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EARTHWORMS AND LEGUMES CONTROL LITTER DECOMPOSITION IN A PLANT DIVERSITY GRADIENT

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Abstract. The role of species and functional group diversity of primary producers for decomposers and decomposition processes is little understood. We made use of the "Jena Biodiversity Experiment" and tested the hypothesis that increasing plant species (1, 4, and 16 species) and functional group diversity (1, 2, 3, and 4 groups) beneficially affects decomposer density and activity and therefore the decomposition of plant litter material. Furthermore, by manipulating the densities of decomposers (earthworms and springtails) within the plant diversity gradient we investigated how the interactions between plant diversity and decomposer densities affect the decomposition of litter belonging to different plant functional groups (grasses, herbs, and legumes). Positive effects of increasing plant species or functional group diversity on earthworms (biomass and density) and microbial biomass were mainly due to the increased incidence of legumes with increasing diversity. Neither plant species diversity nor functional group diversity affected litter decomposition. However, litter decomposition varied with decomposer and plant functional group identity (of both living plants and plant litter). While springtail removal generally had little effect on decomposition, increased earthworm density accelerated the decomposition of nitrogen-rich legume litter, and this was more pronounced at higher plant diversity. The results suggest that earthworms (*Lumbricus terrestris* L.) and legumes function as keystone organisms for grassland decomposition processes and presumably contribute to the recorded increase in primary productivity with increasing plant diversity.

Key words: decomposers; functional groups; "Jena Experiment"; legumes; litter type; Lumbricidae; Lumbricus terrestris; *nitrogen; soil fauna; species richness.*

INTRODUCTION

Biodiversity affects ecosystem functioning and services (Balvanera et al. 2006, Cardinale et al. 2006, Hector and Bagchi 2007); however, the interrelationships between the diversity of organisms performing two fundamental processes for life on Earth, photosynthetic production of plant biomass and plant residue recycling (decomposition), are still poorly understood (McCann 2000, Hooper et al. 2005). With increasing plant diversity aboveground productivity generally increases (Roscher et al. 2005, Spehn et al. 2005). In contrast, belowground responses are often idiosyncratic (Wardle et al. 1999, Gastine et al. 2003), but positive effects on nutrient availability (Oelmann et al. 2007) and decomposition processes (Zaller and Arnone 1999, Spehn et al. 2000) have been documented. Each of these processes is likely to be modified by decomposers because decomposition and nutrient mineralization are essentially driven by microbial and animal activity in soil (Schlesinger 1977, Swift and Heal 1979). Ultimately, decomposer activity is likely to feed back to primary producers because they depend on nutrients made available by microorganisms and decomposer animals (Bardgett 2005).

Plants affect the decomposer subsystem via the quality and quantity of the litter produced (Groffman et al. 1996, Wardle 2002), but also via the amount and composition of root exudates (Bais et al. 2004, Li et al. 2004) and by competing with microorganisms for nutrients (Fransen et al. 1999). The extent to which decomposers respond to changes in quantity and quality of resources inputs belowground has been little explored; however, there is increasing evidence that the composition of plant communities differentially affects decomposer species suggesting that they occupy different niches (Cragg and Bardgett 2001, Partsch et al. 2006).

Simultaneous manipulation of both plant diversity and decomposer densities allows the investigation of relationships among plant diversity, decomposer performance, and decomposition process. Taking advantage of the "Jena Experiment," designed to test for the effects of plant species and functional group diversity and identity

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(Roscher et al. 2004), we investigated the effect of plant species (1, 4, and 16 species) and functional group diversity (1, 2, 3, and 4 groups) on decomposer performance (earthworms and microorganisms) and decomposition processes. Furthermore, by studying the decomposition of litter of different plant functional groups (grasses, herbs, legumes) in subplots where decomposer densities had been manipulated, we investigated the interactions among decomposer densities, litter decomposition, and plant functional group identity.

We manipulated the densities of earthworms (Lumbricidae) and springtails (Collembola), because they are important decomposers in terms of abundance, biomass, and impact on decomposition processes. Earthworms are major ecosystem engineers (sensu Jones et al. 1994) in calcareous grasslands and key decomposers affecting plant performance (Wurst et al. 2003, Partsch et al. 2006), soil physical properties (Lavelle et al. 1997), soil microbial community composition and functioning (Brown 1995, Scheu 2002), nutrient cycling (Parmelee et al. 1989, Edwards and Bohlen 1996), and vegetation development (Scheu 2003, Milcu et al. 2006*b*). Springtails affect plant nutrient availability by grazing on microorganisms, thereby affecting the structure and functioning of the microbial community in the rhizosphere and plant growth and plant performance (Gange 2000, Partsch et al. 2006).

Assuming niche differentiation (complementarity) of decomposer species we hypothesized that (1) increasing plant species and functional group diversity will beneficially affect decomposer community and thereby litter decomposition, (2) increased earthworm densities will increase, whereas reduced springtail densities will decrease litter decomposition, with the effect varying with litter functional group, and (3) these effects will depend on plant species and functional group diversity and identity.

Materials and Methods

The Jena Experiment

The study was implemented as part of the Jena Experiment (Roscher et al. 2004, 2005). The study site is located near the Saale River in the vicinity of Jena (altitude 130 m above sea level), Turingia, Germany. Mean annual air temperature is 9.3°C and annual precipitation is 587 mm. The site was formerly used as typical Central European mesophilic grassland. The soil is an Eutric Fluvisol (FAO-UNESCO 1997) developed from up to 2 m thick loamy fluvial sediments. The target plant community of the experiment is a seminatural species-rich mesophilic grassland (Arhenatherion community). A pool of 60 native plant species was used to establish plots (May 2002) with particular species composition determined by independent random draws with replacement. In this study, we used 46 plots of 20 × 20 m along a gradient of plant species (1, 4, 16 species) and functional group richness (1, 2, 3, 4 groups). There were 16 replicates for mixtures with 1 and 4 plant species diversity and 14 replicates for mixtures with 16 species. Four blocks were established in parallel to the river to account for changes in soil abiotic conditions as a function of distance from the river (Roscher et al. 2004).

Plant functional groups were classified according to three attribute classes: (1) above- and belowground morphological traits, (2) phenological traits, and (3) the ability for N_2 fixation. Seventeen variables of the selected species attributes were analyzed by a multivariate cluster method in order to ascribe species to functional groups (Roscher et al. 2004). According to this analysis the species pool falls into four functional groups: grasses (16 species) containing species of Poaceae and Juncaceae; small herbs (12 species), herbs of small stature, perennial; tall herbs (20 species), herbs with medium to tall stature; and legumes (12 species).

Decomposer treatments were established in each plot (summer 2003) with 1, 4, and 16 plant species, within three randomly selected areas of 2 × 4 m. The three treatments were: (1) control (ambient earthworm and springtail density), (2) increased density of earthworms, and (3) reduced density of springtails.

Manipulation and sampling of earthworm densities

The earthworm enhancement treatment was established because earthworm density was low after establishment of the Jena Experiment, which involved repeated disk cultivation to reduce weed density, a practice which is known to detrimentally affect earthworms (Edwards and Bohlen 1996). Earthworm density was manipulated (enhanced) in 1 × 1 m subplots established in the 1, 4, and 16 plant species diversity treatments. The earthworm subplots were enclosed with PVC shields aboveground (20 cm) and belowground (15 cm). The aboveground shields were sufficient to prevent the escape of large surface active species, such as *Lumbricus terrestris* L., while those belowground were established to prevent or substantially reduce colonization and escape of soil-dwelling earthworm species. The earthworm enhancement plots received 25 adult specimens of *L. terrestris* (fresh mass, 4.2 ± 0.94 g, mean ± SE) per year, roughly doubling the site earthworm biomass per square meter. The addition of *L. terrestris* started in September 2003 and continued in 2004. For determining the effects of plant diversity on earthworm populations, earthworm samples were taken in the control plots (with ambient earthworm densities) by electroshocking twice a year (spring and autumn) starting with spring 2004, using a combination of four octet devices (DEKA 4000, Deka Gerätebau, Marsberg, Germany; Thielemann 1986; see Plate 1). In each enclosure the earthworm extraction was performed for 35 minutes, increasing the voltage from 250 V (10 min) to 300 V (5 min), 400 V (5 min), 500 V (5 min) and 600 V (10 min). Extracted earthworms were determined, counted, and weighed. Because we were not interested

in seasonal variations, earthworm data from spring and autumn 2004 were pooled for statistical analysis.

Manipulation and sampling of springtail densities

For practical reasons we opted for the treatment with reduced densities of springtails. Springtail densities were reduced by spraying a contact insecticide once per month on 2×4 m subplots between April and October (Hortex; chlorpyrifos 2% mass/mass, 40 g in 1 L water, 1 L/plot; Celaflor, Dow Agrosciences, Indianapolis, Indiana, USA). The insecticide was found to strongly reduce springtail density and to have low toxicity to birds, small mammals, predatory beetles, spiders, and earthworms (Brown and Gange 1989). For evaluating colonization of the sites by springtails and the effectiveness of the insecticide application, soil samples (5 cm in diameter, depth 5 cm) were taken in autumn 2004. Springtails were extracted by heat (Kempson et al. 1963), determined, and counted. The numbers obtained serve as abundance measures. The application of Hortex reduced the density of springtails in the insecticide plots by ~50% (average density in the insecticide plots of ~5.000 individuals/m^2; A. Sabais, *personal communication*).

Microbial biomass and respiration

Soil cores (5 cm Ø) from the upper 5 cm of the mineral soil were taken in all treatment plots (April 2004) and sieved (2 mm). Microbial biomass was measured using the substrate-induced respiration (SIR) method (Anderson and Domsch 1978). The microbial respiratory response to the addition of glucose was measured at hourly intervals in an electrolytic O_2 microcompensation apparatus for 24 h at 22°C. Microbial biomass (C_{mic}; μg C/g soil) was measured after the addition of a sufficient amount of glucose as substrate in order to saturate the catabolic activity of microorganisms. The maximum initial respiratory response (MIRR; μg O_2·[g soil dry mass]$^{-1}$·h^{-1}) was calculated as the average of the lowest three readings within the first 11 h and microbial biomass was calculated as $C_{mic} = 38 \times$ MIRR (μg C_{mic}/g soil dry mass; Beck et al. 1997). Soil basal respiration (μL O_2·[g soil dry mass]$^{-1}$·h^{-1}) was calculated as the average O_2 consumption rates of soil unamended with glucose after 15–20 h from the start of the measurements.

Litterbag experiment

Three plant functional groups (grasses, herbs, and legumes) were used to establish four litter treatments (grasses, G; herbs, H; legumes, L; and mixed, M). To reduce the number of litter bags we pooled the small and tall herbs as herbs. Litterbags were built using 4-mm mesh to allow access of soil animals, including large earthworms such as *L. terrestris*. Each litterbag contained 3 g dry mass of plant material of ~3 cm length. Litter of each functional group was obtained by mixing 1 g of senesced litter of three randomly chosen species per plant functional group: grasses (*Festuca rubra, Lolium perenne, Poa pratensis*, $N = 2.0\%$, C:N = 2.6); herbs (*Cirsium oleraceum, Daucus carota, Plantago lanceolata*, $N = 2.3\%$, C:N = 19.6); or legumes (*Lathyrus pratensis, Lotus corniculatus, Trifolium repens*, $N = 3.0\%$, C:N = 15.5). For the mixed litter treatment we used 3 g dry mass litter ($N = 2.4\%$, C:N = 9.3) from a homogenous mixture of all nine plant species. The litter material was collected from the Jena Experiment field site in the previous season (2003), sorted and dried for 3 d at 60°C. Litterbags of each of the four litter treatments were placed on the soil surface of the three decomposer treatments in each diversity plot in February 2004. The litterbags were collected in June 2004, after 4 months of exposure, dried 3 d at 60°C, and weighed.

Statistical analyses

ANOVA as part of the general linear models (GLM; type I sum of squares) was used to analyze in a hierarchical order the effects of block, plant functional group diversity (FG), plant species diversity (S), and presence–absence of legumes (L), grasses (G), small herbs (Sh), tall herbs (Th), and decomposer treatments (D) on earthworm biomass and density, using the SAS 8 statistical package (SAS Institute, Cary, North Carolina, USA). Decomposer manipulations were entered as a single variable with three treatment levels. The *F* values given in the text and tables for the effects of S (log-linear) and FG (linear), and their interactions with other factors refer to those where the respective factor (and interaction) was fitted first (Schmid et al. 2002). No interaction term between S and FG was calculated. The effects of presence–absence of legumes (L), grasses (G), small herbs (Sh), and tall herbs (Th) and their interactions with the decomposer treatments were always fitted after fitting S and FG. As in the litter treatments small and tall herbs were pooled as herbs (H). Interactions between factors that were not significant were excluded from the model in a stepwise manner. Due to the nesting of the decomposer treatments within the diversity treatments, split-plot ANOVA was used for analyzing microbial biomass, respiration, litter mass remaining, and percentage N loss, introducing litter type (grass litter, herb litter, legume litter, mixed litter) as an additional treatment factor. Given the existence of interactions with litter type and for better emphasizing the different effects on litter type (for litter mass remaining and percentage N loss), five different analyses were performed with all four litter types analyzed together and separately. For analyzing quantitative changes in litter nitrogen, the amount of N lost was expressed as percentage of the initial amount and was inspected for treatment effects. Treatments analyzed on the plot scale (Block, FG, S, L, H, Sh, Th) were tested against the residual variability between plots (plotcode). Treatments analyzed on the subplot scale (decomposer treatments) were tested against the residual variability between subplots (plotcode × decomposer

TABLE 1. Split-plot ANOVA table showing the effect of block, functional group diversity (FG), species diversity (S), and presence or absence of legumes (L), grasses (G), small herbs (Sh), tall herbs (Th), and decomposer treatment (D) on earthworm density and biomass, *Lumbricus terrestris* biomass, and microbial respiration and biomass.

Treatment factor	Earthworms						Microorganisms			
	Earthworm density		Earthworm biomass		*L. terrestris* biomass		Microbial respiration		Microbial biomass	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Block	3, 36	10.25***	3, 36	3.59*	3, 36	2.12	3, 26	1.73	3, 26	0.93
FG	3, 36	3.04*	3, 36	0.79	3, 36	0.40	3, 26	1.76	3, 26	2.38†
FG linear	1, 36	4.93*	1, 36	1.91	1, 36	0.85	1, 26	0.05	1, 26	5.00*
S	2, 36	4.02*	2, 36	2.38†	2, 36	1.18	2, 26	2.92†	2, 26	1.80
S log linear	1, 36	7.93**	1, 36	4.61*	1, 36	2.19	1, 26	3.20†	1, 26	3.40†
L	1, 36	9.26**	1, 36	17.94**	1, 36	13.60**	1, 26	2.54	1, 26	6.39*
G	1, 36	5.4*	1, 36	9.19**	1, 36	7.15*	1, 26	0.89	1, 26	0.46
Sh	1, 36	2.14	1, 36	0.01	1, 36	0.11	1, 26	0.09	1, 26	0.01
Th	1, 36	4.71*	1, 36	1.61	1, 36	0.45	1, 26	3.7†	1, 26	2.3
D							2, 108	2.35†	2, 108	2.09

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$.

interaction), while the treatments nested in the subplots (litter type) were tested against the residual variability within subplots. MANOVA (Pillai's trace) was used to investigate effects of block, plant species, and functional group diversity and identity on the relative abundance, relative biomass, and community composition of earthworms. ANCOVAs were used to check if detected significant effects can be explained by covarying variables, such as soil moisture, soil structure (sand, silt, and clay content), earthworm biomass, plant root, shoot and total biomass, plant cover, or presence of specific plant functional group which were fitted before block in the analysis. Tukey's hsd post hoc test was used to compare means of treatments with more than two levels. Data were inspected for homogeneity of variance and were log-transformed if required. Percentage data were arcsine transformed to improve normality.

RESULTS

Effects of plant diversity on the earthworm community

The earthworm community consisted of five species belonging to two functional groups (Bouché 1977), anecic (*Lumbricus terrestris* L.) and endogeic (*Aporrectodea caliginosa* Savigny, *A. rosea* Savigny, *Allolobophora chlorotica* Savigny, and *Octolasion tyrtaeum* Savigny). On average (across all treatments) 96 individuals/m^2 and ~60 g fresh mass/m^2 of earthworms were extracted in 2004. There was a strong block effect with generally higher density and biomass in block 1 (108 ± 8 individuals/m^2, 63 ± 7 g/m^2, mean ± SE) and 2 (123 ± 9 individuals/m^2, 70 ± 8 g/m^2) close to the Saale river compared to block 3 (84 ± 9 individuals/m^2, 49 ± 8 g/m^2) and 4 (72 ± 8 individuals/m^2 and 53 ± 7 g/m^2) distant to the river (Table 1).

Total earthworm density and biomass increased with plant species diversity from 85 ± 9 to 108 ± 9 individuals/m^2 and from 54 ± 6 to 66 ± 7 g/m^2 in monocultures and 16 species mixtures, respectively (Table 1, Fig. 1a). Similarly, total earthworm density increased with plant functional group diversity from 89 ± 7 to 98 ± 12 individuals/m^2 in the 1 and 4 functional group mixtures, respectively. Furthermore, earthworm density and biomass depended on plant functional group identity (Table 1). The presence of grasses reduced total earthworm density (−10.1%) and biomass (−20.6%; Fig. 1b), whereas the presence of legumes increased earthworm density (+30.2%) and in particular earthworm biomass (+51.8%; Fig. 1c). Fitting the effect of legumes (L) before plant species diversity (S) or functional group diversity (FG) eliminated the significant diversity effect (S: $F_{2,36} = 1.10$, $P = 0.364$ and $F_{2,36} = 1.56$, $P = 0.224$ for earthworm biomass and density, respectively) suggesting that the diversity effect was due to the presence of legumes. Analysis of covariance indicated that root and biomass, total plant biomass, or plant cover (data from 2004) did not significantly affect the diversity or legume effect.

Earthworm community composition and diversity were unresponsive to changes in plant species and functional group diversity. Similar to earthworm density and biomass, the relative biomass and abundance of earthworm species mainly varied between blocks (Pillai's trace: $F_{15,96} = 4.17$, $P < 0.001$ and $F_{15,96} = 3.91$, $P < 0.001$, respectively). Relative earthworm biomass varied with the presence of legumes (Pillai's trace: $F_{5,30} = 5.64$, $P < 0.001$). The block effect was mainly due to *A. chlorotica*, with its relative biomass decreasing from 9% in block 1 to ~1% in block 4. The legume effect was mainly due to *L. terrestris*, with its relative biomass decreasing from 73% in plots with legumes to 64% in plots without legumes. *A. chlorotica* biomass also increased marginally ($F_{1,36} = 3.08$, $P = 0.088$) in the presence of legumes. All other earthworm species did not significantly respond to changes in plant species and functional group identity and diversity.

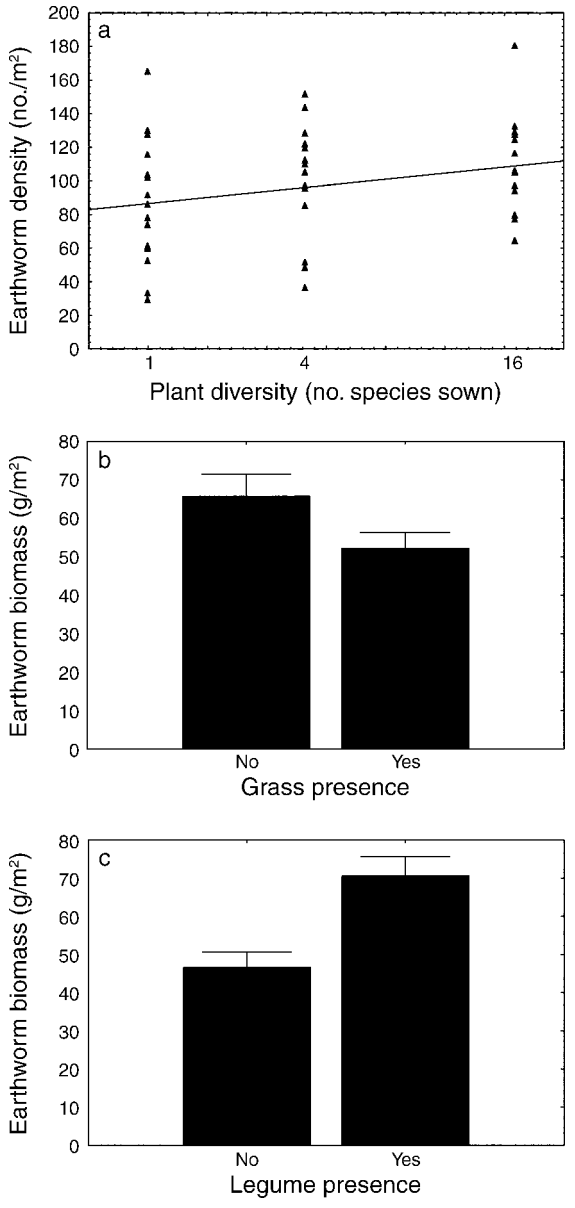

FIG. 1. (a) Density of the earthworm community (in 2004) as affected by the plant species diversity gradient of one ($n = 16$ replicates), four ($n = 14$), and 16 plant species ($n = 14$); and (b) biomass of the earthworm community (mean + SE) as affected by the presence–absence of grasses ($n = 23$) and (c) by the presence–absence of legumes ($n = 23$). For the significance of the effects see Table 1 (S log linear, G, and L).

Effects of plant diversity on microbial biomass and respiration

Microbial biomass increased linearly with increasing plant functional group diversity from 24.0 μg C_{mic}/g soil in monocultures to 26.7, 27.5, and 26.1 μg C_{mic}/g soil at two, three, and four functional groups, respectively (Table 1). However, fitting the presence of legumes (L) before functional group diversity (FG) resulted in a significant legume effect ($F_{1,26} = 6.11$, $P = 0.020$) and rendered functional group diversity nonsignificant ($F_{1,26} = 0.90$, $P = 0.349$), suggesting that the effect of functional groups was due to legumes. No effects of species diversity and decomposer treatments were found (Table 1). Microbial respiration was less responsive; no significant species and functional group diversity and identity or decomposer treatment effects were found (Table 1).

Litter decomposition

Litter mass loss.—The amount of litter that remained in the litter bags strongly depended on block, with higher amounts of litter remaining in block 1 (0.65 ± 0.036 g, mean ± SE) and lower amounts in the blocks farther away from the Saale River (block 2, 0.57 ± 0.038 g; block 3, 0.47 ± 0.036 g; block 4, 0.27 ± 0.038 g). The response of individual litter types followed the same pattern with the exception of grass litter, which differed only between block 2 (1.0 g) and block 3 (1.4 g). Litter identity was also a strong determinant of litter mass loss, with legume litter being decomposed fastest (16.7% remaining) and grasses being slowest (40.5% remaining), and with herbs and mixed litter being intermediate (30.1% and 30.5% remaining, respectively; Fig. 2a).

Decomposer treatments significantly affected the decomposition of litter; in the treatment with increased earthworm density slightly less litter remained in the litterbags (27.2%) than in the control treatment (30.7%; Table 2, Fig. 2b). Furthermore, the interaction between litter type and plant species diversity (litter type × S) suggests that different litter types differentially responded to plant diversity (Table 2).

Plant functional group diversity but not plant species diversity, affected the amount of legume litter remaining. In plots with only one plant functional group, more legume litter remained (18.8%) compared to mixtures containing two (13.6%), three (13.6%), or four (15.6%) plant functional groups. Herb litter decomposition was affected by both plant species diversity and legume presence (significant S × L interaction), with the amount of litter remaining being higher in the absence of living legumes (36.2%) compared to mixtures with legumes (27.0%) in plots with 16 plant species (Table 2). Both functional group effects on legume litter mass loss (FG linear) and the interaction of living plant species diversity and legume presence (S × L interaction) on herb litter mass loss support hypothesis 1. Decomposition of herb and legume litter was accelerated in the increased earthworm density treatment (28.2% vs. 31.6% for herb litter and 14.3% vs. 18.6% remaining for legume litter; Fig. 2c, Table 2). Reduced springtail density treatment had no significant effect on legume litter decomposition (Fig. 2c).

Nitrogen in litter.—After four months of field exposure the concentration of nitrogen in the remaining litter decreased in all types of litter, from an initial 2.0% (C:N = 22.6), 2.3% (C:N = 19.6), 3.0% (C:N = 15.5), and 2.4% (C:N = 19.3) for grasses, legumes, herbs, and mixed

litter, respectively, to 1.5% (C:N = 40.7; grasses), 1.5% (C:N = 29.0; legumes), 1.0% (C:N = 46.1; herbs), and 1.4% (C:N = 32.2; mixed). No block effects on the N loss (as percentage of initial) were found. Generally, N loss varied strongly with the type of litter; it was low in grasses (22.2%) and high in legumes (48.7%), herbs (55.2%), and mixed litter (43.4%; Fig. 3a). Plant species or functional group diversity had no effect on the loss of N (Table 2). However, the percentage N loss was increased in the presence of herbs (44.8% vs. 39.1% of initial) and legumes (44.5% vs. 40.2% of initial).

The interaction between litter type and decomposer treatment (litter type × D; Table 2) suggests that the loss of nitrogen (as percentage of initial) from the different types of litter depended on the decomposer treatment. The loss of N was slightly higher in earthworm enhancement plots (52.2%) than in the control (48.4%) and insecticide treatment (46.0%). Higher loss of N in earthworm enhancement plots was mainly due to increased loss of N from legume and mixed litter (Fig. 3b, Table 2). No interactions between litter type and plant species or functional group diversity were found (Table 2).

Separate analysis of the different litter types showed again an increase in N loss in the presence of legumes (significant for grass, legume, and mixed litter) and herbs (for all litter types) as in the overall analysis (Table 2). In case of legume and grass litter introducing earthworm biomass as covariable in ANCOVA negated the effect of legumes ($F_{1,21} = 3.17$, $P = 0.080$) and herbs ($F_{1,21} = 3.22$, $P = 0.084$), suggesting that legumes and herbs altered litter decomposition via changing earthworm biomass.

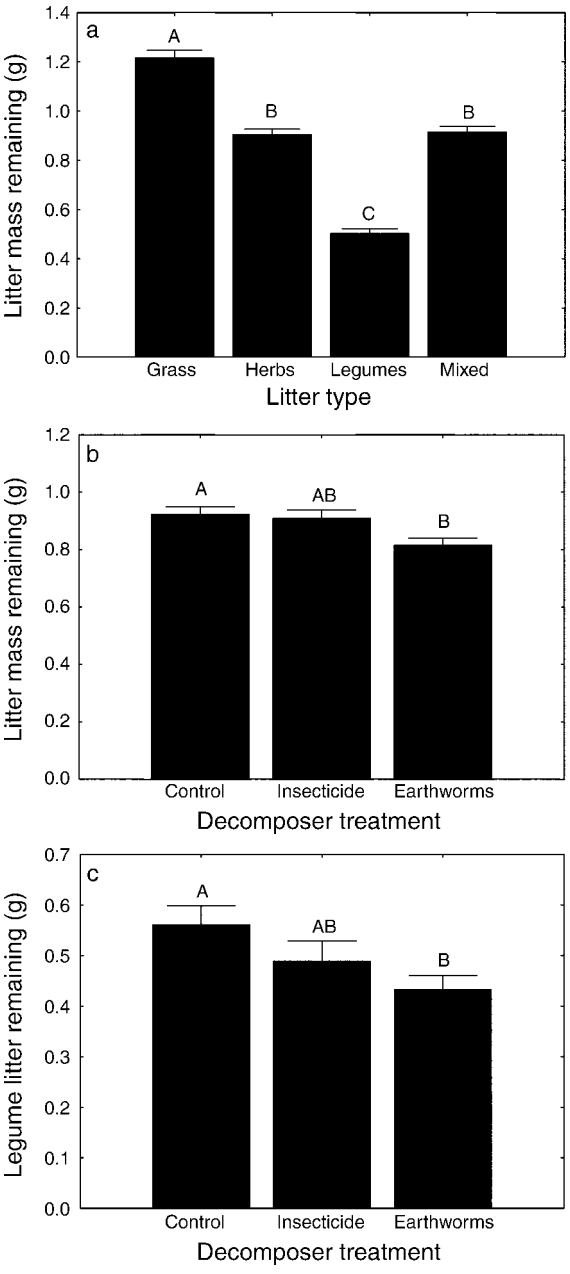

FIG. 2. The amount of litter remaining in litterbags exposed in the field for 4 months as affected by (a) litter type ($n = 138$) and (b) decomposer treatments ($n = 184$). (c) Legume litter remaining as affected by decomposer treatments ($n = 46$). See Table 2 for statistics of these effects. Different letters represent significantly different treatments (Tukey's hsd). Values are given as mean + SE.

DISCUSSION

We investigated the effect of plant diversity on decomposer densities while at the same time assessing the activity of decomposers by manipulating their densities and the litter material. Even though the experiment was carried out only two years after the establishment of the experimental grasslands, the gradient of plant diversity affected the densities and activity of decomposers. However, while both plant species and plant functional group richness positively affected earthworms (biomass and density) and microorganisms (activity), this effect was mainly due to an increased presence of legumes in plots of higher diversity, i.e., the sampling effect (Aarssen 1997, Loreau et al. 2002). The rapid response of decomposers to nitrogen-rich resources was reflected in increased decomposition rates of legume litter, which also increased with plant diversity. The interactions among decomposer manipulations, decomposition, and the effects of plant diversity were often complex and are discussed in the following sections.

Earthworms

In the present study earthworm biomass and density increased with plant species richness, which is consistent with some previous studies (Zaller and Arnone 1999, Spehn et al. 2000); other authors, however, found an idiosyncratic or no response (Wardle et al. 1999, Gastine et al. 2003, Hedlund et al. 2003). An increase in plant species diversity is often associated with increased plant biomass production (Tilman et al. 2001, Roscher et al.

TABLE 2. Split-plot ANOVA table showing the effect of block, functional group diversity (FG), species diversity (S), and presence–absence of legumes (L), grasses (G), herbs (H), decomposer treatment (D), and their interactions on the remaining litter mass and nitrogen, as well as separate analyses of individual functional groups of litter (grass litter, herb litter, legume litter, and mixed litter).

		F value for litter mass remaining					*F* value for N amount remaining (% decrease of initial)				
Source	df	Overall	Grass	Herb	Legume	Mixed	Overall	Grass	Herb	Legume	Mixed
Block	3, 22	7.6***	3.7*	14.4***	18.9***	1.9	2.1	1.9	0.7	2.6	1.1
FG	3, 22	0.8	0.1	0.4	2.9	0.9	0.1	0.4	0.2	0.3	1.0
FG linear	1, 22	0.9	0.1	0.1	5.1*	0.1	0.1	0.6	0.1	0.7	0.7
S	2, 22	0.9	1.4	0.6	2.4	2.1	0.1	0.1	0.2	0.1	1.4
S log linear	1, 22	1.7	0.1	0.1	2.1	4.1†	0.1	0.1	0.1	0.1	0.4
G	1, 22	3.9	3.6†	1.2	2.4	2.0	0.4	0.4	1.1	0.1	0.5
H	1, 22	3.3	2.4	0.9	2.0	2.6	11.6**	12.0**	5.3*	5.9*	12.3**
L	1, 22	1.7	0.4	0.5	0.5	0.1	7.9**	8.7**	1.7	7.2*	8.7**
S × G	2, 22	1.2	1.6	0.4	2.6	0.5	0.3	0.2	0.2	0.4	0.2
S × H	2, 22	0.7	1.5	0.1	2.1	0.1	1.0	0.8	2.2	0.9	1.7
S × L	2, 22	2.3	1.1	3.8*	0.5	2.2	0.1	0.1	0.2	0.7	0.3
Plotcode	22, 427	4.9***	3.2***	2.3**	2.2**	1.9*	7.7***	3.1***	2.6***	2.1**	2.8***
D	2, 44	6.1**	2.0	3.7*	6.7**	1.5	4.7*	1.6	0.8	5.4**	5.7**
D × FG	2, 44	0.6	0.5	1.3	1.8	0.6	0.9	1.0	0.9	0.8	0.2
D × S	2, 44	0.2	1.9	0.2	0.2	1.6	2.6*	1.4	1.1	0.5	1.1
Plotcode × D	44, 427	1.5**					1.5*				
Litter type	3, 427	217.9***					413.5***				
Litter type × D	6, 427	0.6					2.4*				
Litter type × FG	9, 427	1.1					1.4				
Litter type × S	6, 427	2.5*					0.7				

Notes: "Overall" indicates that different litter types (legumes, grass, herbs, and mixed) were analyzed together. "Mixed" litter bags contained different litters mixed together, i.e., legume, grass, and herb litter. Plotcode represents the residual variability between plots.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$.

2005, Spehn et al. 2005). Including plant cover, root, shoot, or total biomass as covariables did not cancel the plant diversity effect on earthworm biomass or density suggesting that the effect was not due to increased biomass production. Rather, the increase in earthworm biomass and density with increasing plant species diversity was driven by the quality of resources associated with the presence of legumes.

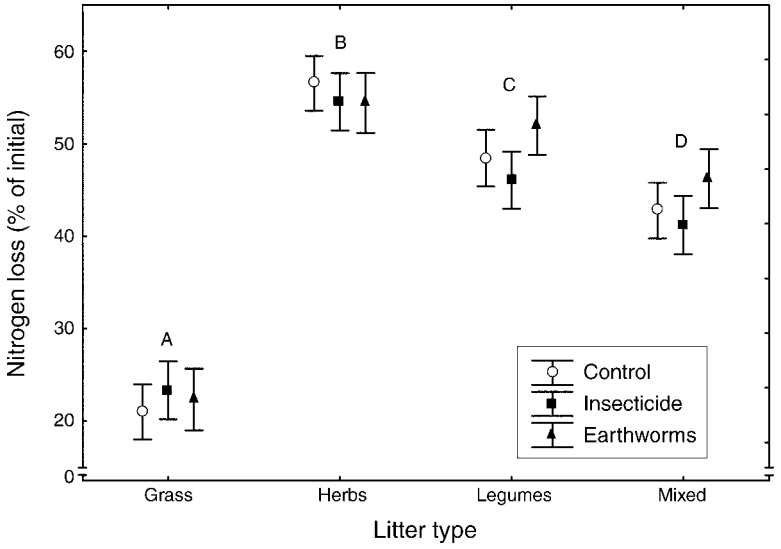

FIG. 3. Litter nitrogen loss (% of initial) as affected by litter type × decomposer treatment interaction ($n = 46$). See Table 1 for statistics of these effects (litter type × D). Different letters represent significantly different effects of litter type (Tukey's hsd). Values are given as mean ± SE.

PLATE 1. Photograph taken during the earthworm sampling campaign in spring 2004. Four octet devices have been simultaneously used to sample and reduce earthworm densities in the reduced-earthworm-density enclosure (bottom enclosure). The earthworm-enhancement enclosure is also visible (top enclosure). Photo credit: A. Milcu.

Earthworm activity and performance is usually considered to be regulated by soil abiotic factors and the amount of plant detritus entering the soil subsystem (Edwards and Bohlen 1996). The biomass and density of two of the five earthworm species (*L. terrestris* and *A. chlorotica*) was increased in the presence of legumes indicating that they benefited from legume resources. Aboveground net primary productivity is assumed to be affected by both complementarity and sampling effect (Roscher et al. 2005). Our results suggest that belowground decomposers (earthworms and microorganisms) only respond to the presence of legumes, suggesting a sampling effect (sensu Aarssen 1997) type of response. The positive effect of legumes might be assumed to be caused by legume leaf litter entering the soil; however, Milcu et al. (2006*a*) found earthworms also benefit from legumes without legume leaves entering the soil. This suggests that earthworms also exploit belowground resources of legumes, potentially dead roots with associated rhizobia rich in nitrogen.

The decrease in earthworm biomass and density from block 1 to 4 is parallel to the gradient in soil texture, i.e., the concentration of sand decreases from 45% in block 1 to 5% in block 4 with a concomitant increase in concentrations of silt and clay from 43% and 16% to 65% and 23%, respectively (Kreutziger 2006). Schulmann and Tiunov (1999) showed that *L. terrestris* benefits from the presence of sand, which facilitates the grinding of organic matter during the gut passage. It is known that heavy textured soils sustain lower populations of earthworms than light textured soils (Edwards and Bohlen 1996). The strong abiotic control of earthworm species at our experimental site and the relatively short time since the experiment had been established might be responsible for the limited response of the earthworm community to the experimental manipulations.

Microbial biomass and springtail effects

Currently, there is no consensus whether increased C substrate diversity (associated with plant diversity) has positive (Zak et al. 2003), negative, or no effect (Dehlin et al. 2006) on microbial functioning. Our results for microorganisms do not support our hypotheses of increasing activity or biomass with plant diversity, but underline the importance of key functional groups of plants for microbial biomass because the presence of legumes and not higher levels of plant production, associated with higher functional group diversity, explained the functional group effect on microbial biomass. Above- and belowground inputs of nitrogen-rich substrates presumably increased nitrogen availability (Oelmann et al. 2007) and thereby reduced N limitation of microorganisms.

Increased earthworm density was expected to reduce microbial biomass by effectively competing with microorganisms for organic matter resources (Scheu and Schaefer 1998). Springtails can also have strong effects

on microflora by grazing on microorganisms; however, the response to springtail exclusion is poorly understood, and both reduction and increase (compensatory growth) in microbial biomass have been reported (Lussenhop 1992, Griffiths and Bardgett 1997). The lack of significant earthworm and springtail effects on microorganisms might have been due to the short time elapsed since the establishment of the decomposer treatments (1.5 years) and the long agricultural history (~40 years) of the site.

Litter decomposition

Increasing plant species diversity may affect decomposition rates by (1) changes in quality and quantity of the litter that enters the decomposer subsystem, (2) changes in microclimate due to increased vegetation density in diverse plant communities, and (3) by changing the activity, density, and structure of the decomposer community. Results of our study suggest that changes in the density and structure of decomposer community and litter quality rather than quantity are most important. In contrast to Hector et al. (2000), changes in microclimate (via changes in vegetation cover and density) did not significantly affect litter decomposition (as suggested by fitting plant cover and biomass as covariables) nor increased diversity and the associated increased litter input. The results suggest that decomposers (in particular earthworms) and litter type significantly affect litter mass loss and litter N loss. Furthermore, the decline in the amount of N in the litter was strongly affected by the functional group identity of living plants; potentially, however, legumes increased decomposition of litter via increasing earthworm biomass.

Despite the close interrelationships between plant community composition and the functioning of the decomposer community, the contribution of macroinvertebrates to litter decomposition has been largely neglected in diversity experiments. The use of very small mesh size in most of the diversity experiments could have masked the importance of large decomposer invertebrates, such as earthworms (Wardle et al. 1999, Knops et al. 2001). Litterbags with big mesh size have the potential disadvantage of overestimating decomposition rates; however, they include litter incorporation into the soil and the fragmentation of plant residues by macro-decomposers, which are intrinsic to litter decomposition.

Macro-decomposers selectively picked the legume litter rich in nitrogen. It is known that litter-feeding earthworms selectively feed on certain litter types, with a preference for nitrogen-rich litter, thereby affecting both litter removal and nitrogen mineralization (Edwards and Bohlen 1996). In the laboratory it has been documented that different decomposer taxa (earthworms and Collembola) compete for nitrogen-rich litter resources (Scheu et al. 1999). Interestingly, the presence of legumes reduced the amount of nitrogen lost from litter, and this might have been due to less pronounced N limitation of microorganisms in the presence of legumes. Indeed, significantly higher amounts of soil nitrate and total dissolved nitrogen were found in plots containing legumes at our experimental site (Oelmann et al. 2007).

Burial of nitrogen-rich litter and accelerated nitrogen mineralization suggest that decomposer invertebrates, earthworms in particular, significantly affect nitrogen cycling and thereby also plant productivity and plant community composition. Long-term experiments manipulating the amount and composition of litter are necessary to further evaluate the implications of these findings. Results from our experiment suggest that if anecic earthworms are present, accumulation of litter rich in nitrogen with increasing productivity as hypothesized by Knops et al. (2001) is unlikely.

The strong block effect on litter decomposition and litter N loss suggests strong abiotic control of litter decomposition, i.e., by soil texture and moisture. However, changes in litter decomposition were likely caused by soil texture mediated changes in the activity of earthworms. The comparatively small effects of plant diversity suggest that litter decomposition and the mobilization of nitrogen therein is primarily controlled by decomposers and only secondarily by plant community composition. Higher silt and clay content in soil is known to improve the water holding capacity (Vereecken et al. 1989). Soil moisture increases microbial (Orchard and Cook 1983) as well as earthworm activity (Nordstrom and Rundgren 1974, Edwards and Bohlen 1996) and thereby also litter decomposition (Swift and Heal 1979, Cortez and Bouche 1998); this probably has been the case at our experimental site.

In conclusion, two years after the establishment of the Jena Experiment field site, the results only partially support hypothesis 1, showing positive (for earthworms and microbial biomass) as well as no effects (for litter decomposition) of plant species or functional group diversity on decomposition or decomposer performance. Furthermore, positive effects on earthworms (biomass and density) and microbial biomass were likely due to increased frequency of a single key plant functional group (legumes) in more diverse communities. This adds to the controversy as to whether sampling effect is widespread in natural communities (Cardinale et al. 2006). Both hypotheses 2 and 3 were generally supported, providing evidence for the importance of decomposers and plant functional group identity (as litter or living plant) for decomposition rates (different litter functional groups decomposed at different rates) and the existence of relatively rapid feedbacks of plant community composition on decomposer communities. In addition, the results show that decomposition of nitrogen-rich litter in calcareous grasslands is strongly controlled (beside environmental factors) by earthworms. Consequently, the results suggest that earthworms (*L. terrestris*) and legume species represent closely inter-

linked keystone taxa controlling microbial activity and decomposition processes in calcareous grasslands.

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