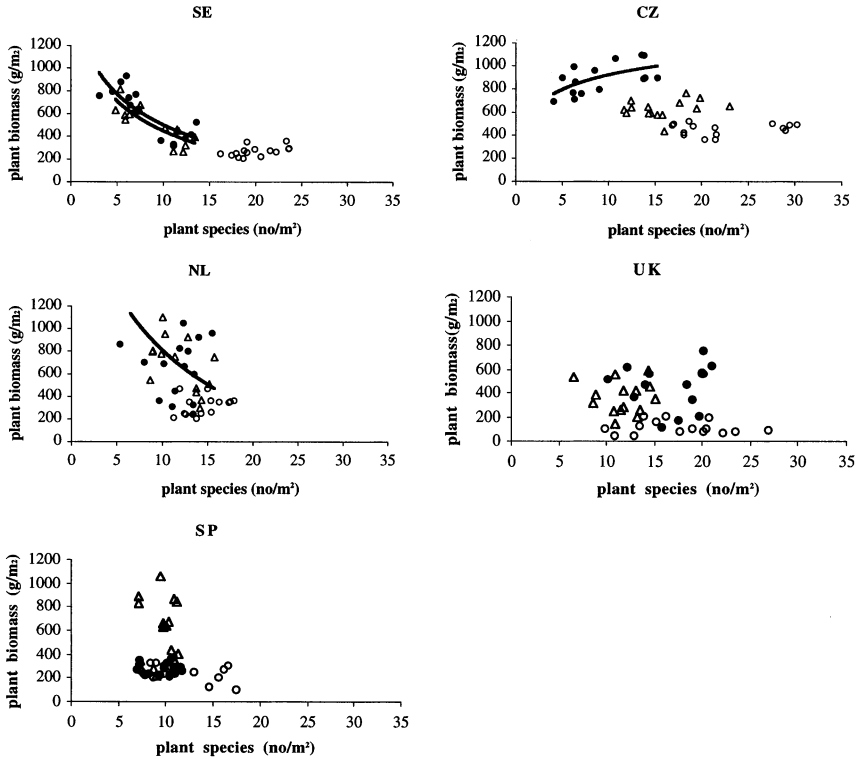


Fig. 3. Plant diversity (no. species m^{-2}) related to plant biomass (g m^{-2}) of the five field sites. Samples taken 1996 (○), 1997 (●) and 1998 (△). Significant linear regressions ($P < 0.05$) of log biomass and species number of each year are indicated with logarithmic curves (as the figure show original data).

tries (2-way ANOVA; Fig. 4). There was no relation between microbial biomass and the development of plant biomass on any of the three sites tested. The microbial biomass of the Swedish site showed a slightly negative relation to a decreasing number of plant species over a two-yr time period (regression, $P < 0.05$, $r^2 = 0.2$; Fig. 5). The bacterial PLFAs and the marker PLFA 18:2ω6 for saprophytic fungi (measured at the SE site only) showed no relations to plant above- and below-ground biomass or plant species diversity. The fatty acid marker NLFA 16:1ω5 of arbuscular mycorrhizal fungal biomass was negatively related to plant above-ground biomass and positively related to plant species number in 1997 (regression: plant biomass $r^2 = 0.8$, $P < 0.001$; plant diversity $r^2 = 0.6$, $P < 0.01$; Fig. 5).

The total number of nematodes increased between 1996 and 1998 at four of the sites but with no response to the sowing treatments (3-way ANOVA, Table 5). The nematodes at the Dutch site showed an opposite pattern and decreased in number. Between 1996 and 1998 there was a positive relation between number of nematodes and plant biomass at four sites (Fig. 6, Table 6). However, when each year was analysed separately there was no significant relationship at any site (regression analyses). A more detailed study on the nematode community in the Netherlands, from 1996–98, showed that the high number of nematodes at the start of the experiment was mainly explained by a high proportion of plant-feeding species (Fig. 7). When comparing proportions of feeding types there was a decrease of plant feeders from 1996 to 1998 (2-way ANOVA, $P < 0.001$) at the same time as bacterial ($P < 0.01$), and fungal feeders increased ($P < 0.001$). There were, however, no changes in proportions of functional groups of nematodes in relation to treatments.

Earthworm biomass was not influenced by treatments at any of the sites (2-way ANOVA: countries $F = 50.256$, treatment $F = 0.612$). It was positively related to plant biomass (regression analysis, $P < 0.05$, $r^2 = 0.32$) on the Swedish site only (Fig. 8) with a corresponding negative relationship to the plant species number (regression analysis, $P < 0.05$, $r^2 = 0.48$). Earthworm biomass from the experimental sites in the Netherlands, the UK and the Czech Republic could not be related to the development of the plant community.

The numbers of collembolans and mites were not influenced by the different treatments, plant biomass production or plant species numbers at any of the field sites (data not shown). The abundance of microbivorous nematodes, fungal and bacterial feeding mites could not be related to respective microbial biomass (results not shown).

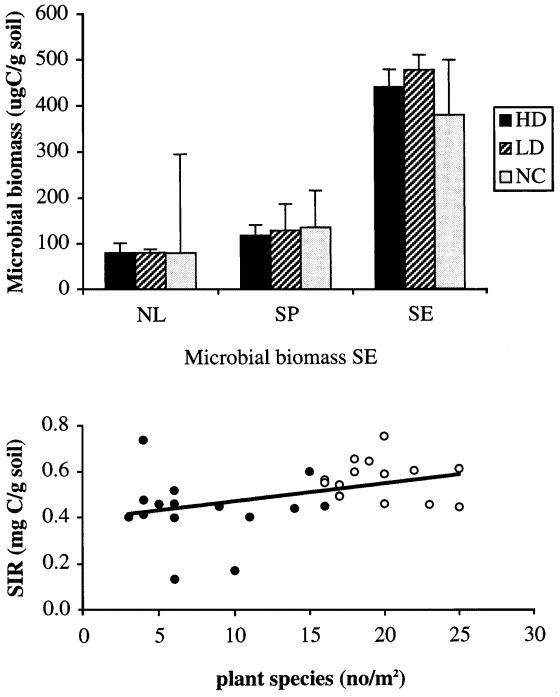
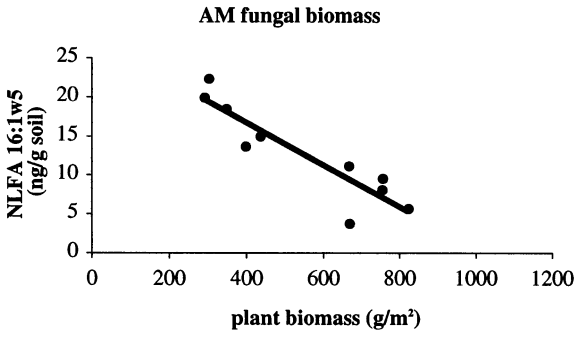


Fig. 4. Top panel: Microbial biomass measured with the substrate induced respiration method (SIR) in Sweden and Spain and with the fumigation technique in the Netherlands. Error bars indicate standard deviation of mean values. Bottom panel: A regression analysis of microbial biomass at the Swedish site between 1996 and 1997 towards plant species numbers. Data are from the Swedish field site in September 1996 (○) and September 1997 (●).



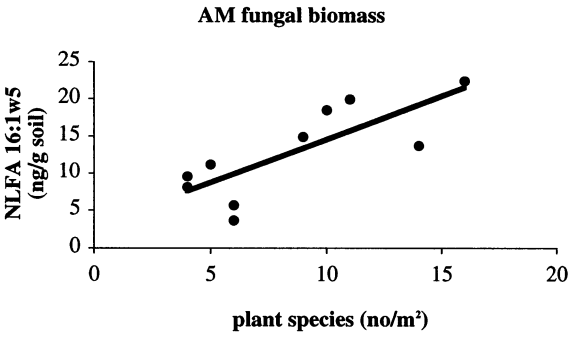


Fig. 5. Plant biomass and plant species number in a regression analyses towards biomass of arbuscular mycorrhizal fungi (AM fungi). Data from the Swedish field site in September 1997.

Table 5. The results of a 3-way ANOVA of number of nematodes found at each field site.

Factor	df	F-value	P
Country	4	25.341	<0.001
Year	1	97.728	<0.001
Treatment	2	2.096	ns
Block	1	4.787	<0.05
Country × Treatment	8	0.957	ns
Treatment × Year	2	1.34	ns
Country × Year	4	5.801	<0.001
Country × Treatment × Year	8	1.246	ns

Discussion

When considering total plant species richness, our results are in disagreement with the hypothesis that in experimental grassland plant communities increase productivity as a result of higher species diversity (Tilman et al. 1996, 2001, Hector et al. 1999). We observed either no significant relationship (3 sites) or when present (Czech Republic and Sweden), the relation was either positive or negative. Our treatments were applied in order to determine their effects on early secondary succession of abandoned land. Consequently, the experimental plots had not been weeded as extensive hand-weeding of plots also introduces hidden treatments, as soil disturbance that may affect soil mineralisation (Wardle 2001) and other abiotic and biotic soil processes, which could in turn affect productivity. On average the high diversity sown communities suppressed non-sown plants more than the low diversity sown treatments (Van der Putten et al. 2000, Lepš et al. 2001).

During grassland secondary succession plant species richness depends on the absence or presence of productive dominant species (Bazzaz 1996). At our CLUE field sites, the establishment and persistence of sown species indeed led to a decrease in total species richness with loss of naturally colonizing species (Lepš et al. 2001). Though in addition to the loss of naturally colonized species there were also a few sown species that dominated in the plots. This appeared at e.g. the Swedish site, where the negative correlation between above-ground biomass and species richness most likely was a secondary effect of development of a few dominant sown species rather than a direct effect of species richness per se.

Our results support the hypothesis that the response of ecosystem functioning to changing diversity relates more to species traits than to species numbers

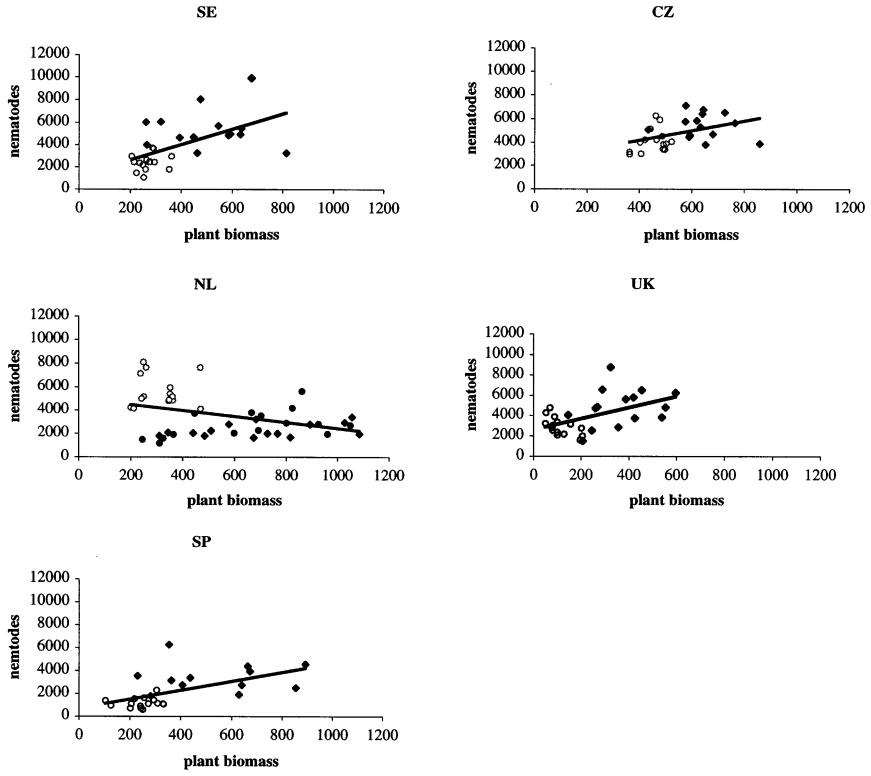


Fig. 6. A regression analysis of above-ground plant biomass (g m^{-2}) and nematode numbers (number 100 g^{-1} soil) from all field sites. Samples from 1996 (○), 1997 (●) and 1998 (◆). Lines indicate significant regressions ($P < 0.05$).

(Chapin et al. 1997, 2000). The effect of the sown species that established in the plots was only clearly visible at the UK site, where the addition of legumes increased the plant biomass over the non-sown plots. The influence of functional group traits on productivity has been reviewed by Diaz and Cabido (2001), who found a high number of field studies to be correlated to functional group traits rather than to species diversity. This is a plausible explanation as all sites in our study included the same functional groups but with different number of species. When comparing the two sown treatments we found significantly more biomass produced in HD plots in the Netherlands when considered over all three field seasons. At the other four sites the above-ground biomass was not affected by the number of sown species in each functional group. In naturally colonized plots there were higher probabilities of a functional group missing or being present in a low proportion.

Table 6. Regression analyses of number of nematodes per 100 g soil to plant biomass (g m^{-2}) of each plot regarded across the years 1996 and 1998.

Country	regression	r^2	slope
CZ	$p<0.05$	0.176	4.10
SP	$p<0.01$	0.325	3.86
UK	$p<0.01$	0.270	5.52
NL	$p<0.05$	0.142	−2.55
SE	$p<0.01$	0.332	6.92

Soil community reponses to identity and productivity of plants

We found no evidence that total microbial biomass or specific groups of microorganisms vary in relation to short-term (maximally three years) experimentally imposed differences in plant production. In the laboratory, microorganisms can respond rapidly to small scale changes in resources and bacteria can double their biomass close to patches of manure (Frostegård et al. 1997) but interspecific competition can alter the result (Mikola 1998). It seems to be a more complex task to predict microbial responses to changed plant diversity and biomass production in field situations. Microbial biomass has been positively related to plant biomass production or additions of organic resources (Wardle et al. 1999b, Broughton and Gross 2000). However, in situations of no visible relation the increased input of organic material into the soil microorganisms can start a faster activity and metabolise carbon when plant production increases (Bossio et al. 1998, Coleman et al. 2002).

The diversity of AM-fungi is important for restoring a species-rich vegetation, as AM-fungi are known to

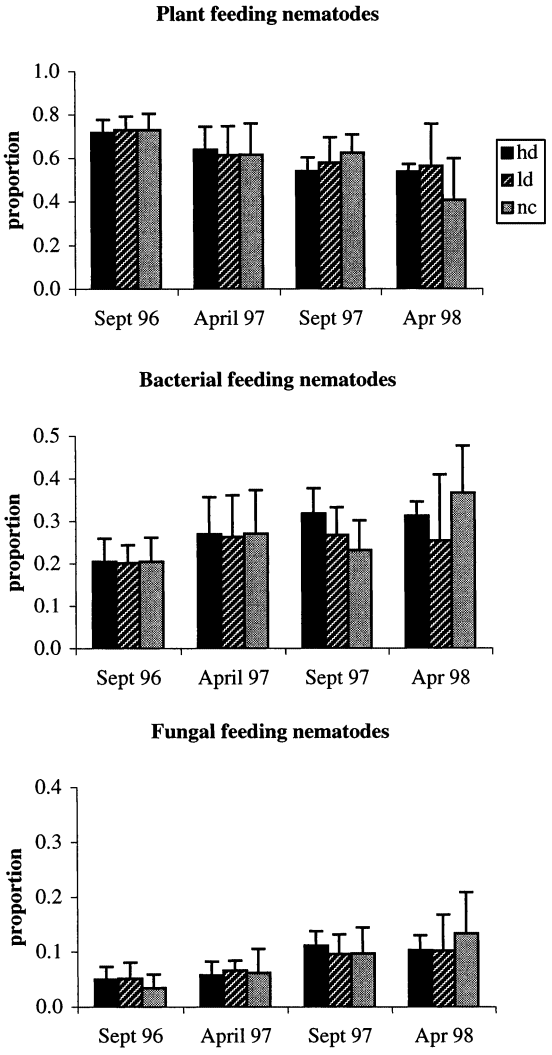


Fig. 7. Proportions of nematode feeding groups estimated from samples taken from treatments in the field site in the Netherlands September 1996 to April 1998. Bars show mean proportions of each of the functional groups and error bars show standard deviation.

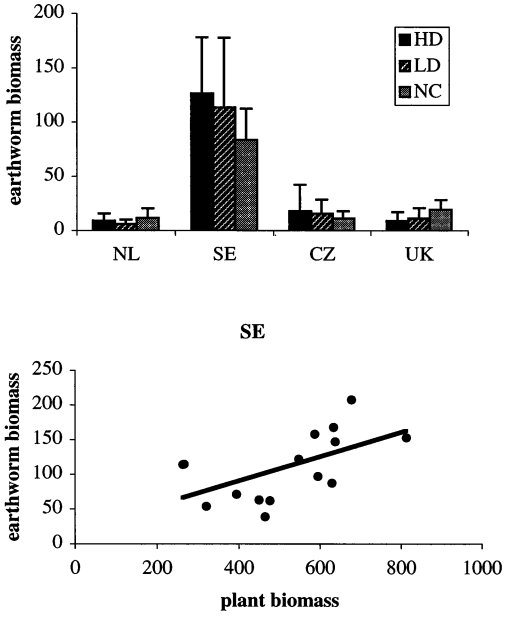


Fig. 8. Top panel: Earthworm biomass (g m^{-2}) shown as mean values of the treatments (error bars indicate standard deviation) at four of the filed sites. Bottom panel: A regression analysis of above-ground plant biomass (g m^{-2}) towards earthworm biomass (g m^{-2}) of the Swedish site.

determine the biomass production and diversity of the plant community (Van der Heijden et al. 1998, Hartnett and Wilson 1999, Marler et al. 1999). However, these experiments investigated effects of mycorrhizal diversity on plant communities. In our experiments we studied how plant species composition or functional group diversity affect AM- and ectomycorrhizal infections of plants. For example, they are reduced in sown treatments at the Swedish CLUE field site (see also Hedlund and Gormsen 2002). In plots where AM fungal biomass was lowest, the biomass of bacterial and saprophytic fungal communities were highest (Hedlund 2002). These shifts in soil microbial composition may have been due to the change in the plant species composition towards a sword dominated by legumes and forbs compared to a more grass dominated community in the naturally colonized plots. The identity of plant species (especially the legumes playing a profound role) was also more important than species diversity for the microbial community in one of the BIODEPTH fields (Spehn et al. 2000). Here, nitrogen fixing plant species were positively correlated to microbial biomass, which was not the case at the Swedish CLUE site. Thus, plant species richness per se does not seem to increase microbial biomass but species identity, i.e. the composition of organic residues of the litter, and interactions between species are probably more important (Wardle and Nicholson 1996, Wardle et al. 1997). In order to find effects of plant species diversity on microbial processes in the soil, the range of plant species diversity, or more probably the range of functional groups of plants, need to be much wider than in the CLUE experimental treatments.

Among the soil invertebrates, the nematodes was the only group that increased in response to changes in plant biomass in more than one site. The other groups (mites, collembolans) showed totally idiosyncratic responses. The opposite response at the Dutch site was due to two root-feeding species that were present in high densities as a result of the crop (maize) cultured before the land was abandoned (Korthals et al. 2001). The hypothesis that an increase in

plant resources can give a bottom-up effect on higher trophic level in the soil food web has been tested and rejected by Mikola and Setälä (1998a). We could not find any relations between trophic groups when comparing e.g. plant biomass with plant-feeding mites and nematodes, or saprophytic mites, collembolans and bacteriovorous nematodes with microbial biomass. No clear evidence have been shown for either top-down or bottom-up regulation of soil food webs and microbial feeding populations are probably limited both by resources and predation (Mikola and Setälä 1998a, b, Coleman et al. 2002).

There were no relations between treatments and nematodes. In fact, in the first three years termination of agricultural practices alone had much larger effects on the nematodes, mites and earthworms than any of the experimental manipulations of the plant assemblages (Korthals et al. 2001, Gormsen et al., unpubl.). Earthworm biomass was positively correlated to plant biomass only at the Swedish site while earthworms at the three other sites were not related to the plant community. This could depend on the relatively higher level of earthworm biomass at the Swedish site compared to the other sites, where other factors than biomass production could restrain the development of the earthworms. Earthworm biomass covaried positively with both plant species richness and biomass in a field experiment by Spehn et al. (2000). In these experiment (Spehn et al. 2000) the biomass of earthworms was also closer to that of the Swedish site and could potentially show a more similar response to the plant community.

Our study shows that plant biomass and the response of the soil community (microbial biomass and soil invertebrate abundance) are idiosyncratically influenced by sowing low and high diversities of mid-successional grassland plant communities. So far, there is very little evidence of effects of plants species diversity treatments on soil communities from biodiversity experiments in the field. In field experiments that has tested these relations, as BIODEPTH (Hector et al. 1999) and field experiments at Cedar Creek (Tilman et al. 1996), the field sites have been subjected to soil sterilization or to top soil removal in order to reduce the work load of weeding. We have chosen not to weed and to leave the top soil intact. Not removing the weeds may obscure the plant species diversity treatments, but our treatments have resulted into clearly different early succession plant communities, some consisting of natural colonizers (mainly weeds) and others of plant communities dominated by low and high diversity mixtures of mid-successional grassland plant species (Van der Putten et al. 2000, Lepš et al. 2001). We observed idiosyncratic effects of the plant community treatments on plant biomass, on soil microbial biomass and on numbers of soil invertebrates, but these idiosyncratic effects were consistent for a range of European climates and site conditions represented in CLUE. Management by mowing of plots will probably delay the effects of the plant communities on the soil community development, since part of the biomass is removed instead of being translocated into the soil community, thus arresting the natural succession, probably maintaining the idiosyncratic responses.

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