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Perspectives in Plant Ecology, Evolution and Systematics 7 (2005) 27–49
Perspectives in Plant Ecology, Evolution and Systematics
www.elsevier.de/ppees

Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth

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Received 15 July 2004; accepted 23 November 2004

Abstract

The influence of plant diversity on belowground ecosystem processes and feedbacks of the latter on plant growth are still largely unexplored. A previous field experiment showed positive effects of plant diversity on diversity and activity of culturable soil bacteria. We examined soil carry-over effects of these experimental communities on subsequent plant growth and the development of soil microbial communities in pots. We inoculated sterile substrate with soil from the field experiment and then sowed three plant species - *Dactylis glomerata*, *Plantago lanceolata* and *Trifolium pratense* - as monocultures or 3-species mixtures into the pots. We measured the phytometers over a period of 14 months. Thereafter, we sampled the soil in 3-species mixtures to analyze microbial communities. The original plant species richness and number of functional groups had positive soil carry-over effects on phytometer germination. Furthermore, in mixtures, species richness also stimulated short-term biomass production of phytometers and catabolic diversity of culturable soil bacteria. The number of plant functional groups had a positive effect on the amount of prokaryotic phospholipid fatty acids (PLFAs) but negative effects on the amount of eukaryotic PLFAs and the activity of culturable soil bacteria. The carry-over effects of plant diversity on phytometer growth disappeared with time. However, specific effects of the presence of particular functional groups or species in the original plant communities remained significant and were sometimes paralleled by effects on soil microbial communities. These results suggest that in the short term, soil carry-over effects of plant diversity are mediated by a general stimulation of soil microbes whereas the longer-term effects of particular plant species are more likely due to compositional shifts in soil microbial communities. Although the mechanisms of soil carry-over effects in biodiversity experiments remain to be determined, our study demonstrates that they exist and probably involve soil microbial communities. This 'memory' of soils may be an important factor in ecosystem development.
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Keywords: Biodiversity; Ecosystem functioning; Grassland ecosystems; Plant–soil interactions; Soil training; Soil microbial diversity; PLFA; BIOLOG; Productivity

Introduction

Changes in plant diversity are known to affect aboveground ecosystem functioning (e.g. Tilman et al., 1997; Hector et al., 1999; Spehn et al., 2000a; van der Putten et al., 2000; Tilman et al., 2001; reviewed in

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doi:10.1016/j.ppees.2004.11.002

Schmid et al., 2002a), but it is increasingly recognized that changes in plant diversity also have implications for belowground ecosystem functioning, including diversity of belowground communities of other organisms (e.g. Hooper et al., 2000; Korthals et al., 2001; van der Putten et al., 2001; Wardle et al., 2002; Zak et al., 2003; Heemsbergen et al., 2004). Because these belowground communities may in turn affect the decomposition of organic material and thus the mineralization of nutrients (e.g. Bardgett and Shine, 1999; Wardle, 2002; Heemsbergen et al., 2004), they may cause feedback effects on the plant community (Klironomos, 2002; Bever, 2003). In this paper, we describe such feed-back phenomena between plant diversity, soil microbial communities and subsequent plant growth.

Plant diversity may influence the belowground ecosystem in various ways. It can for example enhance net primary productivity (NPP; reviewed in Schmid et al., 2002a). NPP is expected to increase soil-carbon input via enhanced turnover of plant biomass and enhanced root exudation and may therefore influence carbon-limited microbial communities in the soil (Niklaus et al., 2003; Zak et al., 2003). These effects of NPP on microbial biomass may be positive (Myrold et al., 1989; Insam et al., 1991; Zak et al., 1994, 2003) or not (Groffman et al., 1996; Niklaus et al., 2003). High plant diversity may lead to high litter diversity, which in turn supports a greater diversity of decomposers and detritivores (Sulkava and Huhta, 1998; Hansen, 2000). In forest soils, plant-species specific fungi have been found to decompose litter (Widden, 1986), implying that a greater diversity of litter types would support a greater fungal diversity. Diverse mixtures of litter have also been found to support more diverse mite communities due to an increased variety of food resources and greater habitat complexity (reviewed in Bardgett, 2002). Hence, the abundance, activity, and composition of decomposer communities may vary markedly with different plant species (Bardgett et al., 1998; Wardle et al., 1999) or specific plant functional groups such as legumes, which can positively influence microbial biomass due to enhanced litter quality, i.e. litter with a lower C/N ratio (Spehn et al., 2000b; Scherer-Lorenzen et al., 2003). Furthermore, plant species can regulate the development of beneficial rhizobacteria through the release of specific sugars and amino acids into the root zone (Burr and Caesar, 1984; Kowalchuk et al., 2002). Hence, higher plant diversity may produce a higher biochemical diversity of root exudates and therefore select for more diverse microbial communities (Lavelle et al., 1995).

According to a review by Bonkowski et al. (2000), feedbacks from the soil compartment on plant growth are major drivers of plant growth in the field. For example, rhizobacteria can influence plant growth directly via the production of plant hormones, or indirectly through the inhibition of plant pathogens in the rooting-zone soil (Sturz and Christie, 2003; Westover and Bever, 2001). Biodiversity research thus must include belowground organisms to achieve a full understanding of terrestrial ecosystem functioning (Copley, 2000; Bever, 2003; de Deyn et al., 2003, 2004; Hedlund et al., 2003), and linking above- and belowground biodiversity effects will be a major challenge for future work (Broughton and Gross, 2000; Hooper et al., 2000; Joshi et al., 2004; Wardle et al., 2004).

At the Swiss site of the European BIODEPTH project, previous studies found that plant diversity in grassland stimulated activity and functional diversity of culturable soil bacteria (Stephan et al., 2000) as well as earthworm population density and biomass (Spehn et al., 2000b). In a comparable grassland field experiment in Minnesota, higher plant diversity led to higher N-mineralization rates and, via enhanced plant biomass, to higher microbial biomass and fungal abundance (Zak et al., 2003). Thus, we hypothesized that soils that develop under high plant species richness or high plant functional diversity may have a positive influence on future plant growth because of increased activity and diversity of beneficial soil microorganisms, although negative effects of soil microbes are of course also conceivable (van der Putten, 2003). We established a soil-inoculation experiment in pots to examine whether carry-over effects from soils 'trained' under different plant diversities in the field experiment at the Swiss BIODEPTH site influence subsequent growth of 'phytometer' plants (Joshi et al., 2001) and the development of soil microbial communities. Because biotic effects can propagate by population dynamic processes, we expected that they should be stimulated with small inocula of 8% of the total soil mass, an expectation which indeed was supported by subsequent soil analyses reported here. To maximize the chances to observe potentially different responses in plant growth to soil training, we used three different phytometer species, *Dactylis glomerata* L., *Plantago lanceolata* L. and *Trifolium pratense* L., belonging to the three functional groups grasses, herbs and legumes. Furthermore, to correct for potential effects of phytometer diversity, we grew the phytometer species in monocultures and in three-species mixtures. To test whether the soil inocula and the phytometers had feedback effects on the development of soil microbial communities in the new soils, we re-sampled these at the end of the 14-months phytometer growth experiment.

For the phytometer growth study, we addressed the following main questions: (1) Do inocula from soils trained with high plant species richness or with high plant functional diversity positively affect the growth of phytometers? (2) Are there soil carry-over effects of particular plant species or plant species compositions on the growth of the phytometer species? (3) Do inocula from soils trained with legumes positively affect the

growth of phytometers? (4) For how long are soil carry-over effects on phytometer growth detectable? Specific questions concerning soil carry-over effects on microbial communities were: (5) Do soil microbial communities still show a signal of the original soil samples taken from species-rich or species-poor plant communities, or have they converged after 14 months of phytometer growth? (6) Do soil microbial communities still show a signal from the original plant communities containing particular species or particular functional groups (e.g. legumes)? (7) Is the diversity of soil microbes related to phytometer growth in the pots?

Material and methods

Origin of soil inocula and phytometer seeds

To study the effects of 'soil training' (i.e. exposure of soil to artificially established plant-diversity levels for a specific time period) on growth of subsequent generations of plants during 14 months, and on microbial communities developed after 14 months of plant growth, we used soils from the Swiss field experiment of the European BIODEPTH project. This field experiment was carried out at Lupsingen near Basel (47°27′N, 07°41′E, 440 m a.s.l.) and has been previously described in detail (Diemer et al., 1997; Hector et al., 1999; Joshi et al., 2000; Spehn et al., 2002; Pfisterer et al., 2004; Spehn et al., 2005). Here, we only give a brief summary of the experimental protocol.

In spring 1995, the calcareous nutrient-rich soil was planted with experimental plant communities ranging in species richness from 1, 2, 4, 8 to 32 species and in functional diversity from 1, 2 to 3 functional groups (grasses, legumes, non-leguminous herbs). Every diversity level had several different species (monocultures) or species combinations (mixtures) and each of these had two replicates arranged in two blocks. In total, there were 64 experimental plots with 32 different communities derived from a pool of 48 local grassland species belonging to 13 plant families (Joshi et al., 2000). There was no obvious spatial gradient in soil nutrients conceivable across the field site at the beginning of the experiment in spring 1995 (Diemer et al., 1997). The range of values across the whole field were: pH 6.3–7.6, total C 3.12–4.59%, total N 0.32–0.45%, soluble NO_3^- 56–89 mg kg^{-1}, soluble PO_4^{3-} 0.7–2.7 mg kg^{-1}, K^+ 3–10 mg kg^{-1} and Mg^{2+} 4–23 mg kg^{-1} (Joshi, unpubl. data; Hector et al., 2005).

In July 2000, we took soil inocula from each of the 64 plots of the field experiment. Each inoculum was composed of five soil samples taken with a bulb planter (0–5 cm depth, 5 cm diameter) at random positions within each 8 × 2-m plot. The soil was homogenized by sieving (<8 mm), which also removed earthworms and roots from the soil. As a control, we used soil from adjacent species-rich grassland surrounding the experimental plots and sown at the same time as these with a mixture of all 48 species. We collected seeds of two of the phytometer species (*P. lanceolata* and *D. glomerata*) at the experimental site and purchased seeds of a third phytometer species (*T. pratense*). These three phytometer species were chosen because they are common species in the grassland surrounding the original field site and because they are representative of three functional groups (grasses, *D. glomerata*; legumes, *T. pratense*; non-leguminous herbs, *P. lanceolata*). Before sowing, the seeds were surface-sterilized with 6% sodium hypochlorite to avoid microbial contamination of the inoculated substrates.

Experimental setup

In mid-August 2000, sterile soil/sand mixtures were prepared. We autoclaved the soil, taken from the surrounding meadow of the original field experiment, twice for 20 min at 121 °C within a time period of 12 h. The newly purchased, washed sand was sterilized (110 °C) for 12 h. Each of the 64 soil inocula was then mixed with sterile soil and sand in the volumetric proportions 8:8:84 (inoculum soil:sterile soil:sterile sand) in separate 17.5 × 17.5-cm pots. Thus, 50% of the soil, which was the sole nutrient source for the growing plants, originated from the inoculum, so that besides microbial carry-over effects initial nutrient carry-over effects caused by the original plant diversity treatments might have occurred as well. However, in the third year of the original field experiment, there was neither an overall effect of species richness on soil inorganic (soluble) nitrogen concentration across all BIODEPTH sites nor a significant site *x* diversity interaction indicating no significant influence of plant species richness at any particular experimental site (Spehn et al., 2005). The only observed effect was that the number of plant functional groups led to a decrease in soil nitrogen in the third year of the BIODEPTH experiment (Spehn et al., 2005) and this might have reduced our chances to observe stronger positive effects of original plant functional diversity via soil microbial communities on subsequent phytometer growth. For each inoculum, we prepared four pots in which the three phytometer species were then sown individually and as a three-species mixture. We sowed 30 seeds per pot in the monocultures and 10 seeds per species in the three-species mixtures. Sowing was completed within 10 days.

The 4 × 64 pots were put into two snail-proof boxes ('box', 1 × 7 m rectangle in Fig. 1) in an experimental garden of the University of Zurich (47°32′N, 08°34′E). Each box contained 128 pots with soil inocula from each

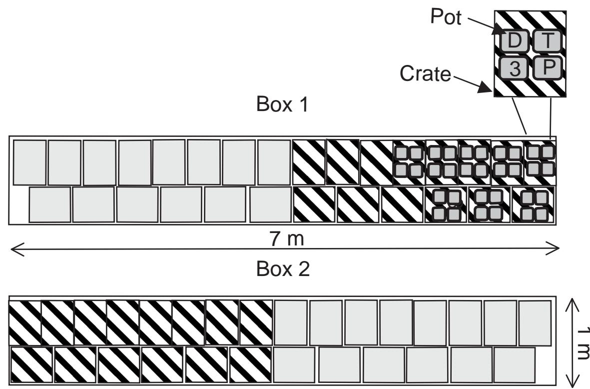

Fig. 1. Experimental setup. Phytometer monocultures: D, *Dactylus glomerata;* P, *Plantago lanceolata*; T, *Trifolium pratense*; 3, phytometer mixture. Pots with soil inocula from block 1 of the original field experiment are hatched, those from block 2 are in gray.

of the 32 different experimental plant communities grown in each block at the field site in Lupsingen, planted with the three monocultures and the three-species mixture (4 × 32 pots). Both boxes had two halves (Fig. 1). In one half there were pots with inocula from the first block of the field experiment and in the other half pots from the second block. Within the halves, the pots were distributed randomly but grouped to 4–6 within crates (14 crates per half of a box; Fig. 1). The crates and boxes ensured that the pots were isolated from the underlying and surrounding soil, respectively. The boxes were covered with a fine mesh towel, to keep off material input from the surrounding environment. We watered each pot separately by hand with an equal amount of water, taking care to avoid splashing between pots; we did not fertilize the pots. For setting up the experiment and during all measurements, we wore gloves and sterilized all the instruments before use with 70% ethanol.

Measurements and harvesting of the phytometers

At the end of August 2000, 2 weeks after sowing, we determined the germination rate in each pot (separated to plant species in the mixtures) and reduced the seedlings randomly to 18 plants per pot resulting in a density of 555 plants m^{-2}, which was similar to the density of seedlings in the original field experiment (Diemer et al., 1997). Weed individuals were counted and removed periodically until the first harvest (November 2000). We measured the leaf length and width of one randomly chosen plant of every species (i.e. three measurements in pots with three-species mixtures) after 1, 3 and 10 months of growth. The leaf width of *T. pratense* was estimated by measuring the length of the middle leaflet. We harvested the aboveground plant biomass in each pot three times by cutting the plants at a height of 1 cm after 3, 10 and 14 months. In the mixtures, the harvested plant material was separated to species. At each harvest, the number of surviving individuals per species was noted. Plant biomass samples were dried at 80 °C for 24 h and weighed. We counted the number of flowers per plant species and pot after 10 and 14 months of growth. The level of herbivory, i.e. plant damage caused by insects and slugs, and pathogen attack, i.e. mainly presence of mildew and rust, were classified into four categories: none (0%), low (1–30%), middle (31–60%) and heavy (61–100%) damage. These variables were assessed at the pot level in June 2001.

Analysis of soil samples from the soil-training experiment

Soil sampling and preparation

In October 2001, after 14 months of phytometer growth, we took soil samples from the 64 pots containing the phytometer mixtures. The 10 soil cores (depth 0–5 cm, diameter 1 cm) taken in each pot were pooled and sieved (<2 mm). We used aliquots of the homogenized and pooled soil samples to determine abiotic soil characteristics, substrate-utilization patterns of culturable soil bacteria (fresh soil), and profiles of phospholipid fatty acids (PLFA) to indicate the presence of different groups of soil microorganisms. Soil samples for the PLFA analysis were stored at −80 °C until processing.

Abiotic soil characteristics

Approximately, 5 g of fresh soil was dried at 105 °C to constant weight to estimate the water content (Allen, 1989). We measured the pH (0.01 M $CaCl_2$) of these soil samples with a Hamilton Single Pore®Plast electrode (pH meter 761, Knick, Berlin, Germany). The percentages of carbon and nitrogen per soil dry mass were measured by dry combustion using an automated carbon–hydrogen–nitrogen analyzer (LECO CHN-900, LECO Corporation, St. Joseph, Mich., USA).

Substrate utilization patterns of culturable soil bacteria

To extract bacteria from soil, 100 mg fresh soil was shaken (Vortex, full speed) for 20 min in 1 ml of Millipore water and then allowed to settle for 3 min. An aliquot (150 µl) of the supernatant was then diluted 100-fold in Millipore water before transferring 100 µl to each well of the BIOLOG EcoPlates™ (Biolog, Hayward, Calif.). We used Millipore water in both the extraction step (instead of 0.2% Tetrasodium-pyrophosphate; Stephan et al., 2000) and dilution step (instead of 0.9% NaCl; Stephan et al., 2000) according to

preliminary tests, where we compared these two methods.

Each plate had 96 wells containing in triplicates 31 carbon sources and a control (water). These substrates contained a redox dye (tetrazolium) that highlights the carbon sources used by the microbes. Active culturable soil bacteria oxidize the substrate and form NADH, which can be quantified by its reduction of the colorant with a spectrophotometer (Microplate Reader 3550, BIO-RAD, Hercules, CA, USA) at 595 nm. We measured the plates after 24, 48, 72 and 96 h incubation at room temperature. For every incubation time, we subtracted the mean absorbance value for the control and took the mean of each substrate per plate to calculate the catabolic activity in the use of that carbon source (A_i) (Balser et al., 2002). Negative A_i values were set to zero. Average well colour development (AWCD) was calculated as the sum of activities (A_i) per plate, divided by the 31 carbon sources (Garland, 1996). For every soil sample, A_i values were used to calculate the Shannon diversity index as measure of catabolic diversity according to Begon et al. (1990). Total AWCD of plates, AWCD of specific groups of carbon sources (alcohols, $AWCD_{alc}$; amines, $AWCD_{a}$; amino acids, $AWCD_{aa}$; carbonic acids, $AWCD_{ca}$; carbohydrates, $AWCD_{c}$; phosphorylated C-sources, $AWCD_{ph}$; polymers, $AWCD_{p}$) and every particular carbon source were analyzed for soil carry-over effects of the field experiment. Well colours were already fully developed after 48 h. Therefore, we refer to the data at 24 and 48 h only.

Phospholipid fatty acid (PLFA) analysis of soil microbial communities

PLFA profiles were examined according to a standard protocol (University of Wisconsin, Madison; www.ecosystem-microbiology.wisc.edu/methods). All chemicals used were of analytical grade. Glassware was washed with hexane to avoid lipid contaminations. Fatty acids were extracted with 10:5:4 volumes of methanol, chloroform and 0.1 M phosphate buffer (pH 7.0). Four grams of freeze-dried soil samples were weighed into 30-ml Teflon tubes. Thereafter, 3.6 ml buffer, 4 ml chloroform and 8 ml methanol were added in that order. Soil samples were extracted in the dark at room temperature for 1 h by shaking the tubes horizontally. The samples were centrifuged at 2000 rpm (25 °C) for 30 min. We decanted the supernatant containing the fatty acids into 30-ml screw-cap glass tubes, added 3.6 ml buffer and 4 ml chloroform and shook the glass tubes immediately for 1 min. The samples were kept at room temperature in the dark over night to allow separation of the two phases. The supernatant was removed with a vacuum aspirator and the bottom phase evaporated with chloroform under nitrogen gas at 30 °C in the dark. This fraction, which contained the fatty acids, was resuspended in 2 ml chloroform, transferred to a clean 15-ml screw-cap glass tube and dried with nitrogen gas again. The extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids using silicic acid columns (Supelco, Bellefonte, PA, USA). The polar-lipid fraction (phospholipids) was eluted with methanol and dried with nitrogen gas. The polar lipids were then subjected to saponification and methylation according to the MIDI protocol (MIDI, Newark, Delaware, USA).

Individual fatty acid methyl esters were identified and quantified using the MIDI Sherlock Microbial Identification System (MIDI, Newark, Delaware, USA). The results for each individual fatty acid were expressed as a percentage of the total amount of fatty acids (mol%) found in a given sample. A total of 38 PLFA were detected and identified in the different soil samples. The Shannon diversity index was calculated from these 38 PLFAs as a relative measure of microbial diversity (Porazinska et al., 2003; Wardle et al., 2003). Furthermore, 32 of the 38 fatty acids were grouped into eukaryotic (subgroups fungi, VAM, protozoa) and prokaryotic (subgroups Gram-negative, Gram-positive) PLFAs (Table 1) and were analyzed with ANOVA (see below). In addition, we calculated and analyzed the ratios of eukaryotic/prokaryotic (euk/prok), fungal/prokaryotic (fung/prok) and Gram-positive/Gram-negative PLFAs. We follow standard nomenclature rules when referring to different fatty acids (Frostegard et al., 1993). Using the 20 fatty acids that were present in proportions $>0.5\%$ we calculated summary variables by principal component analysis (PCA) (Balser and Firestone, in press): principal component 1 (PC_1) and principal component 2 (PC_2). These were then also analyzed with ANOVA.

Statistical analysis

The data were analyzed with analysis of variance (ANOVA) using the software Genstat 5 (Payne et al., 1993). Variables that were repeatedly measured during the experiment (plant biomass, leaf length and width, number of flowers) were analyzed with repeated-measures ANOVA. The species identity of the phytometers ('phyto_spec') and whether they were grown in monoculture or mixture ('phyto_div') as well as the soil-training by species richness (decomposed into log-linear contrast 'log-lindiv' and deviation), by functional group number (decomposed into linear contrast 'lindiv' and deviation), and by legume presence or absence ('legumes') in the original field experiment were considered fixed factors. All terms were fitted sequentially in this order (Table 2), meaning that a term J fitted after terms..., H, I is measuring if there are differences between levels of J when all previous terms ..., H, I are kept constant (see Schmid et al., 2002b for justification

Table 1. Overview of the 38 fatty acids identified in PLFA analysis and their grouping according to their diagnostic meaning into eukaryotic (subgroups fungi, VAM, protozoa) and prokaryotic (subgroups Gram-negative, Gram-positive) PLFAs. Eukaryotic PLFA comprised 38% and prokaryotic PLFA comprised 70% of the total PLFA (percentages do not add up to 100% because some PLFAs were assigned to both groups)

Fatty acid (FA)	Taxonomic group	Reference
16:1w5c	Eukaryotes: arbuscular mycorrhiza, fungi	Olsson et al. (1999)
18:2w6,9c, 18:0 ANTE	Eukaryotes: fungi	Paul and Clark (1996)
20:4w6,9,12,15c	Eukaryotes: protozoa	Paul and Clark (1996)
18:1 w7c	Prokaryotes and eukarotes: Gram-negative bacteria, arbuscular mycorrhiza, fungi	Zelles (1999), Olsson et al.(1999), Ohtonen et al. (1999)
16:0 10 methyl	Prokaryotes: Actinomycetes, Gram-positive bacteria	Zelles (1999)
17:0 10 methyl	Prokaryotes: Actinomycetes, Gram-positive bacteria	Zelles (1999)
TBSA 10Me 18:0	Prokaryotes: Actinomycetes, Gram-positive bacteria	Zelles (1999)
10:0 2OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
12:0 2OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
15:0 3OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
16:1 2OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
16:1w7c, 15 ISO 2OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
16:1w9c	Prokaryotes: Gram-negative bacteria	Zelles (1999)
17:0 CYCLO	Prokaryotes: Gram-negative bacteria	Paul and Clark (1996), Kieft et al. (1994)
17:1w5c	Prokaryotes: Gram-negative bacteria	Zelles (1999)
17:1w8c	Prokaryotes: Gram-negative bacteria	Zelles (1999)
18:1 2OH	Prokaryotes: Gram-negative bacteria	Zelles (1999)
18:1w5c	Prokaryotes: Gram-negative bacteria	Zelles (1999)
19:0 CYCLO w8c	Prokaryotes: Gram-negative bacteria	Kieft et al. (1994)
11methyl 18:1w7c	Prokaryotes: Gram-positive bacteria	Zelles (1999)
15:0 ANTEISO	Prokaryotes: Gram-positive bacteria	Zelles (1999)
15:0 ISO	Prokaryotes: Gram-positive bacteria	Zelles (1999)
16:0 ISO	Prokaryotes: Gram-positive bacteria	Paul and Clark (1996)
16:1 ISO G	Prokaryotes: Gram-positive bacteria	Zelles (1999)
17:0 ANTEISO	Prokaryotes: Gram-positive bacteria	Zelles (1999)
17:0 ISO	Prokaryotes: Gram-positive bacteria	Zelles (1999)
17:1 ISO I, ANTEI B	Prokaryotes: Gram-positive bacteria	T.C. Balser (pers. comm.)
18:0 ISO	Prokaryotes: Gram-positive bacteria	Zelles (1999)
18:1 w9c	Prokaryotes: Gram-positive bacteria	Zelles (1999)
19:1w11c, 19:1w9c	Prokaryotes: Gram-positive bacteria	T.C. Balser (pers. comm.)
20:0 ISO	Prokaryotes: Gram-positive bacteria	Ratledge and Wilkinson (1988)
20:1,w9c	Prokaryotes: Gram-positive bacteria	Zelles (1999)
12:0	—[a]	Ratledge and Wilkinson (1988)
15:0	—[a]	Niklaus et al. (2003)
16:0	—[a]	Ratledge and Wilkinson (1988)
17:0	—[a]	Niklaus et al. (2003)
18:0	—[a]	Ratledge and Wilkinson (1988)
20:0	—[a]	Ratledge and Wilkinson (1988)

[a]Straight-chain saturated fatty acids not assigned to a taxonomic group.

of this approach in biodiversity experiments). In some additional analyses, we used legume frequency ('legu-freq', denoting the percentage of legumes sown in the different treatments at the original field experiment) instead of legume presence or absence as contrast variable. Separate analyses were done to test presence/absence effects of particular plant species grown in the field experiment (individual species contrasts; Rosenthal and Rosnow, 1985; see Tables 6–8 for number of replicates per individual plant species). The spatial variation among the pots, i.e. effects of boxes ('box'), halves within boxes ('half'), and pots (in repeated-measures analyses only) as well as the spatial variation within halves within boxes was estimated, the latter by fitting the *x*- and *y*-coordinates of each pot in the experimental garden (Table 2). The spatial variation

Table 2. Skeleton analysis of variance for the measured variables in the soil-training experiment

	Source of variation	d.f.	Mean square	Variance-ratio
S	Spatial variation	5		
	Box	*1*	*B*	*B/H*
	Half within box	*2*	*H*	*H/P*
	x-Coordinate	*1*	*X*	*X/P*
	y-Coordinate	*1*	*Y*	*Y/P*
A	Phytometer treatment	3		
	phyto_div	*1*	*PD*	*PD/PDxM*
	phyto_spec within phyto_div	*2*	*A*	*A/AxM*
B	Original diversity treatment	30		
	Species richness	*4*	*S*	*S/M*
	log_lindiv (log-linear effect of species richness)	*1*	LL	*LL/M*
	Deviation from log-linearity	*3*	*SD*	*SD/M*
	Number of functional groups	*2*	*F*	*F/M*
	lindiv (linear effect of functional group diversity)	*1*	*FL*	*FL/M*
	Deviation from linearity	*1*	*FD*	*FD/M*
	Legumes	*1*	*L*	*L/M*
	original_mix	*23*	*M*	*M/PDxM*
A × B	Phytometer treatment × Original diversity treatment	93	A.B	A.B/P
P	Pot = Residual	124	P	
	Total	255		

Effects are always adjusted for effects that precede them. The interaction terms are only indicated by the summary row 'A × B' (but see the use of the phyto_div x original_mix [PD × M] and phyto_spec × original_mix [A × M] interactions as denominators in variance ratios).

(except *x*- and *y*-coordinates) and the identity of the particular plant mixture in the original field experiment ('original_mix') were treated as random factors (see Schmid et al., 2002b). Because the different species mixtures in the original field experiment were randomly assembled from the pool of 48 species, the original_mix mean square was used as the appropriate error term to test soil-training effects of diversity treatments (Joshi et al., 2000; Schmid et al., 2002b; Table 2). Interactions between soil-training effects and phytometer treatments (identity and monoculture vs. mixture) were similarly tested against the corresponding interaction terms with original_mix (e.g. log_lindiv × phyto_spec against original_mix × phyto_spec). Because statistical power with this conservative test procedure is moderate even in a large experiment (in our case there were 32 different replicated plant mixtures), we also report marginally significant effects with $0.05 < P \leqslant 0.1$ (Toft and Shea, 1983). Owing to the complexity of the experiment, which was dictated by the nature of the research question, there were a large number of effects tested for each dependent variable. Furthermore, because we were not only interested in demonstrating effects on one integrative variable of phytometer performance, but also on how effects affected different variables and how effects developed over time, there were multiple tests for each effect. That is, we used the analysis of variance as an explorative rather than a confirmatory statistical tool (Schmid et al., 2002b). We did not apply corrections, such as Bonferroni methods, for multiple testing, because they are notorious for their extreme reductions of statistical power under these circumstances (Moran, 2003). Instead of such corrections, we consider the likelihood with which significant results can occur by chance in a large set of statistical tests and compare this with our findings.

Soil training effects on the soil microbial communities at the end of the experiment after 14 months were analyzed as described above; the only exception was the PLFA data for which we ignored species richness in the final analysis because it had little explanatory power. Interactions between soil-training effects and phytometer diversity could not be calculated because soil variables were only measured in the three-species mixtures of phytometers.

Table 2 provides a complete skeleton analysis of variance to show how the variance ratios were calculated. If effects are not listed in tables, significance levels are given in the text. Where necessary, dependent variables were transformed to meet the assumptions of normality and homoscedasticity.

Results

Abiotic soil characteristics

In general, variation in the measured abiotic soil characteristics between the 64 pots containing

Table 3. Overview of significances of effects on variables measured during 14 months of phytometer growth

		A phyto_div	A phyto_spec	B log_lindiv	B deviation	B lindiv	B deviation	B legumes	B original_mix
Two weeks	Germination rate	(*)	**	*		*			
Until 3 months	Weed individuals		**						***
One month	Leaf length	***	***						
One month	Leaf width	*	***					*	***
Three months	Leaf length	*	***				(*)		
Three months	Leaf width	*	***					**	
Three months	Biomass per pot		***		(*)		(*)	*	
Ten months	Leaf length	***	***						*
Ten months	Leaf width	**	***						(*)
Ten months	Flower no.		***					(*)	*
Ten months	Herbivory	*	***						*
Ten months	Pathogens	**	***						
Ten months	Biomass per pot		***						
Fourteen months	Flower no.	***	***						
Fourteen months	Biomass per pot	***	***						

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; (*), $P\leqslant 0.1$; empty cells, not significant.
A, phytometer treatment; B, diversity treatment in the field experiment from which the soil inocula had been taken.

phytometer mixtures was low. However, some significant or marginally significant soil carry-over effects of the original plant communities from which the inocula had been taken were still detectable after 14 months. Soil moisture (exluding one extremely high value, which may have been due to an accident during sample processing) was marginally higher in pots inoculated with soils trained with higher plant species richness ($P = 0.1$) and significantly higher in pots inoculated with soils trained with a higher number of plant functional groups ($P<0.05$). Plant biomass of the phytometer mixture after 14 months was marginally negatively correlated with soil moisture if included in the analysis as a covariate ($P<0.1$), indicating that soil moisture decreased with increasing plant biomass. The soil carbon content was significantly but non-linearly influenced by the original number of plant functional groups (deviation from lindiv $P<0.05$) and marginally positively related to the original frequency of legumes ($P<0.1$). The soil pH was also slightly higher in pots inoculated with soils trained with higher legume frequency ($P<0.1$).

Effects of phytometer diversity and phytometer species identity

The germination rate of phytometer species grown in mixtures was marginally significantly higher (+4.9%) than the average germination rate of phytometer species grown alone (phyto_div in Table 3). Mixtures also both had a significantly higher biomass (+32.2%) and flower number per pot. However, the leaves of phytometer species were 12.7% shorter and 7.7% narrower in mixtures than when grown alone (Table 3), indicating that the higher total biomass led to increased competition and therefore decreased module sizes at the level of leaf populations. Not surprisingly, the comparison among single-species pots showed highly significant differences between the three phytometer species in all variables measured over the 14-months duration of the experiment (phyto_spec, Table 3). However, there were also significant interactions between the original diversity-treatment effects and the phytometer species. In contrast to *P. lanceolata* and *D. glomerata*, the biomass of the legume *T. pratense* decreased with increasing number of species and with the former presence of legumes in a mixture or with increasing number functional groups, respectively. Ten months after the start of the experiment, the biomass of *T. pratense* monocultures was 41% lower if grown on soils of former 32-species mixtures compared with soils stemming from monocultures ($P<0.01$). In addition, *T. pratense* biomass on soils trained with legumes was 22% lower ($P<0.001$). At the end of the experiment, after 14 month, a significant decrease of *T. pratense* biomass with increasing number of functional groups was still detectable ($P = 0.05$).

Effects of soil-training by different plant diversity treatments

Germination of the phytometer species was higher when soil inocula had been trained with higher plant species richness (log_lindiv in Table 3; Fig. 2a) or with a higher number of plant functional groups (lindiv in Table 3; Fig. 2b). On average, 3.0% more phytometer

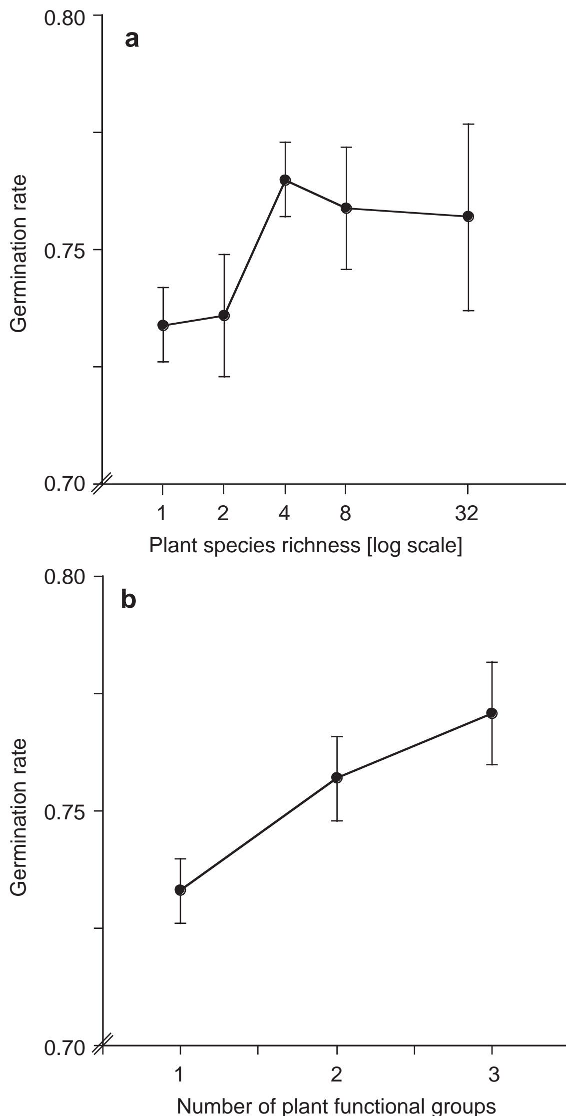

Fig. 2. Soil carry-over effects of (a) original plant species richness and (b) original number of plant functional groups on mean germination rate per pot (means ± 1 SE).

seeds germinated in soils that had been trained with 32 plant species than with monocultures and 4.9% more phytometer seeds germinated in soils trained with three plant functional groups than with one. When the microbial activity of the field soils in 1998 (measured with BIOLOG EcoPlates^{TM} as average well-colour development AWCD; Stephan et al., 2000) was included in the analysis as a covariate, it showed a positive effect on germination rates ($P<0.05$) and explained part of the positive soil carry-over effect of plant-species diversity (log_lindiv reduced to $F_{1,23} = 3.17; P<0.1$ and lindiv reduced to $F_{1,23} = 2.04; P>0.1$). None of the phytometer characters measured at later stages of growth was affected by the original plant diversity, indicating that these carry-over effects on plant growth were short lived (Table 3).

After 14 months of phytometer growth, catabolic activity of the culturable soil bacteria at 24 h averaged over all carbon sources (AWCD) was slightly lower in pots inoculated with soils trained with a higher number of plant functional groups (Table 4). Three of the seven subgroups of carbon sources, the amino acids ($AWCD_{aa}$), carbonic acids ($AWCD_{ca}$) and polymers ($AWCD_{p}$), were mainly responsible for this decline in activity (Table 4). However, catabolic activity of the culturable soil bacteria after 48 h was not related to the number of plant functional groups (overall AWCD), whereas the subgroups alcohols ($AWCD_{alc}$) and polymers ($AWCD_{p}$) still showed significant or marginally significant soil carry-over effects of the number of functional groups ($P<0.05$ and $P<0.1$, respectively). In contrast to catabolic activity (AWCD), the diversity (Shannon index) of carbon-source utilization of bacteria from the phytometer pots at 24 h was not influenced by any soil-training effect from the original field experiment ($P>0.1$). Diversity of carbon-source utilization at 48 h, however, increased log-linearly with the original plant species richness ($P<0.05$, Fig. 3). It also increased with the plant biomass of the phytometer mixture when this was fitted as a covariate ($P<0.05$). Using this covariate actually increased the significance of the log-linear effect of plant species richness ($P = 0.01$).

Microbial biomass in soils after 14 months of phytometer growth, represented by total PLFA content (Zelles et al., 1992; Hill et al., 1993; Balser et al., 2005), was not influenced by soil carry-over effects. However, the diversity of PLFAs decreased marginally in pots inoculated with soils trained with an increasing number of plant functional groups ($P<0.1$). The prokaryotic PLFAs showed a positive and the eukaryotic PLFAs a negative linear soil carry-over effect of plant functional groups (see Table 5; Fig. 4a and b, respectively). Gram-negative bacteria were mainly responsible for the linear increase of prokaryotic PLFAs with increasing number of plant functional groups, whereas all eukaryotic subgroups (fungi, VAM, protozoa) decreased linearly with the number of plant functional groups. Therefore, the eukaryotic/prokaryotic and fungi/prokaryotic ratios decreased linearly with the number of plant functional groups (Fig. 4c). In a principal component analysis, PC_1 explained 78% of the total variance in 20 fatty acids and was positively related to the original number of plant functional groups ($P<0.05$).

Effects of soil-training by legumes

The presence of legumes in the original field experiment affected the growth of the phytometers for up to 10 months after sowing (Table 3). These general legume effects were all negative (Table 6), although the opposite would have been expected if 'legume-trained' soil

Table 4. Pattern of significant and non-significant soil carry-over effects of plant diversity on catabolic activity of culturable soil bacteria at 24 h incubation 14 months after inoculation in pots with three-species phytometer mixtures

Chemical group	AWCD and C-sources at 24 h	Log-linear effect of species richness	Linear effect of plant functional groups	Deviation from linear effect of plant functional groups	Legume contrast: communities with vs. without legumes
	AWCD (whole ecoplate)	ns	0.059	ns	ns
	Alcohol AWCD	ns	ns	ns	ns
Alcohol, carbohydrate	*i*-Erythritol	ns	ns	ns	**0.019**
Alcohol, carbohydrate	D-Mannitol	ns	ns	ns	ns
Alcohol, phosphorylated	D,L-α-Glycerol phosphate	ns	ns	**0.043**	ns
	Amine AWCD	ns	ns	ns	ns
Amine	Phenylethylamine	ns	ns	ns	**0.013**
Amine	Putrescine	0.061	ns	ns	ns
	Amino acid AWCD	ns	**0.035**	ns	ns
Amino acid	L-Arginine	ns	**0.014**	**0.028**	ns
Amino acid	L-Asparagine	ns	0.068	ns	ns
Amino acid	L-Phenylalanine	ns	ns	ns	ns
Amino acid	L-Serine	ns	**0.032**	ns	ns
Amino acid	L-Threonine	ns	ns	ns	ns
Amino acid	Glycyl-L-glutamic acid	ns	ns	ns	ns
	Carbohydrate AWCD	ns	ns	ns	ns
Carbohydrate	β-Methyl-D-glucoside	ns	ns	ns	ns
Carbohydrate	D-Xylose	**0.006**	ns	ns	ns
Carbohydrate	*N*-acetyl-D-glucosamine	ns	ns	ns	ns
Carbohydrate	D-Cellobiose	ns	ns	ns	ns
Carbohydrate	α-D-Lactose	ns	ns	ns	ns
Carbohydrate, alcohol	*i*-Erythritol	ns	ns	ns	**0.019**
Carbohydrate, alcohol	D-Mannitol	ns	ns	ns	ns
Carbohydrate, phosphorylated	Glucose-1-phosphate	ns	ns	ns	ns
Carbohydrate, polymer	α-Cyclodextrin	ns	ns	ns	ns

Carbohydrate, polymer	Glycogen	ns	ns	0.057	ns
	Carbonic acid AWCD	ns	0.088	ns	ns
Carbonic acid	D-Galactonic acid γ-lactone	ns	**0.016**	ns	ns
Carbonic acid	D-Galacturonic acid	0.089	**0.022**	ns	ns
Carbonic acid	2-Hydroxybenzoic acid	ns	ns	ns	ns
Carbonic acid	4-Hydroxybenzoic acid	ns	**0.019**	0.058	ns
Carbonic acid	γ-Hydroxybutyric acid	ns	0.074	ns	ns
Carbonic acid	D-Glucosaminic acid	ns	ns	ns	ns
Carbonic acid	Itaconic acid	0.098	**0.038**	ns	ns
Carbonic acid	α-Ketobutyric acid	ns	ns	ns	ns
Carbonic acid	D-Malic acid	ns	ns	ns	ns
Carbonic acid, ester	Pyruvic acid methyl ester	ns	**0.041**	0.100	ns
	Phosphorylated AWCD	ns	ns	0.087	ns
Phosphorylated, alcohol	D,L-α-Glycerol phosphate	ns	ns	**0.043**	ns
Phosphorylated, carbohydrate	Glucose-1-phosphate	ns	ns	ns	ns
	Polymer AWCD	ns	**0.011**	ns	ns
Polymer	Tween 40	ns	**0.008**	ns	ns
Polymer	Tween 80	ns	**0.013**	ns	ns
Polymer, carbohydrate	α-Cyclodextrin	ns	ns	ns	ns
Polymer, carbohydrate	Glycogen	ns	ns	0.057	ns

Table entries are P-values (type-I error probabilities that the effect was significant by chance) from analyses of variance (bold numbers, $P \leq 0.05$; normal numbers, $P \leq 0.1$; ns, not significant).

inocula had increased nitrogen levels in the pots. Soil-training by legumes decreased the leaf width of phytometers after 1 and 3 months. Furthermore, it decreased total biomass per pot after 3 months of growth by 10.4% and number of flowers per pot after 10 months by 40%. These negative soil-training effects of legumes were also observed when we tested for legume frequency instead of legume presence or absence in the plant communities of the original field experiment: leaf width after 1 and 3 months, total biomass per pot after 3 months, and total number of flowers per pot after 10 months all decreased linearly with legume frequency (legufreq in Table 6).

Legume presence in the original field experiment had a marginally ($P<0.1$) negative soil carry-over effect on bacterial utilization of alcohols ($AWCD_{alc}$) and carbonic acids ($AWCD_c$) at 48 h incubation, but did not significantly affect patterns of PLFAs.

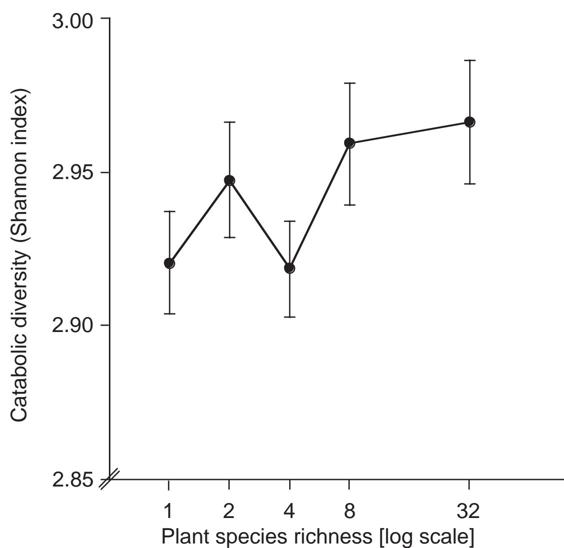

Fig. 3. Relationship between plant species richness in a field experiment and catabolic diversity (Shannon index) of culturable soil bacteria (48 h incubation) 14 months after inoculation in pots with a three-species phytometer mixture (means ± 1 SE).

Effects of soil-training by particular plant communities or particular plant species

Carry-over effects of particular plant species combinations from the original field experiment were observed for up to 10 months in the phytometer measurements (original_mix in Table 3). After 1 month, the leaf width of phytometers varied significantly among the different plant species combinations and the number of weed individuals per pot was influenced up to 3 months. After 10 months, herbivory and the following phytometer characters were still significantly affected by specific plant species combinations from the original field experiment: leaf width, leaf length, total number of flowers per pot (original_mix in Table 3).

Soil inocula trained with particular plant species compositions had no significant effects on the culturable soil bacteria and PLFAs after 14 months of phytometer growth.

In total, 18 plant species that occurred at more than one diversity level in the field experiment were tested for their individual soil-training effects. Seventeen of these plant species had 48 marginally significant ($P\leqslant 0.1$) and 30 significant effects ($P\leqslant 0.05$) on 16 variables measured on the phytometers (see Table 6 for examples). Only one species, *T. repens*, did not affect any of the measured

Table 5. Pattern of soil carry-over effects of plant diversity on PLFA variables 14 months after inoculation in pots with a three-species phytometer mixture

PLFA subgroups (mol%)	Linear effect of of plant functional groups	Deviation from linear effect of plant functional groups	Legume contrast: communities with vs. without legumes
Bacteria, prokaryotes	**0.006+**	ns	ns
Gram-positive bacteria	ns	ns	ns
Gram-negative bacteria	**0.012+**	ns	ns
Eukaryotes	**0.015–**	ns	ns
Protozoa	**0.014–**	ns	ns
VAM	0.073–	ns	ns
Fungi	**0.019–**	ns	ns
Ratios			
Eukaryotes/prokaryotes	**0.007–**	ns	ns
Fungi/prokaryotes	**0.009–**	ns	ns
Gram-pos./Gram-neg.	ns	ns	ns

Table entries are *P*-values (type-I error probabilities that the effect was significant by chance) from analyses of variance (bold numbers, $P\leqslant 0.05$; normal numbers, $P\leqslant 0.1$; ns, not significant). Symbols indicate direction of effects (+, increase; –, decrease). Soil carry-over effects of the number of plant species were not included in the model (see 'Material and methods').

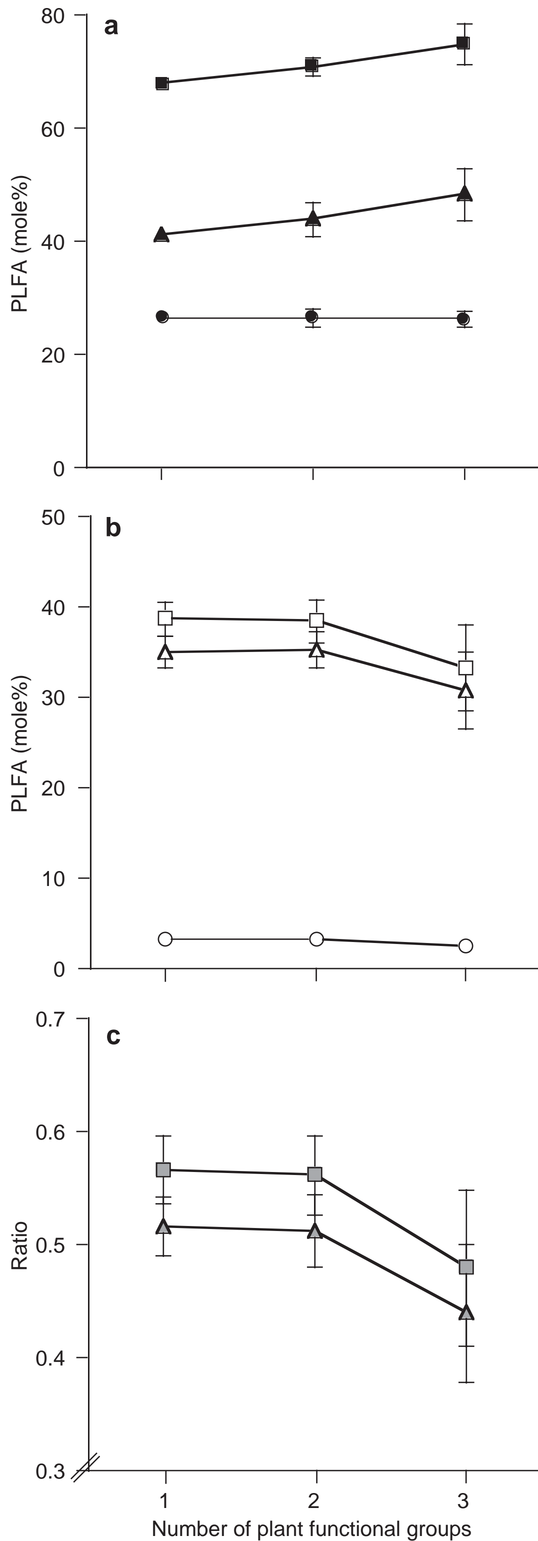

Fig. 4. Relationship between number of plant functional groups in a field experiment and (a) prokaryotic PLFAs (square), partitioned into Gram-negative (triangle) and Gram-positive (circle) bacteria, (b) eukaryotic PLFAs (square), partitioned into fungi (triangle) and protozoa (circle), and (c) ratios of eukaryotic/prokaryotic (square) and fungi/prokaryotic (triangle) PLFAs, all 14 months after inoculation in pots with a three-species phytometer mixture (means ± 1 SE).

phytometer variables. About half of the marginally significant or significant effects might have occurred by chance (type-I errors; 29 for $P \leqslant 0.1$ and 14 for $P \leqslant 0.05$). This means that a substantial number of soil carry-over effects of particular plant species were real but we cannot know which ones (as much as we do not know which effects were missed, i.e. represented type-II errors). We discuss the ones from Table 6 that seem to us to be biologically most plausible or additionally had effects on belowground parameters 14 months after the start of the experiment.

The germination rate of phytometers (assessed after 2 weeks) and their leaf length (assessed after 1 month) were each influenced by particular plant species: *P. lanceolata* had a positive soil carry-over effect on germination rate (+6.3%) and *D. glomerata* had a negative soil carry-over effect on leaf length (−4.8%). The grass *Poa pratensis* L. had a negative soil carry-over effect on the number of weed individuals in phytometer pots. The leaf width of phytometers was marginally decreased if soil inocula came from field plots with *T. pratense*, which corresponds to the significant legume carry-over effect on this character (compare Tables 3 and 6). The leaf width, herbivory level and pathogen level after 10 months of phytometer growth were higher when pots had been inoculated with soils trained by plant communities containing *Lathyrus pratensis* L. (first two measures) or *Lotus corniculatus* L. (third measure). The three grass species *Arrhenatherum elatius* (L.) J. & C. Presl, *Cynosurus cristatus* L. and *D. glomerata* all had negative soil carry-over effects on phytometer leaf width, leaf length, biomass per pot, and number of flowers per pot. In summary, soil carry-over effects of particular plant species, in contrast to those of plant diversity, did not decline in significance with time (Table 6).

Ten of the 18 plant species tested showed at least marginally significant individual soil carry-over effects on catabolic activity and diversity of bacteria in the phytometer pots ($P \leqslant 0.1$, Table 7). However, there were few significant effects ($P \leqslant 0.05$), indicating that some of them may represent random occurrences of significance (type-I errors; Rice, 1989; Moran, 2003). Some of these effects, however, correspond with similarly negative soil carry-over effects on phytometer performance. An example for this was the marginally negative soil carry-over effect of *A. elatius* on catabolic diversity at 24 and 48 h (Shannon index; Table 7).

Four plant species (*A. elatius, L. pratensis*, *T. pratense, Knautia arvensis* (L.) Coult.) had 25 marginally significant ($P \leqslant 0.1$) and 16 significant ($P \leqslant 0.05$) soil carry-over effects on 11 PLFAs (Table 8). Most striking were the consistently negative effects of *A. elatius* and *L. pratensis* and the positive effects of *T. pratense* and *K. arvense* on the abundance of microorganisms, especially eukaryotic ones (protozoa, VAM, fungi), as measured

Table 6. Pattern of soil carry-over effects of particular plant species compositions (original_mix), legume functional group (legumes, legufreq), and particular plant species (columns with species names) on variables measured during 14 months of phytometer growth. Number of plots in the original field experiment with or without (the latter in parentheses) legumes or the particular species are given in the column heads

Variable and time	Original_mix	Legumes 26 (38)	legufreq	Grass, *Arrhenatherum elatius* 22 (42)	Grass, *Cynosurus cristatus* 10 (54)	Grass, *Dactylis glomerata* 20 (44)
Germination rate (after 2 weeks)						
Weed individuals (until 3 months)	<**0.001**					
Leaf width (after 1 month)	<**0.001**	**0.026**–	0.058–			
Leaf length (after 1 month)						0.092–
Leaf width (after 3 months)		**0.005**–	**0.017**–			
Leaf length (after 3 months)						
Biomass per pot (after 3 months)	0.057	**0.022**–	**0.022**–			
Leaf width (after 10 months)						**0.022**–
Leaf length (after 10 months)	**0.031**			**0.035**–	**0.049**–	
Biomass per pot (after 10 months)	0.100			0.055–	**0.019**–	
Flower No. (after 10 months)	**0.004**	0.052–	0.055–	0.080–	0.053–	
Herbivory (after 10 months)	**0.021**					
Pathogens (after 10 months)						
Biomass per pot (after 14 months)				**0.025**–	**0.015**–	
Flower no. (after 14 months)					**0.037**–	**0.043**–

Variable and time	Grass, *Festuca pratense* 20 (44)	Grass, *Festuca rubens* 10 (54)	Grass, *Poa pratensis* 22 (42)	Grass, *Trisetum flavescens* 18 (46)	Legume, *Lathyrus pratensis* 8 (56)	Legume, *Lotus corniculatus* 8 (56)
Germination rate (after 2 weeks)						
Weed individuals (until 3 months)			**0.047**–			
Leaf width (after 1 month)						
Leaf length (after 1 month)						
Leaf width (after 3 months)				**0.048**+		
Leaf length (after 3 months)						
Biomass per pot (after 3 months)						
Leaf width (after 10 months)	**0.007**–	**0.007**+			<**0.001**+	
Leaf length (after 10 months)	**0.024**+					
Biomass per pot (after 10 months)	0.100+					
Flower No. (after 10 months)	**0.048**+	0.081–				
Herbivory (after 10 months)	**0.028**–	**0.045**+			**0.035**+	
Pathogens (after 10 months)						**0.029**+
Biomass per pot (after 14 months)						

Flower no. (after 14 months)	**0.045+**

Variable and time	Legume, *Trifolium pratense* 14 (50)	Non-leguminous herb, *Achillea millefolium* 8 (56)	Non-leguminous herb, *Knautia arvensis* 6 (58)	Non-leguminous herb, *Plantago lanceolata* 12 (52)
Germination rate (after 2 weeks)				**0.042+**
Weed individuals (until 3 months)				
Leaf width (after 1 month)	0.053−			
Leaf length (after 1 month)				
Leaf width (after 3 months)	0.090−			
Leaf length (after 3 months)				
Biomass per pot (after 3 months)				
Leaf width (after 10 months)				0.091−
Leaf length (after 10 months)		**0.047−**	0.052+	
Biomass per pot (after 10 months)		**0.049−**		**0.021+**
Flower no. (after 10 months)		**0.024−**		**0.015+**
Herbivory (after 10 months)			0.054−	
Pathogens (after 10 months)		**0.022+**		
Biomass per pot (after 14 months)				
Flower no. (after 14 months)			0.079+	

Table entries are *P*-values (type-I error probabilities that the effect was significant by chance) from analyses of variance (bold numbers, $P \leqslant 0.05$; normal numbers, $P \leqslant 0.1$; empty cells, not significant). Symbols indicate direction of effects (+, increase; −, decrease). In these analyses the tested terms (columns) were fitted individually, i.e. without previously removing any of the other terms (but notice that in Table 3 legume contrast and original_mix were fitted together in this sequence; see 'Material and methods').

by the PLFAs. Surprisingly, soil training with the legumes *L. pratensis* and *T. pratense*, both known to be associated with N_2-fixing bacteria (*Rhizobium*, Gram-negative), did not positively influence the PLFAs stemming from Gram-negative bacteria. Hence, the biomass of legume associated N_2-fixing bacteria might have been relatively low.

Interactions between phytometer-diversity and soil-training effects

The total phytometer biomass after 3 months of growth increased with the original plant-species diversity in phytometer mixtures (+36.7% from 1 to 32 plant species in original plant community) but not in phytometer monocultures (–5.3%, from 1 to 32 plant species in original plant community; phyto_div × log_lindiv interaction, $P<0.05$). However, pots inoculated with soil trained under high species richness after 3 months had a marginally stronger weed-suppressing effect on phytometer monocultures (−22.8%) than mixtures (−10.4%; phyto_div × log_lindiv interaction, $P<0.1$). After 3 months, soil carry-over effects of legumes on the leaf width of phytometers were more negative in monocultures than in mixtures (phyto_div × legumes interaction, $P<0.05$). After 10 months of growth, however, the direction of this interaction effect was reversed and phytometers showed a greater increase in leaf width in monocultures than in mixtures when grown on soils trained with legumes (phyto_div × legumes interaction, $P<0.05$). These results show that effects of soil training can affect future plant growth differently if plants are grown in monoculture rather than mixture.

Discussion

Effects of phytometer diversity

In mixtures of the three phytometer species, overall germination rate, flower number, and biomass per pot were increased compared with phytometer monocultures. Increased net primary productivity with increasing plant-species richness has been shown in many biodiversity experiments (e.g. Naeem et al., 1994; Tilman et al., 1997; Hector et al., 1999; van Ruijven and Berendse, 2003; see Schmid et al., 2002a for a review). To our knowledge, the observed positive influence of plant-seed diversity on germination rate, however, is reported for the first time here, although we have previously observed it in another biodiversity experiment (Hauser, 1999). This effect, which is unlikely due to reduced resource competition between seeds of different species, indicates that other interactions in the belowground ecosystem compartment might play a role, such as effects of substances released by seeds or microorganisms (see Sturz and Christie, 2003 for a review). Enhanced flower production in the diverse phytometer communities may have been due to smaller interspecific competition compared with intraspecific competition in monocultures.

Soil carry-over effects on abiotic soil characteristics

The original plant diversity and species compositions of the field experiment (1995–2000), which had ‘trained’ the soil inocula used in the phytometer pot experiment had relatively minor effects on abiotic soil conditions measured after 14-months of phytometer growth (2000–2001). There were indications that the original number of plant functional groups affected soil moisture and soil carbon content. In addition, pH and soil carbon content increased slightly with increasing frequency of legumes in the original plant communities. Because the soil inocula were small relative to the total amount of substrate in the pots these abiotic effects were perhaps mediated by microbial communities, as discussed below for the biotic characteristics.

General evidence for biotic soil carry-over effects on plant growth

The original plant diversity and species compositions of the field experiment had several carry-over effects on the growth of phytometer species over the 14-months observation period. We interpret the positive correlation between the microbial activity of the field soils in 1998 (Stephan et al., 2000) and the germination rate of the phytometer species in 2000 as indication that these soil carry-over effects were caused by soil microbes and not by nutrients. Furthermore, the detected negative effects on phytometer growth of soil inocula taken from field plots containing legumes is a further indication that the observed soil carry-over effects were not nutrient-related, because legumes positively affect soil-nitrogen levels (Spehn et al., 2002; Scherer-Lorenzen et al., 2003). Despite the resource-uptake by phytometers, soil carry-over effects persisted over time. Clearly, there are many ways in which plant communities can affect the biomasses or population densities of belowground primary (e.g. microbes, herbivorous nematodes) and secondary consumers (e.g. microbe-feeding nematodes and enchytraeids; Wardle et al., 2003 and references therein). All these offer possibilities for plant diversity, community composition, or the presence of particular plant species to feed back via biotic interactions in the soil compartment on future plant growth and ecosystem functioning. The changing nature of particular soil carry-over effects over time suggests that the phytometer

Table 7. Pattern of soil carry-over effects of particular plant species (columns with species names) on catabolic activity and diversity of culturable soil bacteria 14 months after inoculation in pots with three-species phytometer mixtures

Variable	Grass *Arrhenatherum elatius* 22 (42)	Grass *Dactylis glomerata* 20 (44)	Grass *Festuca pratense* 20 (44)	Grass *Lolium perenne* 22 (42)	Grass *Poa pratensis* 22 (42)	Grass *Trisetum flavescens* 18 (46)	Legume *Trifolium pratense* 14 (50)	Non-leguminous herb *Achillea millefolium* 8 (56)	Non-leguminous herb *Plantago lanceolata* 12 (52)
AWCD (whole ecoplate) 24 h	ns	0.062−	ns	ns	ns	ns	ns	ns	ns
AWCD (whole ecoplate) 48 h	ns	ns	ns	ns	ns	ns	ns	ns	ns
Alcohol AWCD 24 h	ns	ns	ns	ns	ns	0.057 +	0.099−	ns	ns
Alcohol AWCD 48 h	ns	ns	ns	ns	ns	ns	ns	ns	ns
Amine AWCD 24 h	ns	ns	**0.048 +**	ns	ns	ns	ns	ns	ns
Amine AWCD 48 h	0.100−	ns	ns	ns	ns	0.055−	ns	ns	ns
Amino acid AWCD 24 h	ns	**0.019−**	ns	ns	ns	ns	ns	ns	ns
Amino acid AWCD 48 h	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate AWCD 24 h	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbohydrate AWCD 48 h	ns	ns	ns	ns	ns	ns	ns	ns	ns
Carbonic acid AWCD 24 h	ns	0.100−	ns	ns	ns	ns	ns	ns	ns
Carbonic acid AWCD 48 h	ns	ns	ns	ns	0.085−	ns	ns	ns	ns
Phosphorylated AWCD 24 h	ns	ns	ns	ns	ns	0.066 +	0.082−	ns	ns
Phosphorylated AWCD 48 h	ns	ns	ns	**0.002 +**	ns	ns	ns	ns	ns
Polymer AWCD 24 h	ns	0.099−	ns	ns	ns	ns	ns	ns	ns
Polymer AWCD 48 h	ns	ns	ns	ns	ns	ns	ns	ns	**0.048−**
Shannon diversity index 24 h	0.060−	ns	ns	ns	ns	ns	ns	**0.033−**	ns
Shannon diversity index 48 h	0.091−	ns	ns	ns	**0.023 +**	ns	ns	ns	ns

Number of plots in the original field experiment with or without (the latter in parentheses) the particular species are given in the column heads. Table entries are *P*-values (type-I error probabilities that the effect was significant by chance) from analyses of variance (bold numbers, $P \leqslant 0.05$; normal numbers, $P \leqslant 0.1$; ns, not significant). Symbols indicate direction of effects (+1, increase; − decrease). In these analyses the tested terms (columns) were fitted individually, i.e. without previously removing any of the other terms (see 'Material and methods').

Table 8. Pattern of soil carry-over effects of particular plant species (columns with species names) on PLFA variables 14 months after inoculation in pots with three-species phytometer mixtures

Variable	Grass *Arrhenatherum elatius* 22 (42)	Legume *Lathyrus pratensis* 8(56)	Legume *Trifolium pratense* 14(50)	Non-leguminous herb *Knautia arvensis* 6 (58)
PLFA groups (mol%)				
Bacteria, prokaryotes				
Gram-positive bacteria	**0.036**–			
Gram-negative bacteria				
Eukaryotes	**0.009**–	0.054–	0.058+	**0.004**+
Protozoa	0.077–		**0.017**+	**0.018**+
VAM	**0.006**–	0.087–	0.078+	**0.012**+
Fungi	**0.008**–	**0.044**–	0.081+	**0.004**+
Ratios				
Eukaryotes/prokaryotes	**0.021**–	0.052		**0.006**+
Fungi/prokaryotes	**0.019**–	**0.044**–		**0.006**+
Gram-pos./Gram-neg.	0.097–			
Diversity index				
Shannon	**0.039**–			0.081+

Number of plots in the original field experiment with or without (the latter in parentheses) the particular species are given in the column heads. Table entries are P-values (type-I error probabilities that the effect was significant by chance) from analyses of variance (bold numbers, $P \leqslant 0.05$; normal numbers, $P \leqslant 0.1$; empty cells, not significant). Symbols indicate direction of effects (+, increase; –, decrease). In these analyses the tested terms (columns) were fitted individually, i.e. without previously removing any of the other terms (see 'Material and methods').

species may have been influenced by different feedback mechanisms at different stages of their growth.

Soil carry-over effects of plant diversity

The original number of plant species and functional groups showed short-term carry-over effects on the germination rate of phytometers. Both, monocultures and mixtures of phytometers appeared to benefit from the microbial communities developing under soils with higher plant diversity. After 3 months of growth, the original plant species richness still influenced the per-pot biomass of the phytometers in mixtures positively but not in monocultures. The original gradient in microbial diversity may thus have deteriorated more rapidly in phytometer monocultures than in mixtures. Thus, in the same way as the original gradient was created by a diverse plant community, it may have been maintained for longer by a diverse phytometer community. After 10 months, however, biomass production of phytometer mixtures ceased to be influenced by the original plant species richness even though higher plant-species richness still had detectable effects on the diversity of culturable soil bacteria 14 months after the start of the experiment.

Kowalchuk et al. (2002) and Reynolds et al. (2003) suggested that particular plant species promote particular soil-borne microbial populations in the rhizosphere, which would explain the increased microbial diversity in soils from more diverse plant communities. After experiencing a common plant environment of three phytometer species for 14 months, catabolic diversity (as defined by laboratory substrate utilization test) of culturable bacteria was still higher in pots, which had received soil inocula trained for 5 years under high plant-species richness in the original field experiment. Although less than 1% of the soil bacterial community may be culturable (Torsvik and Ovreas, 2002), the culturable bacteria are still considered to be a useful indicator for measuring feedback effects of the plant community on the bacterial decomposer subsystem in soils. The observed positive effect of original plant species richness on the catabolic activity of culturable bacteria was partly due to differences in phytometer biomass (an effect already described by Zak et al., 2003), but even after correcting for the phytometer biomass, the positive effect of original plant species richness persisted. Similarly, Stephan et al. (2000) observed in a previous study a positive linear relationship between the number of plant functional groups and the catabolic diversity and activity of soil bacteria in the original field experiment from which soil inocula were taken. However, with increasing number of functional groups, we observed a slightly linear decrease in the catabolic activity of soil. There was also a negative relationship between the number of plant functional groups and the PLFAs derived from eukaryotic microorganisms (protozoa and fungi) whereas PLFAs derived from prokaryotic microorganisms (primarily Gram-negative bacteria) were positively affected. This may be due to the increase in rhizosphere volume (increase in root mass)

as plant diversity increased. Gram-negative bacteria are dominant in rhizosphere soil. The difference between the positive response of Gram-negative bacteria and the negative response of culturable bacteria in general and eukaryotic microorganisms to increased plant functional diversity may indicate that microbial community composition can shift in complex ways (see Balser and Firestone, in press). In a grassland biodiversity study in California, it was also found that plant functional-group diversity influenced microbial activity and community composition in complex ways; as plant functional group richness increased, microbial activity did not necessarily also increase (T.C. Balser, unpubl. data).

The 'quality aspect' of ecosystem functioning may be better reflected by diversity rather than activity measures of microbial communities (Stephan et al., 2000; Joshi et al., 2004). We observed a positive effect of original plant species number on the catabolic diversity of culturable soil bacteria. Catabolic diversity reflects diversity of carbon-oxidation pathways and therefore functional diversity of soil bacteria (Insam et al., 1996; Ovreas and Torsvik, 1998; Sharma et al., 1998; Staddon et al., 1998), which in turn is related to taxonomic diversity (Haack et al., 1995; Buyer and Drinkwater, 1997; Ibekwe and Kennedy, 1998; Ovreas and Torsvik, 1998). In this case, the results of our experiment parallel those of Stephan et al. (2000), suggesting that the 'diversity memory' of soils is more consistent than the 'activity memory'. However, when we considered the complete microbial diversity as measured by diversity of PLFAs, we could not detect positive soil carry-over effects of plant diversity. Similarly, Wardle et al. (1999) found no relationship between the diversity of PLFA and plant-species richness in a plant removal experiment. The differences between the response of microbial community composition vs. function to changing plant diversity may also reflect the different methods used. Lipid analysis is a measure of in situ microbial community composition, whereas catabolic diversity/substrate utilization is a laboratory assay. Caution is required in interpreting the results since the catabolic diversity measured here may not accurately reflect activity in the soil. However, despite methodological uncertainty, what is most striking in this study is that we were able to demonstrate a carry-over effect on both microbial community composition and function from previous plant growth. This is an important finding and underscores the potential importance of microbial communities as driving variables in an ecosystem, rather than simply being passive carbon or nutrient catalysts (Balser and Firestone, in press).

Soil carry-over effects of legumes

The symbiotic association of the legumes with nitrogen-fixing bacteria had no net beneficial effect on the phytometer species. All detected soil carry-over effects of legumes negatively affected plant growth. The negative soil carry-over effects of legumes on the leaf width of phytometers might have been driven mainly by *T. pratense* (see Table 6). A significant contribution of *T. pratense* on ecosystem functioning was also observed in the field experiment from which the soil inocula had been taken (Spehn et al., 2002). In addition, Taylor and Quesenberry (1996) reported that *T. pratense* could accumulate root-rot when growing for several years in monocultures. Therefore, the negative effects on phytometer growth of soils trained by *T. pratense* could have originated from species-specific soil-borne pathogens (Van der Putten, 2003). In this connection, the increase in protozoa (and to a lesser degree in VAM and fungi) after inoculation with soil trained by *T. pratense* could mean that some of these were pathogenic. The negative effect of these soils on the phytometer species *T. pratense* is a further indication that indeed soil pathogens were involved in the negative effect on phytometer growth. Soil training by two other legume species, *L. pratensis* and *L. corniculatus*, increased herbivory and pathogen levels on phytometers, respectively. In addition, *L. pratensis* negatively influenced the presence of fungi in the soil (see next section on effects of particular plant species). This suggests that different species of legumes contribute to the negative soil carry-over effects of this special plant functional group.

Although these negative effects of soil training by legumes on phytometer growth lasted for up to 10 months, legumes as a functional group had only minor soil carry-over effects on the soil microbial communities developed after 14 months of phytometer growth and assessed with patterns of PLFAs and culturable soil bacteria. In the original field experiment, a positive effect of legume presence on catabolic activity of culturable soil bacteria was observed (Stephan et al., 2000). It appears that soil carry-over effects of legume presence are shorter-lived than those of plant diversity, perhaps because they are of a more quantitative (e.g. amount of fixed nitrogen) rather than qualitative (e.g. occurrence and diversity of particular litter compounds) nature. It is well known that positive effects on ecosystem functioning of legume presence are largely due to their fertilizing effect (e.g. Scherer-Lorenzen et al., 2003), in particular increasing nitrogen availability in soil.

Soil carry-over effects of particular plant species

All diversity effects can be attributed to the traits of individual species and their interactions (Lawton, 2000). In addition to the soil carry-over effects on phytometer growth associated with legume functional group and

legume species there were a number of other carry-over effects of particular plant species grown in the original field experiment (see Table 6). Interestingly, species-specific carry-over effects were more persistent than diversity-related carry-over effects. In fact, several species-specific effects appeared only later in the phytometer experiment and some remained until the end of the 14-months observation period. Species-specific effects at the end of the experiment also influenced soil microbial community composition and in some cases paralleled those on phytometer growth. Soil carry-over effects of particular plant species mainly influenced eukaryotic PLFAs (see Table 8). In contrast to the phytometer growth and performance, these effects were due only to four particular plant species. Most strikingly, soil training with *A. elatius* not only had a negative soil carry-over effect on the aboveground phytometer biomass after 14 months but also reduced soil catabolic diversity, eukaryotic PLFAs (protozoa, VAM, fungi), Gram-positive PLFAs and diversity of PLFAs. Perhaps the negative feedback of this particular plant species grown in the field experiment on phytometer growth was caused by a reduced abundance of VAM in the trained soils (see e.g. van der Heijden et al., 1998). Species-specific soil carry-over effects of the legume *L. pratensis* on fungal PLFAs were also negative, whereas another legume, *T. pratense*, and the non-leguminous herb *K. arvensis* positively influenced eukaryotic PLFAs. These results, however, were not paralleled by effects on phytometer growth in the same direction. In a previous analysis of the soils from our field experiment, Stephan et al. (2000) also found that particular plant-species compositions of experimental communities and the presence of particular species in these were often important for explaining patterns of activity and diversity of culturable soil bacteria. Also, Wardle et al. (2003) pointed out the importance of plant species identity in driving the decomposer sub-system in ecosystems. From the results of our study, we have now substantial evidence for species-specific effects resulting from biotic interactions between plants and soil organisms. According to an expert survey (Schläpfer et al., 1999), biotic variables of ecosystem functioning such as pest control may not show the typical continuous flattening with increasing species richness but initially even increase in slope due to the fact that the number of possible interactions rises faster than the number of species.

Conclusions

Our results provide the first evidence of carry-over effects in soil taken from biodiversity experiments on subsequent plant growth. The soil carry-over effects of original plant diversity played an important role during early phytometer growth, whereas the effects of original plant-species identity influenced phytometer growth over a longer time span. The biological interpretation of the observed effects at present is still speculative and hypothetical. Nevertheless, the negative carry-over effects of legumes and positive carry-over effects of plant diversity, as well as the persistent carry-over effects of particular plant species with time strongly suggest that soil microbial communities play a major role. A further specific indication of biotic soil carry-over effects is the significant correlation between the activity of culturable soil bacteria in the original field experiment and the germination of phytometers in pots. Although the specific mechanisms of soil carry-over effects from biodiversity experiments remain to be determined, our study demonstrates that they exist and involve shifts in soil microbial communities. The observed soil carry-over effects show that feed-back mechanisms between plants and microbial communities can last for a year or more, which seems extraordinary considering the short generation times of microbes, their potential for fast population dynamics and the rapid evolution of microorganisms. It is thus conceivable that the ‘memory’ of soils with regard to microbial communities is a more important factor in ecosystem development than previously assumed. Further experiments will have to be designed to directly determine the nature of the linking mechanism between the above- and below-ground subsystems.

Acknowledgements

We thank Theres Zwimpfer, René Husi, Ryosuke Fujinuma, Michael Bonkowski, Lindsay Turnbull, Peter Edwards and three anonymous reviewers for help in carrying out the experiment, analyzing samples, interpreting data and improving the manuscript, respectively. This research was supported by grants from the Swiss National Science Foundation (Grant No. 31–65224.01 to BS and Grant No. 31-065600.01 to HB) and the German Science Foundation (Grant No. FOR 456—WE 2618/6-1 to Wolfgang Weisser and BS).

References

Allen, S.E., 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

Balser, T.C., Firestone, M.K., in press. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer ecosystem. Biogeochemistry.

Balser, T.C., Kirchner, J.W., Firestone, M.K., 2002. Methodological variability in microbial community-level physiological profiles. Soil Sci. Soc. Am. J. 66, 519–523.

Balser, T.C., Treseder, K.K., Ekenler, M., 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol. Biochem. 37, 601–604.

Bardgett, R.D., 2002. Causes and consequences of biological diversity in soil. Zoology 105, 367–374.

Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol. Biochem. 31, 317–321.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol. Biochem. 30, 1867–1878.

Begon, M., Harper, J.L., Townsend, C.R., 1990. Ecology – Individuals, Populations, Communities. Blackwell Scientific Publications, Oxford.

Bever, J.D., 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol. 157, 465–473.

Bonkowski, M., Cheng, W.X., Griffiths, B.S., Alphei, J., Scheu, S., 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. Eur. J. Soil Biol. 36, 135–147.

Broughton, L.C., Gross, K.L., 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125, 420–427.

Burr, T.J., Caesar, A., 1984. Beneficial plant bacteria. Crit. Rev. Plant Sci. 2, 1–20.

Buyer, J.S., Drinkwater, L.E., 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. J. Microbiol. Methods 30, 3–11.

Copley, J., 2000. Ecology goes underground. Nature 406, 452–454.

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

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

Diemer, M., Joshi, J., Koerner, C., Schmid, B., Spehn, E.M., 1997. An experimental protocol to assess the effects of plant diversity on ecosystem functioning utilized in a European research network. Bull. Geobot. Inst. ETH 63, 95–107.

Frostegard, A., Baath, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.

Garland, J.L., 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. 28, 213–221.

Groffman, P.M., Eagan, P., Sullivan, W.M., Lemunyon, J.L., 1996. Grass species and soil type effects on microbial biomass and activity. Plant Soil 183, 61–67.

Haack, S.K., Garchow, H., Klug, M.J., Forney, L.J., 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61, 1458–1468.

Hansen, R.A., 2000. Effects of habitat complexity and composition on a diverse litter microarthropod assemblage. Ecology 81, 1120–1132.

Hauser, U., 1999. Einfluss von erhöhtem CO_2 und unterschiedlicher Diversität auf die Keimung von Wildkräutern. Diploma thesis, University of Zurich, Zurich, Switzerland.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 1999. Plant diversity and productivity experiments in European grasslands. Science 286, 1123–1127.

Hector, A., Bazeley-White, E., Spehn, E.M., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Jumpponen, A., Koricheva, J., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Palmborg, C., Pereira, J.S., Pfisterer, A.B., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 2005. Ecosystem effects of biodiversity manipulations in European grasslands. Ecol. Arch. 075–001.

Hedlund, K., Regina, I.S., van der Putten, W.H., Leps, J., Diaz, T., Korthals, G.W., Lavorel, S., Brown, V.K., Gormsen, D., Mortimer, S.R., Barrueco, C.R., Roy, J., Smilauer, P., Smilauerova, M., van Dijk, C., 2003. Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags. Oikos 103, 45–58.

Heemsbergen, D.A., Berg, M.P., Loreau, M., van Hal, J.R., Faber, J.A., Verhoef, H.A., 2004. Biodiversity effects on soil processes explained by intespecific functional dissimilarity. Science 306, 1019–1020.

Hill, T.C.J., McPherson, E.F., Harris, J.A., Birch, P., 1993. Microbial biomass estimated by phspholipid phosphate in soils with diverse microbial communities. Soil Biol. Biochem. 25, 1779–1786.

Hooper, D.U., Bignell, D.E., Brown, V.K., Brussaard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., van der Putten, W.H., de Ruiter, P.C., Rusek, J., Silver, W.L., Tiedje, J.M., Wolters, V., 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. Bioscience 50, 1049–1061.

Ibekwe, A.M., Kennedy, A.C., 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiol. Ecol. 26, 151–163.

Insam, H., Mitchell, C.C., Dormaar, J.F., 1991. Relationship of soil microbial biomass and activity with fertilization

practice and crop yield of 3 ultisols. Soil Biol. Biochem. 23, 459–464.

Insam, H., Amor, K., Renner, M., Crepaz, C., 1996. Changes in functional abilities of the microbial community during composting of manure. Microb. Ecol. 31, 77–87.

Joshi, J., Matthies, D., Schmid, B., 2000. Root hemiparasites and plant diversity in experimental grassland communities. J. Ecol. 88, 634–644.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Terry, A.C., Troumbis, A.Y., Lawton, J.H., 2001. Local adaptation enhances performance of common plant species. Ecol. Lett. 4, 536–544.

Joshi, J., Otway, S.J., Koricheva, J., Pfisterer, J., Roy, B.A., Scherer-Lorenzen, M., Schmid, B., Spehn, E.M., Hector, A., 2004. Bottom-up effects and feed-backs in simple and diverse experimental grassland communities. In: Weisser, W., Siemann, E. (Eds.), Insects and Ecosystem Function. Springer, Berlin, pp. 115–134.

Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microbiol. 60, 3292–3299.

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

Korthals, G.W., Smilauer, P., van Dijk, C., van der Putten, W.H., 2001. Linking above- and below-ground biodiversity: abundance and trophic complexity in soil as a response to experimental plant communities on abandoned arable land. Funct. Ecol. 15, 506–514.

Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., van Veen, J.A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek Int. J. General Molecular Microbiol. 81, 509–520.

Lavelle, P., Lattaud, C., Trigo, D., Barois, I., 1995. Mutualism and biodiversity in soils. Plant Soil 170, 23–33.

Lawton, J.H., 2000. Community Ecology in a Changing World. Ecology Institute, Oldendorf/Luhe.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403–405.

Myrold, D.D., Matson, P.A., Peterson, D.L., 1989. Relationships between soil microbial properties and above-ground stand characteristics of conifer forests in Oregon. Biogeochemistry 8, 265–281.

Naeem, S., Thompson, L., Lawler, S.P., Lawton, J.H., Woodfin, R.M., 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368, 734–737.

Niklaus, P.A., Alphei, J., Ebersberger, D., Kampichler, D., Kandeler, E., Tscherko, D., 2003. Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. Global Change Biol. 9, 585–600.

Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., Trappe, J., 1999. Ecosystem properties and microbial community changes in primary succession on a glacier forefront. Oecologia 119, 239–246.

Olsson, P.A., Thingstrup, I., Jakobsen, I., Baath, F., 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biol. Biochem. 31, 1879–1887.

Ovreas, L., Torsvik, V., 1998. Microbial diversity and community structure in two different agricultural soil communities. Microb. Ecol. 36, 303–315.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego.

Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunicliffe Wilson, G., Welham, S.J., White, R.P., 1993. GENSTAT 5 Reference Manual. Clarendon Press, Oxford.

Pfisterer, A.B., Joshi, J., Schmid, B., Fischer, M., 2004. Rapid decay of diversity–productivity relationships after invasion in experimental plant communities. Basic Appl. Ecol. 5, 5–14.

Porazinska, D.L., Bardgett, R.D., Blaauw, M.B., Hunt, H.W., Parsons, A.N., Seastedt, T.R., Wall, D.H., 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecol. Monogr. 73, 377–395.

Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids. Academic Press, London.

Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant microbe soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Rosenthal, R., Rosnow, R.L., 1985. Contrast Analysis. Cambridge University Press, Cambridge.

Scherer-Lorenzen, M., Palmborg, C., Prinz, A., Schulze, E.-D., 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84, 1539–1552.

Schläpfer, F., Schmid, B., Seidl, I., 1999. Expert estimates about effects of biodiversity on ecosystem processes and services. Oikos 84, 346–352.

Schmid, B., Joshi, J., Schläpfer, F., 2002a. Empirical evidence for biodiversity–ecosystem functioning relationships. In: Kinzig, A., Pacala, S., Tilman, D. (Eds.), Functional Consequences of Biodiversity: Experimental Progress and Theoretical Extensions. Princeton University Press, Princeton, pp. 120–150.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W., Tilman, D., 2002b. The design and analysis of biodiversity experiments. In: Loreau, M., Naeem, S., Inchausti, P. (Eds.), Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press, Oxford, pp. 61–75.

Sharma, S., Rangger, A., von Lutzow, M., Insam, H., 1998. Functional diversity of soil bacterial communities increases after maize litter amendment. Eur. J. Soil Biol. 34, 53–60.

Spehn, E.M., Joshi, J., Diemer, M., Schmid, B., Körner, C., 2000a. Aboveground resource use increases with plant species-richness in experimental grassland ecosystems. Funct. Ecol. 14, 326–337.

Spehn, E.M., Joshi, J., Schmid, B., Alphei, J., Körner, C., 2000b. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224, 217–230.

Spehn, E.M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M.C., Dimitrakopoulos, P.G., Finn, J.A., Jumpponen, A., O'Donnovan, G., Pereira, J.S., Schulze, E.D., Troumbis, A.Y., Körner, C., 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98, 205–218.

Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Jumpponen, A., Koricheva, J., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Palmborg, C., Pereira, J.S., Pfisterer, A.B., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 2005. Ecosystem effects of biodiversity manipulations in European grasslands. Ecol. Monogr. 75, 37–63.

Staddon, W.J., Duchesne, L.C., Trevors, J.T., 1998. Impact of clear-cutting and prescribed burning on microbial diversity and community structure in a Jack pine (*Pinus banksiana* Lamb.) clear-cut using Biolog Gram-negative microplates. World J. Microbiol. Biotechnol. 14, 119–123.

Stephan, A., Meyer, A.H., Schmid, B., 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. J. Ecol. 88, 988–998.

Sturz, A.V., Christie, B.R., 2003. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res. 72, 107–123.

Sulkava, P., Huhta, V., 1998. Habitat patchiness affects decomposition and faunal diversity: a microcosm experiment on forest floor. Oecologia 116, 390–396.

Taylor, N.L., Quesenberry, K.H., 1996. Red Clover Science. Kluwer Academic Publishers, Dordrecht.

Tilman, D., Knops, J., Wedin, D., Reich, P.B., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277, 1300–1302.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. Science 294, 843–845.

Toft, C.A., Shea, P.J., 1983. Detecting community-wide patterns - estimating power strengthens statistical inference. Am. Nat. 122, 618–625.

Torsvik, V., Ovreas, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Curr. Opin. Microbiol. 5, 240–245.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.

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

van der Putten, W.H., Mortimer, S.R., Hedlund, K., van Dijk, C., Brown, V.K., Leps, J., Rodriguez-Barrueco, C., Roy, J., Len, T.A.D., Gormsen, D., Korthals, G.W., Lavorel, S., Regina, I.S., Smilauer, P., 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., Vet, L.E.M., Harvey, J.A., Wackers, F.L., 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol. Evol. 16, 547–554.

van Ruijven, J., Berendse, F., 2003. Positive effects of plant species diversity on productivity in the absence of legumes. Ecol. Lett. 6, 170–175.

Wardle, D.A., 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton.

Wardle, D.A., Bonner, K.I., Barker, G.M., Yeates, G.W., Nicholson, K.S., Bardgett, R.D., Watson, R.N., Ghani, A., 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecol. Monogr. 69, 535–568.

Wardle, D.A., Bonner, K.I., Barker, G.M., 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Funct. Ecol. 16, 585–595.

Wardle, D.A., Yeates, G.W., Williamson, W., Bonner, K.I., 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102, 45–56.

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

Westover, K.M., Bever, J.D., 2001. Mechanisms of plant species coexistence: roles of rhizosphere bacteria and root fungal pathogens. Ecology 82, 3285–3294.

Widden, P., 1986. Functional relationships between Quebec forest soil microfungi and their environment. Can. J. Botany 64, 1424–1432.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., Martin, C.W., 1994. Plant production and soil-microorganisms in late-successional ecosystems - a continental-scale study. Ecology 75, 2333–2347.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fert. Soils 29, 111–129.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem. 24, 317–323.