Biol Fertil Soils (2015) 51:197–205
DOI 10.1007/s00374-014-0963-2

ORIGINAL PAPER

The influence of fertilizer addition, cutting frequency and herbicide application on soil organisms in grassland

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Received: 28 June 2014 / Revised: 10 September 2014 / Accepted: 19 September 2014 / Published online: 7 October 2014

Abstract In Europe, grasslands are among the most important agricultural systems which are subject to continuous management intensification, potentially threatening ecosystem services. We analysed the impact of management of permanent grassland, i.e. fertilizer addition (−NPK and +NPK), cutting frequency (one and three cuts per year) and herbicide application targeting either dicotyledons or monocotyledons, on microorganisms and soil animals. Microbial biomass decreased in treatments cut once with fertilizer addition. However, in treatments cut thrice, microbial biomass did not decline with fertilizer addition, suggesting that cutting frequency alleviates the detrimental effect of fertilizer input. Fungal but not bacterial phospholipid fatty acid biomarkers varied with sward composition, indicating fungi more sensitively respond to changes in grassland management and sward composition than bacteria. The abundance of dipterans and beetles increased with fertilizer addition but decreased with sward-cutting frequency. Epigeic earthworms benefited from a reduced proportion of monocotyledons. The results indicate that microbial parameters more sensitively respond to intensification of management practices in grassland than to soil animals, in particular, early after changes of management practices.

Keywords Soil microflora · Soil fauna · Fertilizer · Plant functional groups · Cutting frequency · PLFA

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Introduction

In Europe, grasslands are among the most important agricultural systems, covering about 22 % of the EU-25 (European Union, with 25 member states, 2004–2006) land area (Stoate et al. 2009). Since the 1960s, management of grasslands in Europe has been intensified continuously, enhancing productivity (Isselstein et al. 2005). Nitrogen (N) fertilizer is applied to increase aboveground plant biomass, and swards are cut earlier and more frequently (Walker et al. 2004; LeBauer and Treseder 2008). Further, herbicide applications reduced unwanted plants. At the same time, changes in land use are among the most important drivers for present and expected global decline in biodiversity (Virágh 1987; Vitousek et al. 1997; Sala et al. 2000) and ecosystem functioning (Suding et al. 2005; Harpole and Tilman 2007). A variety of groups of invertebrates function as drivers of ecosystem services, such as low herbivore pest load, pollination and decomposition (Tscharntke et al. 2005; Isaacs et al. 2009). However, besides negative effects on diversity, increased plant productivity is associated by higher arthropod abundance in an aboveground system (Siemann 1998; Haddad et al. 2000). Similarly, in the belowground system, microarthropod abundance and biomass have been found to increase with N fertilization, presumably, due to increased plant biomass and associated increased microbial biomass acting as a resource for soil decomposer animals (Cole et al. 2005; van der Wal et al. 2009). In contrast, N fertilization decreases the amount of carbon (C) allocated by plants into roots and via root exudates into the rhizosphere (Marschner et al. 1996; Bazot et al. 2006). Variations in the release of root C are likely to be responsible for changes in soil microbial biomass in grassland systems of different diversity (Eisenhauer et al. 2010) but effects of N addition on microorganisms remain little understood (Treseder 2008).

Frequent cutting of grassland increases the mortality of aboveground invertebrates and stimulates emigration of the surviving mobile fauna (Thorbek and Bilde 2004; Humbert et al. 2012). On the other hand, cutting increases microbial biomass, presumably due to stimulating root exudation (Mawdsley and Bardgett 1997; Guitian and Bardgett 2000), and this likely beneficially affects decomposers and thereby also higher trophic-level invertebrates.

Increased plant species number in N-limited grassland systems is associated by increased C sequestration in soil (Tilman et al. 2006). However, the increase in C sequestration is unlikely due to plant diversity per se, rather, it likely results from changes in functional traits of one or more plant functional groups. For example, N-fixing legumes play an important role in enhancing soil C and N storages (Fornara and Tilman 2008; De Deyn et al. 2009). Also, microbial biomass and the abundance of collembolans (Collembola) and earthworms (Lumbricidae) are increased in the presence of legumes (Gastine et al. 2003; Salamon et al. 2004). However, soil microorganisms also have been found to respond little to changes in plant functional group composition (Marshall et al. 2011), but rather to changes in plant diversity (Eisenhauer et al. 2013), and fungal communities have been shown to vary with grass species identity (Mouhamadou et al. 2013).

The scarce studies on effects of grassland management practices on soil organisms and their inconsistent results call for further investigations. Therefore, we studied variations in community structure of soil microorganisms and animals in a temperate grassland system as affected by NPK fertilizer addition, cutting frequency and sward composition, i.e. variations in plant functional groups. We hypothesized that (1) fertilizer addition increases, (2) more frequent cutting decreases and (3) herbicide-induced increase in the abundance of dicotyledons increases the abundance of soil biota. We expected the beneficial impact of fertilizer addition to be due to increased resource availability, i.e. increased plant productivity, the negative effect of frequent cutting to be due to disturbances overriding beneficial effects of increased root exudation, and the positive effect of higher frequency of dicotyledons to be due in particular to increased abundance of legumes.

Methods

Study site and experimental design

The experiment was carried out within the framework of the 'GRASSMAN' (Grassland Management) experiment, investigating effects of grassland management on ecosystem functioning (Petersen et al. 2012). The study site is located in the Solling, a small mountain range in the north of Germany (51° 44′ N, 9° 23′ E; 490 m a.s.l.). The mean annual temperature is 6.9 °C, and the mean annual precipitation is 1028 mm (average of 1969–1990; German Weather Service). Until 2 years before the start of the experiment, the grassland had been used for cattle grazing and received 80 kg N ha^{-1} y^{-1}. Vegetation is classified as nutrient-poor montane mesic Lolio-Cynosuretum, and the soil is characterized as Haplic Cambisol.

The experiment was set up in 2008 in a full factorial design comprising the factors sward composition (three levels differing in the proportion of plant functional groups), cutting frequency (one and three cuts per year) and fertilization (with and without fertilizer addition). Each treatment level was replicated six times. For setting up the experiment, the site was mown and plots of a size of 15×15 m were established. Sward compositions were manipulated by applying herbicides targeting either dicotyledons or monocotyledons, resulting in sward compositions reduced in dicotyledons (DR) or monocotyledons (MR); plots not treated with herbicides served as control (Ctr). In 2010, the DR sward comprised 78.0, 21.8 and 0.2 %; the MR sward 49.3, 48.7 and 2.0 %; and the Ctr sward 65.7, 32.8 and 1.5 % of grasses, herbs and legumes, respectively (T. From unpubl. data). Fertilization treatments included NPK fertilizer addition (+NPK) and no fertilizer addition (−NPK). Fertilization started in 2008 with the addition of N and was continued in 2009 by the addition of N, which was split into two equal applications per year, while the combined P and K fertilizer was applied once per year, resulting in the application of 180-30-100 kg N-P-K ha^{-1} y^{-1} on respective plots. Half of the plots were mown annually once in July, the other half thrice in May, July and September.

Micro organisms

In May 2010, three soil cores (5 cm Ø) per plot were taken, mixed and sieved to determin soil microbial biomass (C_{mic}) and phospholipid fatty acids (PLFA). C_{mic}, basal respiration and specific respiration were determined using SIR (substrate-induced respiration), i.e. the respiratory response of microorganisms to glucose addition (Anderson and Domsch 1978). Respiration rates were measured using an automated O_2 microcompensation system (Scheu 1992). Soil basal respiration (μl O_2 g^{-1} soil dry wt h^{-1}) was calculated as the mean of O_2 consumption rates between 14 and 22 h (without addition of substrate) after attachment of the vessels to the measurement system. Subsequently, samples were supplemented with 6 mg glucose g^{-1} soil dry wt (Beck et al. 1993), and the mean of the three lowest values within 2–10 h after addition of glucose was taken as the maximum initial respiratory response (MIRR). C_{mic} (μg C g^{-1} soil dry wt) was calculated as 38×MIRR (Beck et al. 1997). From basal respiration and microbial biomass, microbial specific respiration (μl O_2 mg^{-1} C_{mic} h^{-1}) was calculated.

Fatty acid analysis

From the six replicates, four were selected at random for fatty acid (FA) analysis. PLFAs were extracted following

Frostegård et al. (1991) and analysed by gas chromatography using Clarus 500 (PerkinElmer, Waltham, USA). PLFAs a15:0, i15:0, i16:0 and i17:0 were used as marker FAs for Gram-positive bacteria; cy17:0 and cy19:0 as marker FAs for Gram-negative bacteria; 18:2ω6,9 as a saprotrophic fungal marker and 16:1ω7 as an unspecific bacterial marker (Ruess and Chamberlain 2010).

Invertebrate animal abundance

In May 2010, one small (5 cm Ø) and one large soil core (20 cm Ø) per plot were taken for extracting soil meso- and macrofauna, respectively. Samples were divided into sward, hereinafter termed litter layer and 0–3 cm soil layer and extracted using a modified heat extractor (Kempson et al. 1963). Invertebrates were filtered into collection vials and stored in 70 % ethanol until determination and counting.

Statistical analysis

The effect of fertilizer addition, sward composition and cutting frequency on C_{mic}, basal respiration, specific respiration, ratios of FAs (fungal to bacterial PLFA ratio and Gram-negative to Gram-positive PLFA ratio) and total bacterial and fungal PLFA were analysed by individual ANOVAs. Animal taxa were analysed using general linear model (GLM), accounting for missing values. Earthworms and collembolans were first analysed by multivariate analysis of variance (MANOVA) and individual taxonomic groups with (protected) ANOVAs. Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was performed using CANOCO for Windows 5 (Microcomputer Power, Ithaca, NY, USA) (Ter Braak and Šmilauer P 2012), with experimental treatments included as supplementary variables. Prior to the analyses, the data were inspected for homogeneity of variance using Levene test; log transformation generally improved homogeneity of variance, and therefore we uniformly used log-transformed data for statistical analyses. Means given in text and tables are based on non-transformed data.

Results

Microorganisms

C_{mic} was on average 922 (±188 SD) μg C g^{-1} dry wt but significantly varied with fertilizer addition and cutting frequency. Fertilized treatments cut once per year had lower C_{mic}, whereas this was not the case if swards were cut thrice (Table 1; Fig. 1). Neither basal respiration nor microbial specific respiration varied significantly between treatments (Tables 1 and 2).

Fungal PLFA in MR sward significantly exceeded that in DR and Ctr swards. Similarly, the fungal to bacterial PLFA ratio in MR sward significantly exceeded that in DR and Ctr (Tables 1 and 2). Neither bacterial PLFAs nor Gram-negative to Gram-positive PLFA ratio significantly varied between treatments (Tables 1 and 2).

Detritivorous animals

The total abundance (including soil and litter layers and hereafter just named abundance) of epigeic earthworms varied with sward composition and was higher in MR than in DR swards, with the Ctr being intermediate (Tables 3 and 4). The abundance of dipterans (Diptera) was significantly higher in fertilized as compared to non-fertilized treatments (Tables 3 and 4; Fig. 2a). Further, the abundance of dipterans in treatments cut once per year exceeded that in treatments cut thrice per year with the difference being more pronounced in DR as compared to Ctr and MR swards (interaction between sward composition and cutting frequency; Table 3). The abundance

Table 1 GLM table of *F* and *P* values on the effect of fertilizer addition, sward composition and cutting frequency on microbial biomass (C_{mic}), basal respiration (BR), microbial specific respiration (Mic SR), bacterial and fungal PLFAs and fungal to bacterial and Gram-negative to Gram-positive PLFA ratios. Significant effects are given in italics. None of the interactions were significant (not shown)

	Block		Fertilizer		Sward		Cut		Fertilizer×cut	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
C_{mic}	0.67	0.649	*7.31*	*0.009*	0.34	0.711	*6.78*	*0.012*	*7.21*	*0.010*
BR	0.75	0.593	2.55	0.116	0.96	0.389	2.30	0.135	3.04	0.087
Mic SR	1.12	0.036	0.68	0.414	2.29	0.111	0.65	0.425	0.86	0.359
Bacterial PLFAs	*17.55*	*<0.0001*	2.66	0.113	0.63	0.542	2.61	0.117	1.10	0.302
Fungal PLFA	*9.62*	*0.000*	0.87	0.359	*6.01*	*0.006*	0.40	0.534	2.04	0.163
Fungal to bacterial PLFA ratio	*3.88*	*0.019*	2.29	0.141	*6.52*	*0.004*	0.03	0.854	1.08	0.306
Gram-negative to Gram-positive PLFA ratio	*8.13*	*0.000*	0.16	0.692	2.21	0.127	0.41	0.526	0.04	0.845

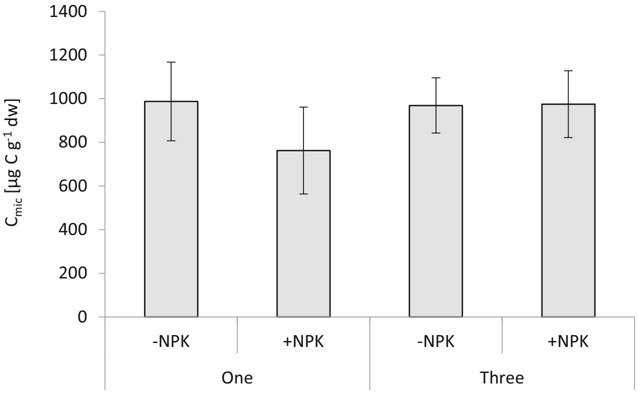

Fig. 1 Effect of the addition of fertilizer and cutting frequency on soil microbial biomass (C_{mic}); means with standard deviation; for statistical analyses, see Table 1

of oribatid mites (Oribatida) in trend was higher in non-fertilized as compared to fertilized treatments (Tables 3 and 4; Fig. 2b). Collembolans mainly comprised not only Isotomidae (42±24 %) and Onychiuridae (31±25 %) but also Symphypleona (14±16 %), Entomobryidae (9±13 %) and Hypogastruridae and Neanuridae (together 3±4 %). Neither the abundance of total collembolans nor that of individual collembolan groups, with exception for the Entomobryidae, was significantly affected by experimental treatments but varied markedly between blocks (Table 1). Entomobryidae reached higher abundance in non-fertilized treatments, with 2693 (±2596) ind. m^{-2} in comparison to 1527 (±1936) ind. m^{-2} in fertilized treatments (ANOVA, $F_{1,45}$=5.24, P=0.027).

Predatory animals

The majority of beetles (Coleoptera) were larvae (73±12 %) and comprised mainly predatory taxa including Staphylinidae (78±19 %), Cantharidae (10±17 %), Elateridae (7±8 %) and Carabidae (4±6 %). Adult beetles also comprised mainly not only predators such as Staphylinidae (83±17 %) and Carabidae (8±12 %) but also herbivores such as Curculionidae (5±9 %) and Nitidulidae (4±10 %). The abundance of beetles in treatments with fertilizer addition and only one cut per year exceeded that in treatments without fertilizer addition cut thrice (significant interaction between fertilizer addition and cutting frequency; Table 3).

Spiders (Aranea) comprised mainly Linyphiidae (96±7 %) and some Lycosidae (3±6 %) and Tetragnathidae (1±4 %). For centipedes (Chilopoda), the interaction between fertilizer, sward and cutting frequency was significant (Table 3). In fertilized treatments, centipede abundance increased in the order MR<DR<Ctr, with abundances being generally lower in treatments cut once per year. In fertilized treatments, the MR sward cut once had the lowest abundance with 16 (±17) ind. m^{-2} and the Ctr sward cut thrice per year, the highest abundance with 57 (±93) ind. m^{-2}. In non-fertilized treatments, abundances increased in swards cut once per year in the order DR<Ctr<MR, whereas abundances declined in this order in treatments cut thrice per year. In treatments cut thrice, this effect was more pronounced, reaching a maximal abundance of 118 (±86) ind. m^{-2} in the DR sward and a minimum of zero in the MR sward. However, because of the low number of individuals, interpreting these differences remains ambiguous.

Depth distribution

The majority of animal taxa were more abundant in the litter than in the soil layer. Only in centipedes, the abundance in soil (58±45 %) exceeded that in litter. Generally, the percentage of animals in the litter layer decreased in the following order oribatid mites≥spiders>beetles>dipterans>gamasid mites (Gamasida)>earthworms>collembolans>centipedes (Fig. 3).

Dipterans predominantly occurred in the litter layer, and this was most pronounced in treatments cut thrice ($F_{1,50}$= 4.88, P=0.032). Oribatid mites also mainly occurred in the litter layer but this was more pronounced in non-fertilized treatments ($F_{1,48}$=5.99, P=0.018). The percentage of beetle

Table 2 Means of microbial biomass (C_{mic}; µg C g^{-1} dry wt), basal respiration (BR; µl O_2 g^{-1} dry wt h^{-1}), microbial specific respiration (Mic SR; µl O_2 mg^{-1} C_{mic} h^{-1}), bacterial and fungal PLFAs (nmol g^{-1} dry wt), fungal to bacterial and Gram-negative to Gram-positive PLFA ratios affected by fertilizer addition, sward composition and cutting frequency. Significant effects are given in italics

	Fertilizer		Sward			Cut	
	−NPK	+NPK	Ctr	DR	MR	One	Three
C_{mic}	*977±153*	*868±206*	893±178	936±188	938±203	*871±219*	*972±138*
BR	6.25±1.76	5.68±1.57	5.86±1.49	6.35±2.00	5.66±1.48	5.69±1.60	6.22±1.73
Mic SR	6.36±1.27	6.61±1.23	6.59±1.18	6.80±1.53	6.05±0.85	6.61±1.21	6.37±1.29
Bacterial PLFAs	51.52±13.65	47.62±13.16	46.50±11.16	50.92±15.85	51.15±13.36	47.09±13.47	51.66±13.24
Fungal PLFA	0.60±0.38	0.70±0.42	*0.49±0.22*	*0.58±0.37*	*0.87±0.47*	0.62±0.33	0.69±0.45
Fungal to bacterial PLFA ratio	0.011±0.006	0.014±0.007	*0.010±0.003*	*0.011±0.005*	*0.017±0.008*	0.013±0.005	0.013±0.008
Gram-negative to Gram-positive PLFA ratio	0.51±0.08	0.51±0.06	0.52±0.07	0.48±0.09	0.53±0.05	0.50±0.08	0.52±0.07

Table 3 GLM and MANOVA (for total Lumbricidae and Collembola) table of *F* and *P* values on the effect of fertilizer addition, sward composition and cutting frequency on the abundance (in soil and litter) of animal taxa. Significant effects are given in italics

	Block		Fertilizer		Sward		Cut		Fertilizer × cut		Sward × cut		Fertilizer × sward × cut	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Lumbricidae	*2.15*	*0.030*	0.13	0.879	*3.28*	*0.014*	0.05	0.952	0.94	0.399	1.16	0.331	1.64	0.204
Epigeic	1.03	0.409	0.24	0.628	*4.67*	*0.014*	0.06	0.805	0.04	0.849	0.70	0.503	0.18	0.832
Endogeic	*3.40*	*0.010*	0.01	0.939	1.56	0.221	0.05	0.816	1.91	0.173	1.62	0.208	0.98	0.423
Diptera	*5.10*	*0.001*	*4.64*	*0.036*	1.30	0.282	*8.73*	*0.005*	1.73	0.195	*3.64*	*0.033*	0.10	0.907
Oribatida	*3.50*	*0.009*	3.46	0.069	0.56	0.573	0.00	0.957	1.03	0.316	1.02	0.367	1.08	0.349
Collembola	*153.81*	*0.000*	1.17	0.343	1.12	0.345	1.38	0.253	0.92	0.479	0.57	0.833	1.06	0.404
Coleoptera	1.05	0.398	*13.16*	*0.001*	2.20	0.121	1.01	0.319	*6.46*	*0.014*	1.08	0.347	0.81	0.450
Chilopoda	0.29	0.917	0.95	0.334	0.35	0.707	1.09	0.301	1.56	0.217	*4.70*	*0.013*	*3.58*	*0.035*
Aranea	0.98	0.437	1.40	0.243	2.73	0.075	0.01	0.922	1.86	0.179	1.02	0.368	2.21	0.120
Gamasida	2.11	0.080	1.55	0.219	1.67	0.199	0.01	0.937	0.49	0.488	0.29	0.750	2.43	0.098

larvae in the litter layer of swards cut once increased in the order MR>Ctr>DR, whereas in treatments cut thrice, it declined in this order ($F_{2,51}=4.52$, $P=0.016$ for the interaction between sward composition and cutting frequency, data not shown).

Discussion

We analysed the impact of management of permanent grassland, i.e. fertilizer addition, cutting frequency and herbicide application, targeting either dicotyledons or monocotyledons on biomass, activity and composition of microorganisms and abundance of soil animals. Microbial biomass and soil arthropods (dipterans and beetles) responded to cutting frequency and fertilizer addition, whereas fungal abundance and earthworm abundance, to sward composition.

In swards cut once, microbial biomass decreased with fertilization, supporting earlier findings that soil microorganisms in grassland are negatively affected by fertilization (de Vries et al. 2007), presumably due to reduced release of root C into the rhizosphere (Marschner et al. 1996), which has been suggested to be responsible for changes in soil microbial growth (Eisenhauer et al. 2010). However, in swards cut thrice, microbial biomass did not decline with fertilizer addition, suggesting that cutting frequency alleviates the detrimental effect of fertilizer input, presumably due to higher root exudation (Mawdsley and Bardgett 1997; Guitian and Bardgett 2000). Interestingly, fungal PLFA but not bacterial PLFAs varied with sward composition, supporting earlier findings that fungi more sensitively respond to changes in plant functional groups than bacteria (Lange et al. 2014). In our study, a higher fungal biomass was associated with MR, as compared to the DR and Ctr swards. In line, Smith et al. (2008) found the fungal to bacterial ratio to be higher in

Table 4 Mean abundance (in soil and litter) and standard deviation (ind. m^{-2}) of taxa as affected by fertilizer addition, sward composition and cutting frequency. Significant effects are given in italics

	Fertilizer		Sward			Cut	
	−NPK	+NPK	Ctr	DR	MR	One	Three
Lumbricidae	431±228	416±181	*450±250*	*384±201*	*431±211*	363±154	492±234
Epigeic	113±80	140±107	*114±122*	*90±80*	*175±122*	115±81	140±109
Endogeic	322±194	262±151	336±214	294±182	256±161	244±134	350±202
Diptera	*665±863*	*934±1213*	740±610	823±1466	849±990	*1082±1388*	*525±433*
Oribatida	26348±16464	20980±20002	24122±18173	17912±11614	28920±22416	23693±19658	23792±17345
Collembola	32561±25747	39242±27638	31447±23622	35630±22877	40350±32717	33545±26892	37756±26746
Coleoptera	*906±308*	*1209±481*	934±255	1177±551	1080±424	1125±523	998±309
Aranea	324±167	382±194	310±163	328±164	422±203	342±149	366±212
Chilopoda	55±71	36±51	49±69	48±60	40±57	40±60	51±63
Gamasida	7710±5973	9417±7197	9273±6894	7053±6136	9185±6777	8686±7033	8406±6292

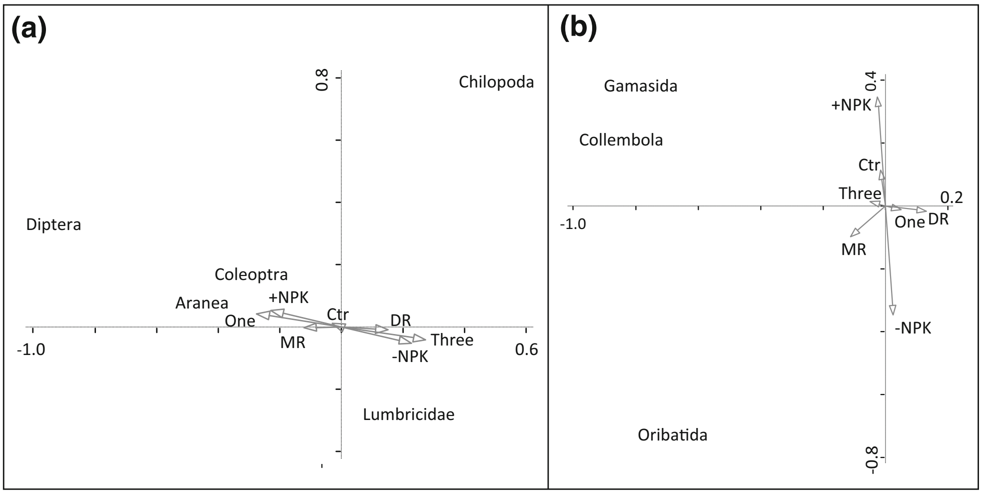

Fig. 2 Principal components analysis (PCA) with experimental treatments included as supplementary variables for the soil **a** macrofauna and **b** mesofauna; eigenvalues of the first and second axis of 0.477 and 0.209 (**a**) and of 0.625 and 0.220 (**b**), respectively

swards dominated by forbs including legumes, whereas Lange et al. (2014) found legumes to negatively affect fungal biomass. Further studies are needed to uncover the mechanisms how sward composition affects the relationship between bacteria and fungi in soil.

As expected, fertilizer addition increased the abundance of some taxa of soil invertebrates, namely beetles and dipterans, but the opposite was true for entomobryid collembolans and in trend also for oribatid mites. However, in general, the effect of fertilizer addition on the abundance of most soil invertebrate taxa was minor, e.g. total collembolans and all other collembolan groups except Entomobryidae did not respond significantly to experimental treatments. Earlier studies reported inconsistent response of soil invertebrates to N fertilization with increases or decreases in abundances typically being limited to certain taxa and most taxa being unresponsive (Bardgett and Cook 1998; Cole et al. 2005, 2008; Eisenhauer et al. 2013). This contrasts the aboveground systems where fertilizer induced higher plant biomass and increased plant nutrient concentrations, generally resulting in increased abundances of arthropods, in particular, that of herbivore taxa (Haddad et al. 2000; Siemann 1998). Dipterans either increased or did not respond to mineral fertilizer addition (Frouz 1999). In our study, dipterans increased significantly with fertilization, suggesting that they benefited from a higher resource supply, which is consistent with the findings of Frouz (1999) that the biomass of dipteran larvae increased with increased plant litter input in unmown meadows. Similarly, in our study, the abundance of dipterans decreased with higher cutting frequency, suggesting that it was mainly due to detritivorous taxa. Interestingly, other groups of mainly detritivorous taxa, i.e. entomobryid collembolans and oribatid mites, respond in the opposite way with the abundance decreasing with fertilization. Most entomobryid collembolans and oribatid mite species are secondary decomposers relying on microorganisms as food (Schneider et al. 2004; Chahartaghi et al. 2005; Maraun et al. 2011), the decline in abundance in these groups therefore likely was due to reduced microbial biomass in fertilized treatments.

Epigeic earthworms were more abundant in the MR than in the DR sward. Feeding on litter, epigeic earthworms heavily

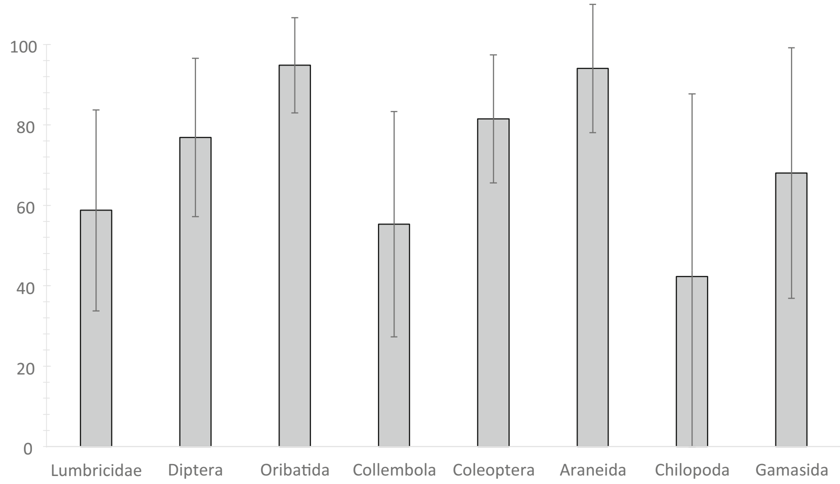

Fig. 3 Individuals of soil animal groups in the litter layer as percentages of the total, i.e. of those in both the litter and 0–3 cm of the soil; means with standard deviation

rely on high-quality food resources such as legume litter (Gastine et al. 2003). Therefore, they likely benefited from a reduced proportion of grasses and increased proportion of herbs, in particular legumes, in the MR sward. Supporting this conclusion, root N concentrations of herbs at the study site were higher than those of grasses (Leuschner et al. 2013).

The majority of beetles at our study sites comprised Staphylinidae, which typically live as generalist predators, feeding on small arthropods (Bohac 1999). As the abundance of microarthropods was little affected by experimental treatments, variations in the abundance of staphylinid beetles likely were due to different abundances of dipteran larvae. Indeed, the abundance of beetles increased parallel to that of dipterans in fertilized treatments as well as in treatments cut only once per year. However, beetles may also have benefited from denser vegetation in treatments with fertilizer addition and only one cut per year as Staphylinidae prefer shaded microhabitats (Bohac 1999; Dauber et al. 2005). Further, lower cutting frequency may have improved overwintering conditions of Staphylinidae (Collins et al. 2003). The more pronounced response of beetles and dipterans to experimental treatments as compared to other taxa studied likely was due to the fact that both are good dispersers, enabling them to aggregate at sites with favourable environmental conditions and ample food supply.

In accordance to our hypotheses, some soil animal groups significantly responded to the studied variations in grassland management. However, in general, soil animals responded little to the studied management practices and variations in sward composition. Potentially, the low responsiveness was due to the relatively short time the experiment was lasting, as it was set up only 2 years before taking samples. A number of studies stressed the need for long-term studies lasting several years, allowing evaluation of management effects on soil organisms (Wardle et al. 1999; Gastine et al. 2003). Eisenhauer et al. (2012) emphasized the time lag between changes in plant diversity and community composition and the response of the belowground system, due to buffering by the pool of soil organic matter. Generally, the importance of soil history for current soil processes and soil community structure and functioning, i.e. the legacy of soil, is increasingly recognized (Elgersma et al. 2011; de Vries et al. 2012; Allison et al. 2013). Also, it has to be considered that abundances of arthropods vary considerably with season and year due to changes in biotic and abiotic conditions (Boyer et al. 2003; Yang et al. 2008), which we did not record in the present study.

As indicated by the significant response of microorganisms, with microbial biomass responding to the interaction of cutting frequency and fertilizer addition, and fungal abundances responding to sward composition, microorganisms are more sensitively responding to changes in grassland management than to soil invertebrates. The more sensitive response of microorganisms also is reflected by the fact that fertilizer addition and cutting frequency interactively affected microbial biomass, with higher cutting frequency lessening the detrimental effect of fertilizer addition. Notably, the response of soil invertebrates was most pronounced in taxa at the base of the food web such as (detritivorous) dipterans, earthworms, entomobryid collembolans and in trend oribatid mites, suggesting that management effects diminish at higher trophic levels and the direction of effects varies among taxa, presumably due to changes in the availability of food resources. The results indicate that microbial parameters more sensitively respond to intensification of management practices in grassland than to soil animals, at least early after changes of management practices. Studies lasting several years are needed to clarify if this also holds in the long term.

Acknowledgments The study formed part of the Cluster of Excellence ‘Functional Biodiversity Research’ funded by the Ministry of Science and Culture of Lower Saxony and the ‘Niedersächsisches Vorab’. We thank Mark Maraun for advice in data analysis, Christel Fischer for help in sorting mesofauna and Guido Humpert for support in the laboratory.

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