Influence of plant diversity on soil organic carbon storage and microbial transformation of organic carbon in soils

Dissertation

Zur Erlangung des akademischen Grades doctor rerum naturalium
(Dr. rer. nat.)

Vorgelegt dem Rat der Chemisch-Geowissenschaftlichen Fakultät der
Friedrich-Schiller-Universität Jena

von Diplom Geoökologin Maike Habekost

geboren am 11.10.1979 in Hannover

Gutachter

1. Prof. Dr. Roland Mäusbacher, Institut für Geographie, FSU Jena
2. Prof. Dr. Stefan Scheu, FB Biologie, TU Darmstadt
3. PD Dr. Gerd Gleixner, Max-Planck-Institut für Biogeochemie, Jena

Tag der öffentlichen Verteidigung: 10.12.2008

Table of Contents

List of Figures

List of Tables

1 Introduction

1.1 Global change and carbon cycle

It is widely accepted that global changes, e.g. climate change, occur worldwide. According to the IPCC report (IPCC, 2007), climate warming is projevted to drive major changes in ecosystem structure and function, species interactions and ecosystem goods and services . A further concern the assumption that an increase in average temperature of about 1.5 - 2.5 °C, which is very likely in most scenarios, will increase the extinction risk of plant and animal species by 20 - 30 %. One essential driving force behind climate change is the increase of carbon dioxide concentration from pre-industrial times mainly due to burning of fossil fuels and changes in land use (IPCC, 2007). There is strong evidence that human activities are perturbing the carbon cycle to a significant extent and that negative consequences of the resulting climate change are likely. As carbon dioxide is one of the main determinants of climate change further understanding of the carbon cycle is of major importance.

The terrestrial biosphere plays a central role in the global carbon cycle. The soil carbon pool is the biggest carbon pool; presumably 1500 gt or approximately two thirds of the terrestrial carbon is stored in the soil (Amundson, 2001). In grassland ecosystems the soil carbon pool is particularly important because up to 98 % of the total organic carbon storage can be found sequestered below ground (Hungate et al., 1996). Soils have an enormous potential to act as carbon sinks and to mitigate human induced increases of atmospheric carbon dioxide. In a meta-analysis Guo and Gifford (2007) found that carbon stocks increased after land use change from crop to pasture while the reversed land use change usually lead to a decline in carbon stocks.

In general, carbon storage is suggested to be influenced by selective preservation of recalcitrant compounds, physical protection against decomposition and interactions with mineral surfaces (Torn et al., 1997; von Lützow et al., 2008). Further, the amount of soil carbon is related to soil texture (Schimel et al., 1994; Tan et al., 2004) and particularly the soil clay content drives the amount of soil organic carbon (Schimel et al., 1994; Telles et al., 2003). Furthermore, in addition to soil abiotic factors also biotic factors influence carbon storage. Atmospheric carbon will be retained in plant biomass increasing the carbon reservoir of the vegetation (Schulze, 2006). Aboveground biomass enters the soil labile carbon pool via roots, root exudates and litter input. The distribution and the amount of

input depend on the aboveground vegetation. In temperate grasslands three quater of the root biomass is found within the upper 0.3 m of the soil (Jackson et al., 1996; Jobbagy et al., 2000). According to the high root input in the top soil layer, the storage in the top 0.2 m of grasslands accounts for 40 % of total storage of the upper 1 m (Jobbagy et al., 2000). Although a high proportion of roots can be found in the top soil, the roots of many grasses and herbs grow to 1 m depth or even deeper (Craine et al., 2003). As a consequence, a large proportion of roots enters the soil to a considerable depth leading to high storage deeper in the soil. In grasslands at least 60 % of the whole carbon storage can be found between 0.2 m and 1 m depth (Jobbagy et al., 2000). However, decomposition of organic material is depth-dependent with higher rates in the upper soil layers than in deeper soil layers (Gill et al., 2002). Input of roots, litter and partly decomposed plant material, which is relatively labile, is decomposed by macro-, meso- and microorganisms and sequestered as soil organic matter associated to mineral soil particles in a more sustainable way. Both carbon pools, the labile and the more sustainable pool, can be separated by density fractionation (Six et al. 2002; Gregorich et al. 2006). Microorganisms are suggested to play a centrale role in the transformation of organic inputs (Ekschmitt et al., 2008). However, a better understanding of microbial-mediated soil organic matter transformation is needed (De Deyn et al., 2008). Similar to root input and decomposition, microbial biomass and diversity of soil microbial communities are lower in the sub soil than in the top soil (Ekschmitt et al., 2008). This depth distribution can be largely attributed to the decline in substrate availability (Fierer et al., 2003). Additional to organic carbon, nitrogen which often is a limiting nutrient for plant growth is also important for soil microorganisms (Spehn et al., 2000; Billings et al., 2008) and has an effect on decomposition of organic matter and carbon storage.

Beyond soil abiotic factors controlling carbon storage the vegetation strongly impacts the amount, variety and transformation of organic inputs and therefore carbon storage. Apart from the impact of land use change on carbon storage it can be assumed that changes in the aboveground vegetation, e.g. changes in plant diversity and plant functional group composition, impact belowground diversity (Hooper et al., 2000; Wardle et al., 2004) and thereby carbon storage. Currently, the links between the above- and belowground compartments are not well understood. Particularly, it is still under debate how plant diversity influences belowground processes like carbon storage (Catovsky et al., 2002; Steinbeiss et al., 2008a), decomposition (Hector et al., 2000; Scherer-Lorenzen, 2008) and

soil macro-, meso- and microorganisms (Stephan et al., 2000; Zak et al., 2003; Laossi et al., 2008; van der Heijden et al., 2008).

Diversity comprises a broad spectrum of biotic scales and can generally be described as the number of entities, the evenness of their distribution, the differences in functional traits and their interactions (Millenium Ecosystem Assessment, 2005; Diaz et al., 2006). Besides the number of species the number of functional groups is an important diversity measure. A functional group encompasses a set of species with similar morphological, physiological and phenological traits and it can be assumed that species within a functional group provide similar ecosystem services and react similar to environmental changes (Hooper et al., 2005). Functional group richness is of specific interest since human impacts on the environment not only cause a general decline in the number of species but also in the number of different functional groups. Furthermore, the dominance among different functional groups may be shifted (Loreau et al., 2001). Additionally, the presence or absence of distinct functional groups like grasses or legumes might be important for ecosystem functioning (Diaz & Cabido 2001). Ecosystem functioning comprises the flow of energy and materials through an ecosystem. Examples for ecosystem functioning are primary production or nutrient cycling (Diaz & Cabido 2001; Hooper et al., 2005; Millenium Ecosystem Assessment, 2005).

Changes in plant diversity is suggested to have considerable impacts on most if not all of the biotically controlled parameters and processes driving carbon storage (Figure 1-1).

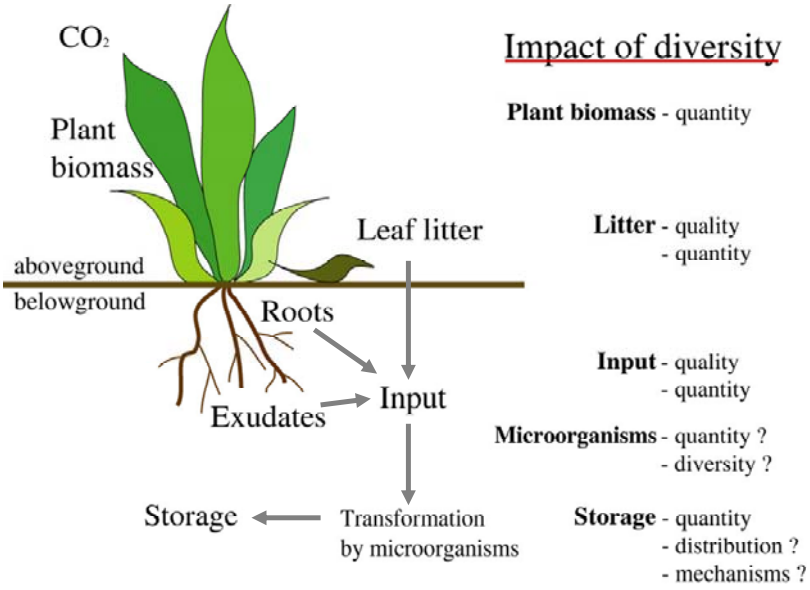

Figure 1-1: Parameters and processes effecting soil organic matter storage and the impact of plant diversity on these parameters and processes

Increasing species richness often leads to enhanced aboveground biomass production (Hector et al., 1999; Tilman et al., 2001; Flombaum et al., 2008) which in turn is likely to result in higher input of above- and belowground organic matter resembling major parameters for carbon storage (Rasse et al., 2005). Furthermore, the microbial transformation of inputs is assumed to lead to sustainable storage and might be changed by aboveground diversity as well. In low diverse plant communities, phenological development of the stand is relatively synchronous, potentially leading to vegetation gaps during the growing season. These vegetation gaps are suggested to indirectly influence microbial transformations by affecting soil abiotic conditions and the microclimate through changes in evapotranspiration (Scherer-Lorenzen, 2008) and have direct impacts on the litter supply of the soil microbial community. Additionally, it is most likely that a more diverse plant community leads to more heterogeneous resources entering the soil, which in turn might influence microbial diversity, e.g. through promoting greater resource partitioning and niche complementarity among the soil organisms (Hooper et al., 2000). In addition, increased inputs at higher plant diversity is assumed to promote particularly those organisms that are primarily regulated by resource availability (Mikola et al., 1998). However, the question if more diverse input at higher plant diversity leads to changes in soil microbial biomass and community structure requires further investigation.

As soil abiotic factors drive carbon and nitrogen transformation and their storage in soils, effects of plant diversity on storage might be hidden. Therefore, it is most promising to study the impact of plant diversity on carbon storage under experimental conditions where soil abiotic parameters are less variable and better defined like in the Jena Experiment; a grassland diversity experiment in the north of Jena. Overall, the Jena Experiment seeks to investigate the interactions between plant diversity and ecosystem functioning and focuses on trophic intreractions and element cycling. In 2002, randomly assembled plant communities of European grassland species ranging from 1 - 60 species and from 1 - 4 functional groups were established on a former agricultural field.

A previous study on the impact of plant diversity on soil organic carbon in experimental grasslands showed that an increase in sown species richness leads to higher organic carbon storage in the top soil (Steinbeiss et al., 2008a). Root biomass production tended to rise with increasing species richness and was an important determinant for storage in the top soil. However, additional mechanisms beyond a simple input driven one must be important because another study revealed that a higher input did not necessarily lead to higher carbon

storage (Steinbeiss et al. 2008b). Furthermore, these studies revealed that species richness was also an important determinant for soil organic carbon storage. Based on these results, this thesis aimed to investigate the impact of plant diversity on soil organic carbon and nitrogen storage in the top and sub soil and focusses on microbial transformation of organic inputs to soils. Improved understanding of the impact of plant diversity on storage mechanisms will provide valuable information concerning global change predictions and mitigation strategies.

1.2 Outline of the thesis

The thesis is composed of four studies and is intended to test the following hypotheses:

- Plant diversity is assumed to increase the amount and depth distribution of root inputs. Furthermore, plant diversity might lead to changes in the substrate use efficiency of soil microbial communities. Since both parameters are important determinants for carbon and nitrogen storage we hypothesised that plant diversity positively influences the soil organic carbon and total nitrogen storage in the top and the sub soil profile and therefore might mitigate consequences of climate change.
- Based on previous results on carbon and nitrogen storage in the top soil we further hypothesised that increased storage at higher diversity levels is mainly caused by increased transformation of organic inputs. This implies that increased plant diversity would lead to sustainable sequestration of organic carbon and nitrogen and consequently ensure ecosystem functioning.
- Quality, quantity, variety and timing of carbon and nitrogen inputs, which are prerequisites for microbial nutrition, are different at varying plant diversity levels. Therefore, we hypothesised that plant diversity affects belowground microbial community biomass and composition.

To test whether plant diversity increases soil organic carbon and nitrogen storage in the top and sub soil soil, samples up to 1 m depth were taken from plots with different plant diversity in 2007 (Chapter 2). In both soil segments increased root biomass at higher diversity might be an important driving factor for carbon and nitrogen storage. Besides organic matter input further determinants for storage are discussed. Density fractionation was performed to test if a higher proportion of the organic input is microbially transformed

and therefore sustainable stored at higher diversity levels (Chapter 3). For soil microorganisms and the microbial transformation the quality and quantity of organic matter inputs might be important. The effect of season and vegetation cover on soil microbial biomass and community structure was determined using microbial biomass measures (phospholipid fatty acids and chloroform fumigation extraction) (Chapter 4). Season and vegetation cover were assumed to have an impact on soil microogranisms because they strongly alter plant input and the microenvironmental conditions of the soil. Five years after the establishment of the Jena Experiment analyses of microbial phospholipid fatty acids were used to study the effect of plant diversity on soil microbial abundance and community structure (Chapter 5). In this study the whole diversity gradient and two adjacent meadows and arable plots were analysed and it was tested which plant diversity parameter (number of species, number of functional groups and the presence of functional groups) is most important for microbial biomass and community structure. Finally, the conclusions of the main results were drawn and the general impact of plant diversity on carbon and nitrogen storage and microbial transformation of organic inputs are discussed (Chapter 6).

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2 Organic carbon and nitrogen storage in soil depth profiles of experimental grasslands with varying plant diversity

Chapter source: Habekost *et al.*, 2008. Organic carbon and nitrogen storage in soil depth profiles of experimental grasslands with varying plant diversity. Submitted to Biogeochemistry

Abstract

Land use change from arable land to grassland is known to increase carbon and nitrogen stocks in soil. However, the role of plant diversity for soil carbon and nitrogen storage is still unclear. Therefore, we investigated the effect of plant diversity on soil organic carbon and nitrogen storage in The Jena Experiment, a large grassland biodiversity experiment in Germany that was established in 2002 on an agricultural field. Three independent soil cores to 1 m depth were taken on 20 plots at the beginning of the experiment and in 2007. After five years, soil organic carbon and total nitrogen stocks had increased by 675 g m^{-2} (sd = 114 g m^{-2}) and 93 g m^{-2} (sd = 12 g m^{-2}). The gain of soil organic carbon and nitrogen was concentrated in two depth segments. In 0 - 20 cm depth we found an addition of 736 g m^{-2} (sd = 266 g m^{-2}) of organic carbon and 72 g m^{-2} (sd = 25 g m^{-2}) of nitrogen. The storage was positively affected by plant diversity and soil texture, but only the effect of soil texture was significant. Root biomass was neither significant for soil organic carbon nor for total nitrogen storage in this depth segment. In 60 - 90 cm depth, 162 g m^{-2} (sd = 415 g m^{-2}) organic carbon and 39 g m^{-2} (sd = 35 g m^{-2}) total nitrogen were sequestered five years after land use change. In this depth segment, root growth and plant diversity were significant drivers for the observed storage. Our results indicate that plant diversity positively affects both, organic carbon and total nitrogen storage in soils. Low C/N ratios of stored soil organic matter suggested that the soil microbial community, that mainly mediates the transformation of organic matter, might drive this storage.

Keywords: soil carbon and nitrogen sequestration, land use change, managed grassland, sown species richness, The Jena Experiment

2.1 Introduction

The terrestrial biosphere is a key component in the global carbon cycle. More than 70 % of carbon from the terrestrial biosphere is stored in soils (Schimel et al. 2001). The understanding of patterns and driving factors of soil organic carbon and associated nitrogen storage is critical for our understanding of the biosphere and the feedback to climate change (Schaphoff et al. 2006; Woodwell et al. 1998). Land use change from arable land to grassland can particularly increase soil carbon stocks (Conant et al. 2001; Han et al. 2007). However, the role of plants and especially the role of plant diversity in this process is still not well understood.

Plant biomass input is a major determinant of the formation of soil organic carbon and nitrogen and roots consequently leave distinct imprints on the relative distribution of carbon and nitrogen with depth (Jobbagy & Jackson 2000). Increasing niche complementarity in more diverse plant mixtures may lead to a deeper root distribution and therefore to a higher soil organic carbon and total nitrogen storage in the sub soil. Beside the amount and spatial distribution of organic matter inputs, the decomposition of these inputs, which is accompanied by decreasing C/N ratios, is a main prerequisite for storage. The decomposition and transformation of organic inputs is essentially mediated by soil microogranisms (Persiani et al. 2008; van der Heijden et al. 2008). The soil microbial community is impacted by both the amount and the quality of inputs. Low amounts of organic matter increased the substrate use efficiency (Wu et al. 1993) and a higher variety of inputs seemed to increase the microbial diversity (Spehn et al. 2000; Stephan et al. 2000). A more diverse and better adapted microbial community, probably including a higher proportion of k-strategists, use organic matter inputs more completely and efficient (Eisenhauer et al. 2008) and in turn have effects on soil organic carbon and nitrogen storage.

The positive effect of plant diversity, especially sown species richness, for soil organic carbon storage in top soils was recently established (Steinbeiss et al. 2008). However, so far it is neither investigated if plant diversity affects soil organic carbon storage in the sub soil nor if the total nitrogen storage in the entire profile is influenced by plant diversity. Therefore, we studied soil organic carbon and total nitrogen stock changes in an established grassland biodiversity experiment (The Jena Experiment) in up to 1m depth.

We hypothesize that plant diversity positively influences the soil organic carbon and total nitrogen storage in the soil profile because plant diversity is supposed to change (I.) the amount and distribution of root inputs and (II.) the substrate use efficiency and diversity of soil microbial communities, which are both important determinants for soil organic carbon and nitrogen storage.

2.2 Materials and Methods

2.2.1 Site description

All samples were collected at the field site of The Jena Experiment, a field experiment investigating interactions between plant diversity and ecosystem processes focussing on element cycling and trophic interactions (Roscher et al. 2004). Before the start of the experiment, the site had been used and managed as an arable field for 40 years. The soil was classified as Eutric Fluvisol (FAO 1998) developed from loamy fluvial sediments. The texture ranged from sandy loam to silty clay with increasing distance to the river Saale flowing close to the experimental site. Due to the varying soil properties, the field site was divided into four blocks located parallel to the river. For soil depth analyses the most intensively investigated block 2 was chosen. The sand content increased in the upper 20 cm of the soil profile from 11 % in the north to 36 % in the south of the block, while the silt content ranged from 67 to 47 %, respectively. The clay content showed almost no spatial trend and stayed in the range of 18 to 22 %. In 20 - 100 cm depth, soil texture was homogenous containing 16 % sand, 59 % silt and 25 % clay.

In May 2002, different mixtures of grassland species belonging to the species pool of Central European meadows were sown on the plots (each 20 m x 20 m). The plant diversity gradient spanned 1, 2, 4, 8, 16 and 60 species and 1, 2, 3 and 4 functional groups (legumes, grasses, tall herbs and small herbs). The investigated block 2 contained 4 replicates of monocultures, 2, 4 and 8 species mixtures, 3 replicates of 16 species mixtures and one plot with a 60 species mixture. The number of plots with the same number of functional groups was highly unbalanced between the functional group levels and was therefore not included in the analyses.

In June and August each year, the plots were mown and the mown biomass was removed from the plots. The plant diversity gradient was maintained by weeding in April and June each year.

2.2.2 Soil sampling and analysis

Soil samples were taken in April 2002 before sowing and in April 2007 on 20 plots of the intensively investigated block 2. Three independent soil cores per plot were collected to a depth of 1 m by using a machine driven soil corer (Cobra, Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) with an inner diameter of 8.7 cm. Soil cores were segmented into 5 cm depth sections and soil samples were dried at 40 °C and sieved to 1 mm particle size (Allard et al. 2005; Ostonen et al. 2005; Stevens & Jones 2006).

Total nitrogen, total carbon and inorganic carbon concentrations of the ground samples were determined by elemental analysis at 1150 °C (Elementaranalysator vario Max CN, Elementar Analysensysteme GmbH, Hanau, Germany). The concentration of organic carbon was calculated by subtracting the total and inorganic carbon concentrations (Steinbeiss et al. 2008). Certified reference soil material was measured every 60 samples. The repeated measurements of the soil standard resulted in a relative standard deviation for soil organic carbon concentration of 1.6 % and for total nitrogen concentration of 2.2 %.

Biodiversity effects on soil organic carbon and total nitrogen stocks were analyzed for 0 - 20 cm, 20 - 30 cm, 30 - 60 cm, 60 - 90 cm and 90 - 100 cm depth segments. These segments corresponded to distinct zones of stock changes and could be compared with experimental data of total root biomass that was determined with this depth resolution.

In 2002, soil bulk density was determined at 6 plots on block 2. The depth of the segments for density measurements ranged from 0 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm, 40 - 60 cm, 60 - 80 cm and 80 - 100 cm. Samples were taken with a metal bulk density ring of 10 cm height, sieved to 2 mm and dried at 105 °C. The soil density was calculated by weight (Hartge & Horn 1992). The chosen plots represented a spatial gradient across the block and an average soil bulk density value per depth segment was calculated for the beginning of the experiment. In 2007, changes in the bulk density were measured for every plot in block 2 with 5 cm depth resolution using the inner diameter of the soil corer for volume calculation. In 0 - 30 cm depth a logarithmic and in 30 - 100 cm depth a linear regression was applied to adapt the depth resolution of both measurements, 2002 and 2007 ($0.8 \leq R \leq 1$).

2.2.3 Root biomass

Standing root biomass was determined in 2006 in 0 - 20 cm, 20 - 30 cm, 30 - 60 cm, 60 - 90 cm and 90 - 120 cm depth. Total root standing biomass (in the following referred to as root biomass) was separated from 50 g sub-samples per depth segment. Soil was removed from the roots by rinsing over a sieve with 0.5 mm mesh size. The separated roots were dried at 70 °C and weighed.

2.2.4 Statistics

Statistical analyses were carried out with SPSS Version 15.0 (SPSS Inc., Chicago, USA). All soil data were normally distributed and showed homoscedasticity. Differences in soil organic carbon and total nitrogen stocks between 2002 and 2007 were compared by paired t-tests. Differences between plots with (12 plots) and without (8 plots) legumes were also tested by t-tests.

In the analyses of variance (ANOVAs, Type I SS) the logarithm of sown species richness was included as a fixed factor. In 0 - 20 cm depth, where soil texture markedly varied between the plots, the sand content was fitted first as a covariate. The root biomass was tested as covariate before the sown species richness in order to distinguish between effects of the input amount and remaining plant diversity effects. The 60 species plot was excluded from the statistical analyses due to the lack of replicates on block 2.

2.3 Results

2.3.1 Soil organic carbon and total nitrogen stocks

Soil bulk density, necessary to calculate soil organic carbon and total nitrogen stocks varied between 0.85 g cm^{-3} and 1.85 g cm^{-3} in dependence of both sand content and depth and remained the same between 2002 and 2007 ($0.210 \leq p \leq 0.991$) (data not shown).

In 2002, soil organic carbon and total nitrogen stocks were high in the plough horizon and rapidly decreased in the horizons below (Table 2-1). The total organic carbon stock in the plough horizon (0 - 30 cm depth) was 7462 g m^{-2} (sd = 109 g m^{-2}), while 11505 g m^{-2} (sd = 192 g m^{-2}) were found in 30 - 100 cm depth. The total nitrogen stock in the plough horizon was 798 g m^{-2} (sd = 12 g m^{-2}) and 1358 g m^{-2} (sd = 21 g m^{-2}) were found in 30 - 100 cm. Similar to organic carbon and total nitrogen stocks, the mean C/N ratio was higher in the plough horizon (9.4 sd = 0.3) and decreased below (8.5 sd = 0.6).

Table 2-1: Soil organic carbon, total nitrogen stocks and stock changes between 2002 and 2007 (sd = standard deviation). P-values evaluate differences between stocks in 2002 and 2007. Asterisks mark significance at the 0.05 (*), 0.01 (**) or 0.001 (***) level. Standard deviation in parentheses.

depth	Corg 2002	Corg 2007	Corg 2007 - 2002		p-value	N 2002	N 2007	N 2007 - 2002		p-value	C/N ratio 2002	C/N ratio 2007
(cm)	(g m^{-2})	(g m^{-2})	(g m^{-2})			(g m^{-2})	(g m^{-2})	(g m^{-2})				
0 – 5	1093 (116)	1426 (167)	333 (112)	(***)	< 0.001	115 (10)	143 (15)	28 (8)	(***)	< 0.001	9.5 (0.4)	10.0 (0.3)
5 – 10	1173 (130)	1419 (155)	246 (53)	(***)	< 0.001	123 (12)	149 (14)	26 (6)	(***)	< 0.001	9.5 (0.3)	9.5 (0.3)
10 – 15	1259 (129)	1392 (158)	133 (71)	(***)	< 0.001	133 (11)	148 (14)	14 (7)	(***)	< 0.001	9.4 (0.4)	9.4 (0.3)
15 – 20	1336 (150)	1362 (167)	25 (84)		0.163	141 (13)	144 (15)	3 (8)	(*)	0.047	9.5 (0.3)	9.4 (0.4)
20 – 25	1343 (149)	1371 (174)	28 (103)		0.232	145 (15)	147 (16)	2 (9)		0.331	9.2 (0.3)	9.3 (0.3)
25 – 30	1259 (166)	1247 (186)	-12 (145)		0.703	140 (15)	138 (17)	-2 (14)		0.502	9.0 (0.5)	9.0 (0.3)
30 – 35	1104 (194)	965 (191)	-139 (210)	(**)	0.006	127 (18)	111 (19)	-16 (19)	(**)	0.001	8.7 (0.6)	8.6 (0.4)
35 – 40	866 (116)	804 (120)	-62 (159)		0.068	103 (11)	94 (13)	-9 (16)	(*)	0.017	8.4 (0.5)	8.5 (0.3)
40 – 45	747 (66)	709 (95)	-38 (84)	(*)	0.041	89 (8)	84 (11)	-5 (12)		0.064	8.4 (0.6)	8.4 (0.4)
45 – 50	697 (87)	690 (72)	-7 (89)		0.776	84 (9)	83 (9)	-1 (10)		0.65	8.4 (0.8)	8.4 (0.4)
50 – 55	662 (86)	647 (78)	-15 (65)		0.342	79 (8)	79 (9)	0 (7)		0.839	8.4 (0.9)	8.2 (0.4)
55 – 60	586 (98)	620 (151)	34 (120)		0.166	69 (8)	77 (10)	9 (6)	(***)	< 0.001	8.5 (0.9)	8.2 (0.5)
60 – 65	564 (109)	598 (147)	34 (123)		0.185	66 (9)	74 (10)	9 (7)	(***)	< 0.001	8.6 (1.2)	8.3 (0.5)
65 – 70	542 (109)	597 (103)	55 (82)	(**)	0.005	65 (9)	73 (12)	8 (7)	(***)	< 0.001	8.3 (1.2)	8.1 (0.4)
70 – 75	537 (106)	577 (116)	39 (86)	(*)	0.039	65 (10)	72 (14)	7 (8)	(***)	< 0.001	8.3 (1.1)	8.0 (0.5)
75 – 80	539 (120)	567 (122)	29 (68)	(*)	0.043	63 (12)	70 (14)	7 (7)	(***)	< 0.001	8.5 (1.0)	8.1 (0.5)
80 – 85	518 (126)	522 (120)	4 (62)		0.578	61 (13)	65 (14)	4 (6)	(**)	0.005	8.4 (0.7)	8.0 (0.4)
85 – 90	496 (121)	498 (115)	2 (81)		0.8	59 (13)	63 (14)	4 (6)	(**)	0.006	8.5 (0.9)	8.0 (0.5)
90 – 95	470 (126)	480 (118)	11 (78)		0.442	56 (14)	60 (14)	4 (5)	(***)	< 0.001	8.4 (0.6)	7.9 (0.5)
95 – 100	453 (104)	429 (100)	-25 (64)		0.1	55 (13)	55 (12)	0 (5)		0.888	8.3 (0.9)	7.8 (0.5)
0 - 100	**16244 (337)**	**16918 (378)**	**675 (114)**	(**)	**0.005**	**1837 (34)**	**1931 (35)**	**93 (12)**	(**)	**0.001**	**8.7 (0.8)**	**8.5 (0.8)**

In total, 16244 g m^{-2} (sd = 337 g m^{-2}) of organic carbon and 1837 g m^{-2} (sd = 34 g m^{-2}) of total nitrogen were found in 0 - 100 cm depth.

From 2002 to 2007, the soil organic carbon stocks increased significantly in 0 - 15 cm depth by 133 g m^{-2} to 333 g m^{-2} and in 65 - 80 cm depth by 29 g m^{-2} to 55 g m^{-2} ($0.001 \leq p \leq 0.043$). A soil organic carbon loss of 38 g m^{-2} to 139 g m^{-2} was observed in 30 - 45 cm depth ($0.006 \leq p \leq 0.068$) with the highest loss between 30 and 35 cm directly below the plough horizon (139 g m^{-2} sd = 210 g m^{-2}).

Integrated to the depth resolution used for statistical analyses (0 - 20 cm, 20 - 30 cm, 30 - 60 cm, 60 - 90 cm, 90 - 100 cm), the change in soil organic carbon stocks between 2002 and 2007 was still significant in 0 - 20 cm depth ($p < 0.001$) (Figure 2-1). Below this depth segment (20 - 30 cm depth) carbon stocks did not change ($p = 0.869$), while in 30 - 60 cm depth the soil organic carbon stock decreased by 225 g m^{-2} (sd = 560 g m^{-2}) ($p = 0.054$), whereas in 60 - 90 cm depth 162 g m^{-2} (sd = 458 g m^{-2}) soil organic carbon were gained ($p = 0.062$). In 90 - 100 cm depth, no change in the carbon stock was observed ($p = 0.715$). Five years of grassland vegetation significantly increased carbon stocks summed over the whole profile from 16244 g m^{-2} (sd = 337 g m^{-2}) to 16918 g m^{-2} (sd = 378 g m^{-2}) ($p = 0.005$).

In the 5 cm segments, total nitrogen stocks significantly increased by 3 g m^{-2} to 28 g m^{-2} within the five years in 0 - 20 cm depth ($0.001 \leq p \leq 0.047$), decreased by 9 g m^{-2} to 16 g m^{-2} ($0.001 \leq p \leq 0.017$) in 30 - 40 cm depth and increased again in 55 - 95 cm depth (increase: 4 g m^{-2} to 9 g m^{-2}, $0.001 \leq p \leq 0.006$) (Table 2-1). Integrated to the depth resolution used for statistical analyses, the increase in total nitrogen between 2002 and

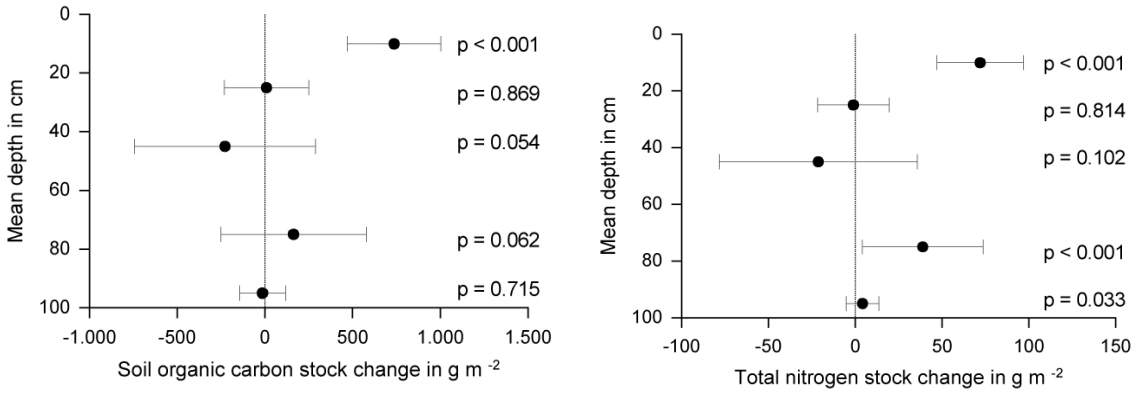

Figure 2-1: Soil organic carbon and total nitrogen stock changes in various depths between 2002 and 2007; error bars represent standard deviations.

2007 was significant in 0 - 20 cm, 60 - 90 cm and 90 - 100 cm depth, where 591 g m^{-2} (sd = 65 g m^{-2}, $p < 0.001$), 417 g m^{-2} (sd = 2 g m^{-2}, $p = 0.001$) and 115 g m^{-2} (sd = 26 g m^{-2}, $p = 0.033$) of the total nitrogen were gained, respectively (Figure 2-1). The total nitrogen loss was not significant in 30 - 60 cm depth (21 g m^{-2} sd = 57 g m^{-2}, $p = 0.102$). Over the whole profile, total nitrogen stocks increased from 1837 g m^{-2} (sd = 34 g m^{-2}) in 2002 to 1931 g m^{-2} (sd = 35 g m^{-2}) in 2007 ($p = 0.001$).

The C/N ratio in 2007 had not changed compared to 2002. The C/N ratio of the stored organic matter was higher in the upper 20 cm of the depth profile compared to the 60 - 90 cm depth (0 - 20 cm: 9.9 sd = 1.4; 60 - 90 cm: 6.7 sd = 3.7).

2.3.2 Effects of plant diversity on soil organic carbon and total nitrogen stocks

In 0 - 20 cm depth, soil organic carbon storage after five years was highest on 16 species plots and lowest in monocultures (Figure 2-3). While plots with monocultures stored 444 g m^{-2} (sd = 121 g m^{-2}), plots with 16 species stored 1003 g m^{-2} (sd = 145 g m^{-2}). Carbon storage in this depth was significantly determined by the soil texture (Table 2-2), which explained 47 % of the variance in the sequential ANOVA. Sown species richness explained 20 % of the variance. Most interestingly, root biomass had no effect on carbon storage in the upper 20 cm.

Table 2-2: Significance and explained proportion of the sum of squares (SS) for changes of soil organic carbon (C_{org}) and total nitrogen (N) stocks five years after establishment of the experimental design gained by sequential analyses of variance components (ANOVA). Asterisks mark significance at the 0.05 (*) or 0.01 (**) level.

	depth	parameter	p-value		F-value	% of SS
C_{org}	0-20 cm	sand content in %	0.002	(**)	15.8	46.8
		root biomass in 2006	0.745		0.1	0.3
		ln sown species richness	0.217		1.7	20.3
	20 - 30 cm	root biomass in 2006	0.123		2.8	13.9
		ln sown species richness	0.242		1.6	31.7
	30 - 60 cm	root biomass in 2006	0.912		0.1	0.1
		ln sown species richness	0.556		0.8	22.3
	60 - 90 cm	root biomass in 2006	0.024	(*)	6.6	21.6
		ln sown species richness	0.078		2.7	35.5
N	0-20 cm	sand content in %	0.009	(**)	10.0	37.5
		root biomass in 2006	0.492		0.5	1.9
		ln sown species richness	0.335		1.3	19.3
	20 - 30 cm	root biomass in 2006	0.387		0.8	3.9
		ln sown species richness	0.133		2.2	43
	30 - 60 cm	root biomass in 2006	0.969		<0.1	<0.1
		ln sown species richness	0.402		1.1	28.7
	60 - 90 cm	root biomass in 2006	0.003	(**)	13.3	26.6
		ln sown species richness	0.006	(**)	5.9	47.3

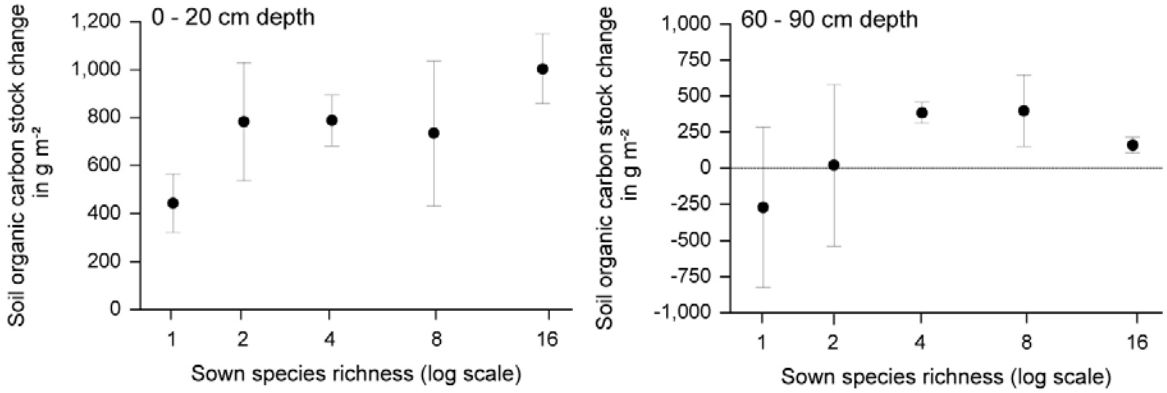

Figure 2-2: Relationship between sown species richness and changes in soil organic carbon stocks between 2002 and 2007 for soil depths 0 - 20 cm (left graph) and 60 - 90 cm (right graph); error bars represent standard deviations.

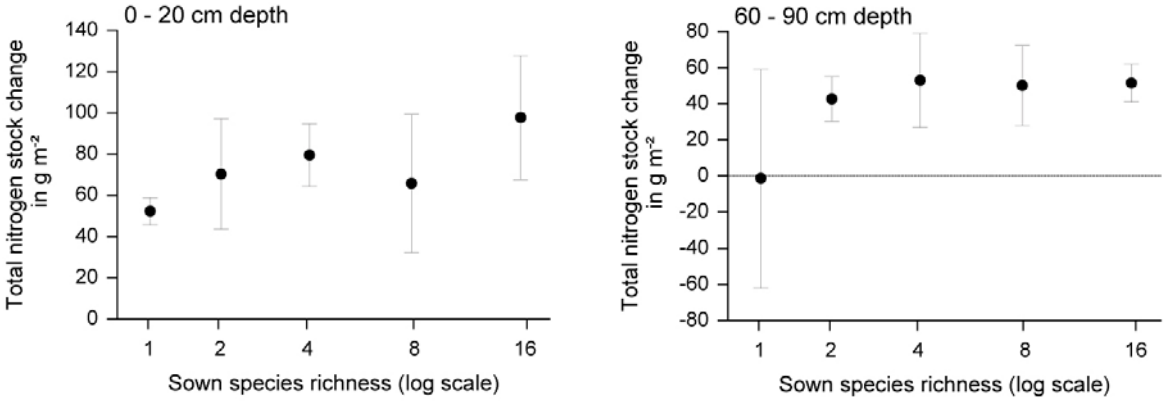

Figure 2-3: Relationship between sown species richness and total nitrogen stock changes between 2002 and 2007 for soil depths 0 - 20 cm (left graph) and 60 - 90 cm (right graph); error bars represent standard deviations.

In the zones of carbon loss at 20 - 30 cm and 30 - 60 cm depth, changes in soil organic carbon stocks were neither dependent on sown species richness (20 - 30 cm: $p = 0.242$; 30 - 60 cm: $p = 0.556$) nor on root biomass (20 - 30 cm: $p = 0.123$; 30 - 60 cm: $p = 0.912$) (Table 2-2). In contrast, in 60 - 90 cm depth, root biomass had a significant impact on soil organic carbon storage ($p = 0.024$) and explained 22 % of the variance between the plots. Sown species richness was statistically not significant ($p = 0.078$), but nevertheless explained 36 % of the variance. In 60 - 90 cm depth, plots with 4 and 8 species stored most of the carbon, i.e. 384 g m^{-2} (sd = 73 g m^{-2}) and 397 g m^{-2} (sd = 248 g m^{-2}), respectively, while plots with monocultures lost 270 g m^{-2} (sd = 554 g m^{-2}) during the investigation period (Figure 2-2).

In the 0 - 20 cm depth segment, the total nitrogen storage was positively correlated with the sown species richness ($R = 0.44$, $p = 0.058$). Plots with 16 species stored significantly more nitrogen ($p = 0.018$) than plots with monocultures (monocultures: 52 g m^{-2}

sd = 6 g m $^{-2}$; 16 species: 97 g m $^{-2}$ sd = 30 g m^{-2}) (Figure 2-3). However, sequential ANOVA showed that only the effect of the sand content was significant and explained 38 % of the variance (Table 2-2). As observed for carbon, the sown species richness was not significantly determining the nitrogen storage ($p = 0.335$), but explained 19 % of the variance (Table 2-2). No effect of root biomass on nitrogen storage could be detected in this depth ($p = 0.492$). The same held true for 20 - 30 cm and 30 - 60 cm depths, where neither root biomass nor sown species richness were significant parameters for the total nitrogen storage (20 - 30 cm: $p = 0.387$, $p = 0.133$; 30 - 60 cm: $p = 0.969$, $p = 0.402$ for root biomass and sown species richness, respectively). Interestingly, in the sub soil, between 60 and 90 cm depth, the root biomass and sown species richness were significant drivers for the total nitrogen storage (root biomass: $p = 0.003$; sown species richness: $p = 0.006$). While root biomass explained 27 % of the variance, approximtaley half of the total variance was explained by sown species richness (47 %). Total nitrogen stock changes were negative on plots with monocultures (1 g m $^{-2}$ sd = 60 g m^{-2}), while total nitrogen stocks on plots with 2 to 16 species increased (Figure 2-3).

The presence or absence of legumes had no effect on soil organic carbon and total nitrogen storage.

2.4 Discussion

Before the establishment of The Jena Experiment the organic carbon and total nitrogen distribution layed within the normal range of agricultural fields (Kirchmann et al. 2004) and confirmed lower total stocks compared to grasslands (Post & Kwon 2000; Vleeshouwers & Verhagen 2002). Our results showed that both soil organic carbon and total nitrogen stocks, increased during five years after conversion from cropland to grassland. We found that plant diversity, tested as sown species richness, and standing root biomass, as a proxy for root biomass input, were important drivers for the amount of soil organic carbon and total nitrogen stored in soils, whereas litter quality, tested as presence and absence of legumes, had no effect on organic carbon and total nitrogen storage. Stored soil organic carbon and total nitrogen were gained and lost in the depth profile at a C/N ratio of 9.7 (organic carbon = 9.74 * total nitrogen - 11.74, $R = 0.87$, $p < 0.001$). This suggests that not root biomass itself but transformed biomass, like microbial remains, were stored in the soil.

In the top soil (0 - 20 cm depth) root biomass did not explain much of the variance of soil organic carbon and total nitrogen storage. In this depth, mainly the soil texture controlled stock changes. This is in line with the general observation that the soil carbon content is positively correlated to silt and clay and negatively to the sand content (Schimel et al. 1994). Obviously, here the physical attachment of transformed organic carbon and total nitrogen to mineral surfaces (Torn et al., 1997) is more important for storage than the total input (Sun et al. 2004). In addition, plant diversity can be assumed to be a second driver for organic carbon and total nitrogen storage, because it explained much of the variance in the sequential ANOVAs. This assumption is supported by a previous study of Steinbeiss et al. (2008), who found a significant effect of plant sown species richness on carbon storage using segmented soil samples from all blocks of The Jena Experiment. Based on these results, we suggested that plant sown species richness mediates other soil and ecosystem parameters, like the composition and activity of micro-, meso- and macro-decomposers (Habekost et al. 2008; Spehn et al. 2000; Stephan et al. 2000) to be responsible for the organic carbon and nitrogen storage in top soils. This is strongly supported by the calculated C/N ratios of stored organic matter in the 0 - 20 cm depth segment of 9.9 being near the Redfield ratio of microbial biomass. It can be assumed that higher and more diverse inputs that occur on plots with higher plant diversity would induce a shift of soil microbial communities towards more diverse communities comprising a higher proportion of k-strategists, which might have a higher carbon use efficiency than resource wasting r-strategists. Consequently different soil microbial communities would affect both decomposition and storage of organic carbon and nitrogen in not carbon limited top soils.

However, the total carbon and nitrogen storage also increased in the sub soil (60 - 90 cm depth) within five years after conversion. In contrast to the top soil, the comparatively low root biomass input was in addition to sown species richness an important factor for soil organic carbon and total nitrogen storage. With decreasing organic matter input the substrate use efficiency of microbial communities increases (Witter & Kanal 1998), coinciding with lower respiratory losses and more efficient growth of microogranisms (Wu et al. 1993). Due to the relative small input compared to the top soil and the resulting relatively high storage rates, we assume that the substrate use efficiency in 60 - 90 cm depth is higher compared to the top soil. The low C/N ratios of stored organic matter suggest complete decomposition of root biomass and hence we assume that carbon and nitrogen storage in the sub soil is not decomposition but input limited. In addition to the

root input, plant diversity is an important factor for carbon and total nitrogen storage in this depth segment, because microbial diversity is mediated by plants similarly as in the top soil (Kramer & Gleixner 2008; Steenwerth et al. 2008). Also for the sub soil it can be assumed that the soil microbial community is more diverse in mixtures with higher sown species richness and tends to be depleted under monocultures. This might explain why monocultures, which have nearly the same root input like 4 and 8 species mixtures, lost soil organic carbon and total nitrogen, while plots with higher sown species richness stored carbon. On higher diverse mixtures, the resource utilization and concomitantly the transformation to soil organic matter points to more long lasting life strategies (k-strategies), whereas in monocultures more resource wasting r-strategists might occur.

Our results suggest that the observed positive effect of plant diversity on carbon and total nitrogen storage in soils is mediated by direct links of above ground diversity to composition, activity and substrate use efficiency of below ground organisms. More detailed studies are necessary to establish and ascertain this link.

2.5 Acknowledgements

The current project (GL 262-6) and The Jena Experiment in general is funded by the Deutsche Forschungsgemeinschaft (DFG) as part of a large research group (FOR 456) with additional support from the Friedrich Schiller University Jena and the Max Planck Society. E.-D. Schulze, M. Scherer-Lorenzen and S. Scheu improved the manuscript with their helpful comments. We thank H. Bessler and C. Engels (Humboldt University Berlin) for the root data, J. Baade (Friedrich Schiller University Jena) and Y. Kreutziger for the bulk density and soil texture data from 2002 and J. Kirschstein and numerous student helpers for their assistance in the soil sampling campaigns and sample preparation. We gratefully acknowledge all the people who were involved in planning, set-up and maintaining of the experiment, especially E.-D. Schulze, W. W. Weisser, B. Schmid, J. Schumacher, C. Middelhoff, C. Roscher and A. Weigelt.

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3 Partitioning of organic carbon and nitrogen in soil density fractions of an experimental grassland with varying plant diversity

Chapter source: Habekost *et al.*, 2008. Partitioning of organic carbon and nitrogen in soil density fractions of an experimental grassland with varying plant diversity. Submitted to Soil Biology & Biochemistry

Abstract

Both, conversion from arable land to grassland and plant diversity increase organic carbon and nitrogen storage in soils. The underlying mechanisms and processes are still not well understood. We investigated soil density fractions to asses the impact of land use change and plant species richness on the contribution of light and heavy fraction to soil organic carbon and nitrogen storage in order to explain if this storage appear only in the fast decomposed light fraction or in the more sustained mineral associated heavy fraction. The study was done at the field site of The Jena Experiment (Germany). Soil samples were taken in 2002 shortly before the establishment of the field site, 2004 and 2006 to 30 cm depth and segmented in 5 cm depth sections. The investigated plant diversity gradient spanned 4, 8 and 16 species and contained plots with and without distinct functional groups (small and tall herbs, legumes and grasses). In 2006, additional soil samples were taken from reference sites with arable land and meadows.

We found that both density fractions were sensitive to land use change. The light fraction increased by 109 g m^{-2} (sd = 51 g m^{-2}) of organic carbon and 5.4 g m^{-2} (sd = 2.8 g m^{-2}) of nitrogen and the heavy fraction increased by 311 g m^{-2} (sd = 101 g m^{-2}) of organic carbon by 38.4 g m^{-2} (sd = 8.2 g m^{-2}) of nitrogen within four years. The heavy fraction contributed with 74 % for organic carbon and 88 % for nitrogen the main proportion to storage. Furthermore, organic carbon storage was like the bulk storage positively affected by plant diversity. An effect of sown species richness on bulk nitrogen stock changes and storage in density fractions was not found. $\Delta^{14}C$ values of both density fractions increased with time and indicated that recent input was not exclusively stored in the light fraction but mainly directly microbially transformed and stored into the heavy fraction.

Keywords: density fractionation, plant diversity, The Jena Experiment, soil organic matter, land use change, carbon sequestration, nitrogen, $\Delta^{14}C$

3.1 Introduction

Understanding the drivers and mechanisms of soil carbon and associated nitrogen storage is essential against the background of global climate change (Lal, 2008). However, studies on soil organic carbon and nitrogen properties of the bulk soil are not sensitive enough to reveal carbon and nitrogen dynamics and storage mechanisms (Schlesinger et al., 2001). It is more promising to use physical fractionation techniques to separate soil fractions with distinct chemical and physical characteristics (Six et al. 2002; Gregorich et al. 2006). Density fractionation has been widely used to separate soil into a light and a heavy fraction, in which soil organic matter is stabilized to different degrees. The light fraction is build up by roots, litter and partly decomposed plant material (Carter et al., 1999; Poirier et al., 2005). It has been identified as a pool of active soil organic matter and as a readily decomposable substrate for soil microorganisms (Magid et al., 2001). Transformation of light fraction by soil microorganisms then leads to a more sustained storage in the heavy fraction, in which soil organic matter is more processed and stabilized (Golchin et al., 1994).

The amount and allocation of soil organic carbon and nitrogen in density fractions differ greatly between different land use types (Whalen et al., 2000). Accordingly, land use change can substantially shift the proportion of organic carbon and nitrogen stored in the respective density fractions through a change in input and decomposition (John et al., 2005); e.g. conversion from arable land to grassland increases the amount of carbon stored in the light fraction (Conant et al., 2001).

Beside the different contributions of density fractions to storage under certain land use types, plant diversity might also have an influence on the distribution of the amount and proportion of organic carbon and nitrogen between density fractions. In experimental grasslands (The Jena Experiment) a positive relationship between species richness and bulk organic carbon storage in the top soil was found (Steinbeiss et al., 2008). Underlying mechanisms and the question if the increased soil organic carbon storage is sustainable are currently subject of discussion. As plant diversity alters carbon and nitrogen inputs above- and belowground (Hector et al., 2000; Tilman et al., 2001; Wardle et al., 2004) this is hypothesized to result in increased root biomass at higher diversity and therefore higher amounts of light fraction. Further, aboveground diversity positively alters the belowground decomposer community, e.g. the soil microbial communities (Spehn et al., 2000; Wardle et al., 2006, Habekost et al., 2008). The soil microbial community mainly mediates the

transformation or organic carbon and nitrogen from the light fraction into the heavy fraction. Therefore, an impact of plant diversity on the proportion that both density fractions contribute to bulk storage might be possible. Plant functional groups, like legumes or grasses, are known to deliver inputs of different quantity and quality (Spehn et al., 2002). Thus, the presence and absence of functional groups might also have an influence on the characteristics and properties of density fractions.

Effects of land use change and plant diversity on soil organic carbon storage in density fractions were studied in an established grassland biodiversity experiment (The Jena Experiment), which was used as arable land before, and on two adjacent meadows and agricultural sites. Using density fractionation of soil from different plant diversity levels we want to answer the following questions: (1.) Is higher bulk storage with increasing plant diversity an effect of increased amounts of light fraction? (2.) Do the quantity and quality of input have an effect on the storage proportions in different density fractions? (3.) Does plant diversity help to store organic carbon and nitrogen more sustainable?

3.2 Materials and Methods

3.2.1 Study site

All samples were collected at the field site of The Jena Experiment, which is situated in the north of Jena, Germany, on the floodplains of the river Saale. The mean annual air temperature was 9.3 °C and the mean annual precipitation amounted to 587 mm (Kluge et al., 2000). The Jena Experiment was planned as a long-term experiment and was established in 2002. Before this time, the field site was used for agriculture and ploughed and fertilized regularly for the last 40 years. The soil was classified as Eutric Fluvisol (FAO, 1998) that developed from loamy fluvial sediments. The texture ranged from sandy loam to silty clay with increasing distance to the river. The field site was divided into four blocks according to the homogeneity of soil properties that run parallel to the river. Further information on soil characteristics and experimental design are given in Roscher et al. (2004). The sown grassland species typically grow in Central European mesophilic grasslands. The sowing density was 1000 seeds per m^2 divided equally among the species of each mixture. According to their morphological and ecological traits all species were categorized into four functional groups (grasses, small herbs, tall herbs, legumes) (Roscher et al., 2004). Density fractionation involved the selection of 24 plots that covered a gradient in sown species richness of 4, 8 and 16 species and represented plots with and

without certain plant functional groups. In June and August, the plots were mown and the cut biomass was removed from the plots. The plant diversity gradient was maintained by weeding.

Four reference sites i.e. two meadows and two arable land sites were additionally analyzed in 2006. The meadows were adjacent to the field site of The Jena Experiment and the arable land sites were directly on the field site of the Jena Experiment situated. The meadows were cut two times a year like the plots of The Jena Experiment. The arable sites were managed according to good agricultural practice but not fertilized.

3.2.2 Soil sampling and analysis

Soil samples were taken in April 2002 (before the sowing), 2004 and 2006 as paired samples with a spatial distance of less than 30 cm to avoid additional spatial variability (Lal et al., 2000). A split tube sampler with an inner diameter of 4.8 cm (Eijkelkamp Agrisearch Equipment, Giesbeck, Netherlands) was used to take soil cores up to 30 cm depth. While in 2002 five independent cores per plot were taken and separated in 5 cm depth segments, three cores per plot were taken in 2004 and 2006 and pooled per depth segment, respectively. After drying at 40 °C, the soil was sieved to 2 mm and remaining plant constituents were removed by hand. In 2004 and 2006, the soil was sieved to 1 mm according to common root removal techniques (Allard et al., 2005; Ostonen et al., 2005; Stevens et al., 2006). No additional mineral particles were removed by this process. Total nitrogen and organic carbon concentration of ground samples were determined by an elemental analyzer at 1150 °C (Elementaranalysator vario Max CN, Elementar Analysensysteme GmbH, Hanau, Germany). Bulk density was determined in 2002 in 0 - 10 cm, 10 - 20 cm and 20 - 30 cm depth. Samples were taken with a metal bulk density ring of 10 cm height, sieved to 2 mm and dried at 105 °C. To adapt soil organic carbon and nitrogen stocks to a 5 cm depth resolution a logarithmic regression was applied to the measured bulk densities. In 2004 and 2006, soil samples were weighed to estimate bulk density.

Soil samples originating from the reference sites (meadows, arable land) were taken in April 2006 and treated the same way as the samples from The Jena Experiment.

3.2.3 Standing root biomass

Three cores (0 - 30 cm depth) per plot were taken using a stainless steel corer and standing root biomass was determined at the pooled sample per plot. Samples were carefully homogenized and standing root biomass was determined from 50 g sub-samples by rinsing with water over a sieve with 0.5 mm mesh size.

3.2.4 Density fractionation

Soil organic matter was separated into a light and a heavy fraction by suspending 15 g of soil in 70 ml sodium ploytungstate solution (Sometu, Berlin, Germany), which was adjusted to a density of 1.6 g cm^{-3} (Golchin et al., 1994). For density fractionation of soil samples from 2002, aliquots of the five independent cores were mixed. Ultrasonication with 450 J ml^{-1} was used to break down macroaggregates, remove the encrustation on plant remains and free the occluded light fraction (Schmidt et al., 1999; Kolbl et al., 2005). The beaker was placed in an ice bath and sonicated using a probe-type ultrasonic disintegrator (Heinemann, Schwäbisch Gmünd, Germany) with an immersion depth of 1.5 cm. To ensure a defined energy input, the tip was calibrated regularly. Particles that adhered to the sonication tip were washed into suspension with polytungstate solution. Then, the samples were centrifuged for 30 minutes at 3500 U min^{-1}. The floating material was completely transferred to a millipore filter funnel fitted with a glass-fibre filter paper and filtered under vacuum. The light fraction was washed with millipore water to remove polytungstate remains, transferred into pre-weighted flasks and freeze-dried. The remaining sample was stirred with a glass stick and refilled with sodium ploytungstate solution. To ensure a complete removal of the light fraction the process was repeated three times. The remaining sample (heavy fraction) was washed five times with millipore water, transferred to a pre-weighted flask and freeze-dried. Both fractions were ground and analyzed for total nitrogen and organic carbon concentration as the bulk samples.

3.2.5 Radiocarbon measurements

Radiocarbon concentrations (^{14}C) were measured at light and heavy fractions. For 2002, 9 plots from The Jena Experiment, representing all diversity levels and covering the block gradient were chosen for measurements. For 2006, all plots and the reference sites were analyzed. Radiocarbon concentrations of the density fractions were determined with accelerator mass spectroscopy, 3MV AMS (High Voltage Engineering Europa,

Amersfoort, Netherlands) after decalcification with HCl and freeze drying (Steinhof et al., 2004). CO_2 evolved during dry combustion of the sample was reduced to graphite by heating a mixture of H_2 and CO_2 with iron powder at 650°C. The graphite was pressed into targets and measured with the AMS facility. All values were corrected for fractionation using $\delta^{13}C$ values. The radiocarbon data were expressed as $\Delta^{14}C$ that is ‰ deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard. The value is corrected for the radioactive decay of this standard since 1950. Average precision for the $\Delta^{14}C$ values was ± 4.2 ‰. To determine the proportion of recent inputs to the density fractions (DF) a $\Delta^{14}C$ mass balance was calculated for light and heavy fraction, respectively.

$$\Delta^{14}C_{calculated} = \frac{\left(amount DF_{2002} * \Delta^{14} C_{2002}\right) + \left(amount DF_{2006-2002} * \Delta^{14} C_{CO_2 air}\right)}{amount DF_{2006}}$$

$\Delta^{14}C$ values of atmospheric carbon dioxide from Schauinsland (Germany), which were measured between 2002 and 2006, were used to calculate $\Delta^{14}C_{CO_2 air}$. If the $\Delta^{14}C_{calculated}$ is the same as $\Delta^{14}C_{measured}$ for the respective fraction, than the whole input between 2002 and 2006 can assumed to be of recent origin.

3.2.6 Statistical analyses

Statistical analyses were carried out with SPSS Version 16.0 (SPSS Inc., Chicago, USA). Differences in soil organic carbon and total nitrogen in the light and heavy fraction between sampling dates were tested with paired t-tests. Differences between management forms were tested with unpaired t-tested. In order to account for differences in soil properties between the respective blocks, the block effect was included as a random factor fitted first in all analyses of variance (ANOVAs, type I SS). The log transformed sown species richness or the presence/absence of certain functional groups was included as fixed factors. The statistical significance was evaluated at the $p \leq 0.05$ level.

3.3 Results

3.3.1 Density fractions of the experimental site in 2002, 2004 and 2006

The mean mass recovery of the density fractionation was 95.2 % (sd = 2.3 %). The recovery of organic carbon was in the same order of magnitude as for nitrogen (organic carbon: 86.2 % sd = 9.2 %; nitrogen: 88.3 % sd = 9 %). The light fraction only accounted for 1 % on average of the fractionated soil mass. Organic carbon and nitrogen

concentration of the density fractions were contrariwise to the masses. Organic carbon and nitrogen concentration were 25 % (sd = 9 %) and 1.3 % (sd = 0.5 %) in the light fraction, respectively and 1.5 % (sd = 0.3 %) and 0.2 % (sd ≤ 0.1 %) in the heavy fraction, respectively. Although the light fraction contributed only to a small proportion to the soil mass, it contributed to11 % (sd = 4 %) of the bulk carbon and to 5 % (sd = 9 %) of the total nitrogen stocks. The main proportion of bulk carbon and nitrogen was found in the heavy fraction (organic carbon: 89 % sd = 4 %; nitrogen: 95 % sd = 9 %).

Bulk soil: In 2002, before the establishment of The Jena Experiment, the amount of bulk organic carbon and nitrogen of the plots chosen for density fractionation showed a depth depending distribution and ranged between 796 g m^{-2} (sd = 111 g m^{-2}) and 1196 g m^{-2} (sd = 197 g m^{-2}) for organic carbon and 86.0 g m^{-2} (sd = 11.8 g m^{-2}) to 128.9 g m^{-2} (sd = 19.8 g m^{-2}) for nitrogen (Figure 3-1 a and b). Within four years of The Jena Experiment soil organic carbon stocks significantly increased in 0 - 15 cm depth ($p \leq 0.001$) and significantly decreased in 20 - 30 cm depth ($<0.001 \leq p \leq 0.010$) (Table 3-1). The rate of organic carbon storage in the top 5 cm was higher in 2002 to 2004 (273 g m^{-2} sd = 145 g m^{-2}) than in 2004 to 2006 (147 g m^{-2} sd = 142 g m^{-2}) ($p = 0.007$). Nitrogen stocks increased between 2002 and 2006 in 0 - 20 cm depth ($<0.001 \leq p \leq 0.013$) and decreased in 25 - 30 cm depth ($p = 0.001$) (Table 3-1).

Light fraction: The main increase of organic carbon of the light fraction was measured in the upper 20 cm of the soil profile ($p \leq 0.001$), while organic carbon of the light fraction

Table 3-1: p-values of soil organic carbon and nitrogen stock changes in 2002 - 2004, 2004 - 2006 and 2002 - 2006.

		Changes of Light fraction			Changes of Heavy fraction			Changes of Bulk		
	Depth	2002 - 2004	2004 - 2006	2002 - 2006	2002 - 2004	2004 - 2006	2002 - 2006	2002 - 2004	2004 - 2006	2002 - 2006
C_{org}	2.5	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
	7.5	0.002	0.053	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.	<0.001
	12.5	0.029	n.s.	<0.001	0.016	n.s.	0.001	0.001	0.031	<0.001
	17.5	n.s.	0.023	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	22.5	n.s.	n.s.	n.s.	n.s.	0.006	0.005	n.s.	0.020	0.010
	27.5	n.s.	n.s.	n.s.	0.009	0.002	0.000	0.010	0.002	<0.001
N	2.5	0.002	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.	<0.001	<0.001
	7.5	n.s.	0.003	<0.001	n.s.	<0.001	<0.001	n.s.	<0.001	<0.001
	12.5	n.s.	0.003	0.004	0.002	<0.001	<0.001	0.011	<0.001	<0.001
	17.5	0.036	<0.001	0.003	<0.001	<0.001	0.021	<0.001	<0.001	0.013
	22.5	0.021	0.012	n.s.	<0.001	<0.001	n.s.	n.s.	<0.001	n.s.
	27.5	0.046	n.s.	n.s.	<0.001	n.s.	0.001	n.s.	0.198	0.001

stayed constant below 20 cm depth throughout the four years of investigation (Figure 3-1 c, Table 3-1). The increase of organic carbon of the light fraction over the whole time span was highest in 0 - 5 cm depth (109 g m^{-2} sd = 51 g m^{-2}), where the amount of organic carbon of the light fraction was more than doubled within 4 years. The proportion that the organic carbon of the light fraction contributed to bulk values increased from 2002 (10.2 % sd = 2.3 %) to 2004 (12.8 % sd = 3.7 %, $p < 0.001$) and to 2006 (15.4 % sd = 4.0 %, $p = 0.009$).

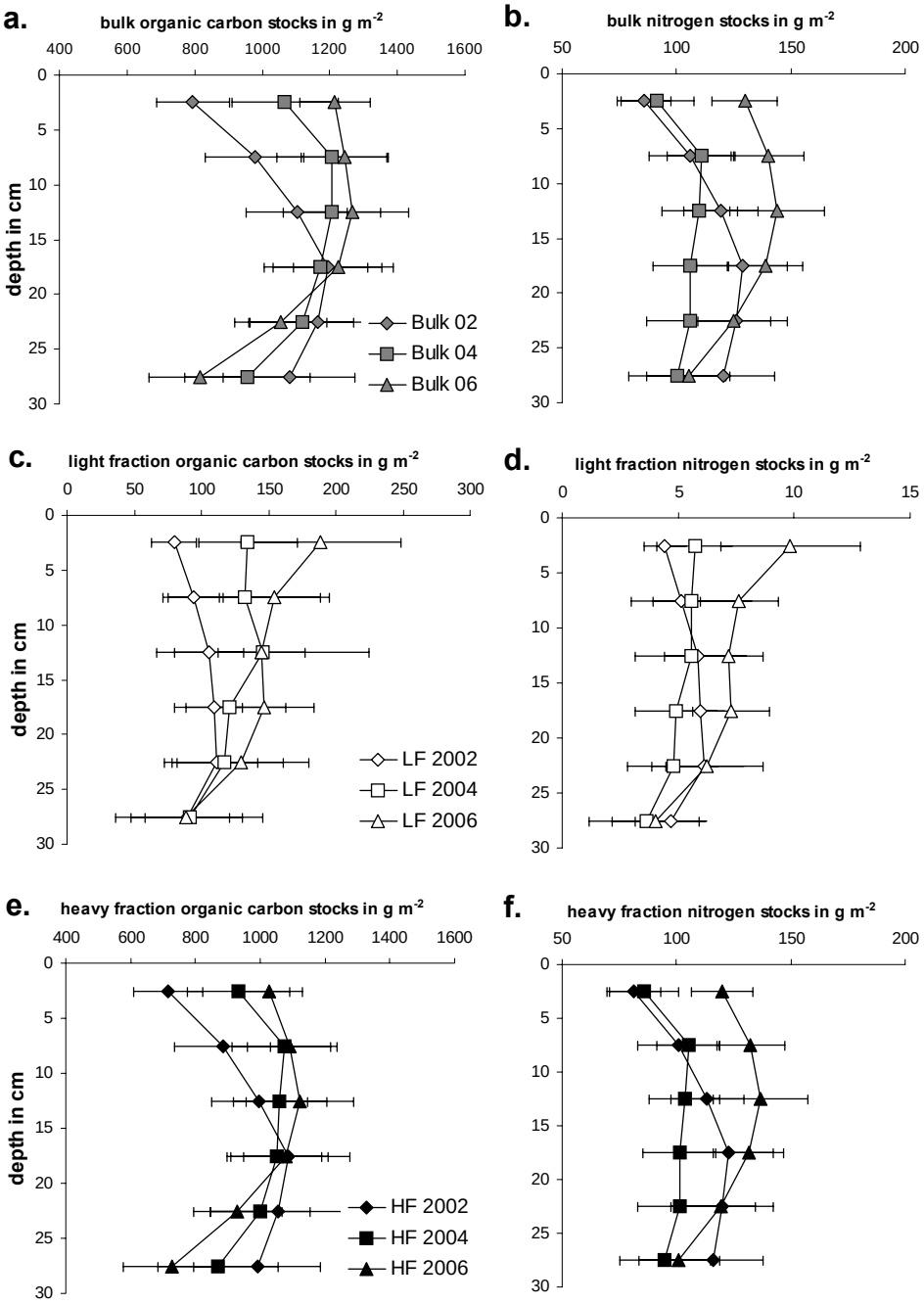

Figure 3-1: Depth distribution of bulk organic carbon and nitrogen and organic carbon and nitrogen of the light and heavy fraction of the experimental site in 2002, 2004 and 2006

Between 2002 and 2006, nitrogen of the light fraction was stored in 0 - 20 cm depth and like for organic carbon nitrogen storage was highest in 0 - 5 cm depth (2002 to 2006: 4.1 g m^{-2} sd = 3.3 g m^{-2}) (Figure 3-1 d). In contrast to organic carbon of the light fraction, which was stored with constant rates over time, the storage rates for nitrogen increased with time. While the amount of nitrogen in 0 - 5 cm depth increased by 25 % during the first two years it increased by 70 % between 2004 and 2006. Also the contribution of nitrogen of the light fraction to bulk stocks significantly increased between 2002 (5.2 % sd = 1.2 %), 2004 (6.3 % sd = 1.7 %) and 2006 (7.6 % sd = 2.1 %) (2002 to 2004: p = 0.004; 2004 to 2006: p = 0.023).

Heavy fraction: The organic carbon of the heavy fraction constituted the major proportion of the bulk stocks and increased between 2002 and 2006 in 0 - 15 cm depth and decreased below 20 cm depth like the bulk stocks (Figure 3-1 e) (Table 3-1). Similar to organic carbon of the heavy fraction nitrogen of the heavy fraction contributed the major proportion to bulk nitrogen stocks. It increased between 2002 and 2006 in 0 - 15 cm depth and decreased below 25 cm depth (Figure 3-1 f) (Table 3-1).

3.3.2 Arable land and meadow sites

Bulk soil: While organic carbon and nitrogen stocks of arable land plots were homogenously distributed with depth (mean organic carbon: 1894 g m^{-2} sd = 301 g m^{-2}; mean nitrogen: 220.5 g m^{-2} sd = 35.6 g m^{-2}) (Figure 3-2 a and b), stocks of the meadow sites were highest in the top 10 cm of the soil and decreased below.

Light fraction: At the grassland sites, the organic carbon of the light fraction decreased with depth from 411 g m^{-2} (sd = 80 g m^{-2}) in 0 - 5 cm (Figure 3-2 c) to 167 g m^{-2} (sd = 13 g m^{-2}) in 10 - 15 cm depth and stayed constant below 15 cm depth. The amount of light fraction that was found in 0 - 5 cm depth of the meadow was twice as much as was found on plots of The Jena Experiment. The amount of nitrogen of the light fraction showed the same pattern as organic carbon (Figure 3-2 d).

Heavy fraction: The amount of organic carbon of the heavy fraction decreased more evenly within the whole soil profile from 3009 g m^{-2} (sd = 59 g m^{-2}) in 0 - 5 cm depth to 1363 g m^{-2} (sd = 191 g m-2) in 25 - 30 cm depth. Organic carbon and nitrogen of the heavy fraction of the arable land sites stayed constant with depth (Figure 3-2 e and f).

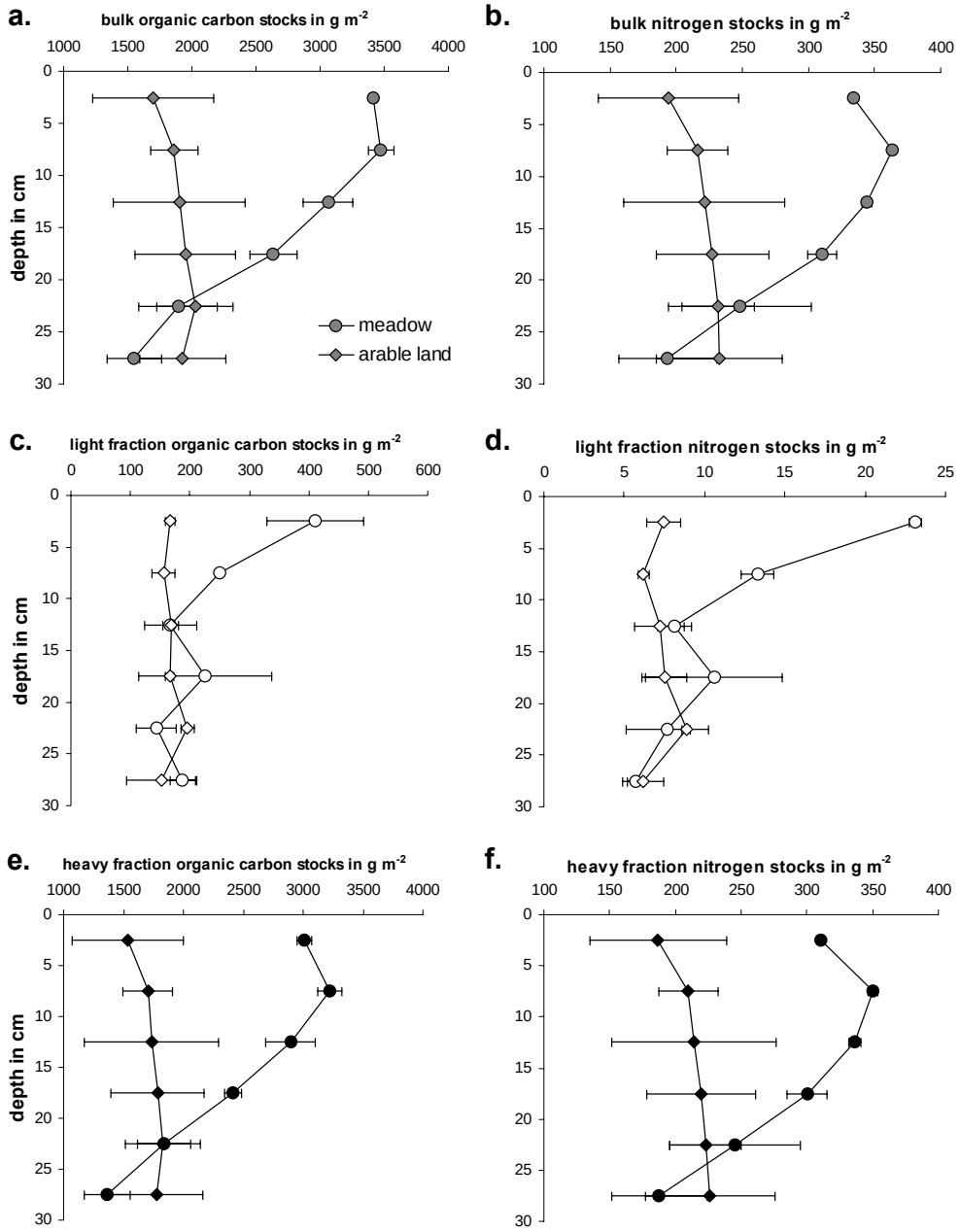

Figure 3-2: Depth distribution of bulk organic carbon and nitrogen and organic carbon and nitrogen of the light and heavy fraction of arable land and meadow sites in 2006

3.3.3 Effects of plant diversity

Bulk soil: From 2002 to 2006, bulk organic carbon stock changes in 0 - 5 cm depth were positively correlated to species richness ($R = 0.45$, $p = 0.034$) (Table 3-2). In the applied ANOVA 19 % of the variance was explained by species richness. Bulk nitrogen stock changes from 2002 to 2006 were not altered by species richness ($p = 0.685$). In 2004, standing root biomass was significantly positively correlated with species richness ($R = 0.38$, $p = 0.049$), number of functional groups ($R = 0.49$, $p = 0.009$) and number of

grass species (R = 0.58, p = 0.002). In 2006, only the correlation between standing root biomass and number of functional groups remained significant (R = 0.48, p = 0.025). In both years, standing root biomass was higher on plots containing grasses compared to plots without grasses (2004: p = 0.015; 2006: p = 0.036) and was reduced if small herbs were present (2004: p = 0.042; 2006: p = 0.001).

Between 2002 and 2006 organic carbon stock changes were higher on plots containing small herbs (459 g m^{-2} sd = 86 g m^{-2}) than on plots without this functional group (373 g m^{-2} sd = 137 g m^{-2}) (p = 0.086). Further, the presence of grasses tended to decrease stock changes of bulk nitrogen between 2002 and 2006 (with grasses: 42.2 g m^{-2} sd = 9.6 g m^{-2}; without grasses 48.0 g m^{-2} sd = 7.0 g m^{-2}; p = 0.108).

Light fraction: Between 2002 and 2004 changes of the organic carbon stocks of the light fraction were not significantly altered by species richness (p = 0.674) (Table 3-2). The same was found for stock changes between 2002 and 2006 (p = 0.946). The light fraction contributed to bulk organic carbon storage from 2002 to 2004 with 20 % (sd = 12 %), 24 % (sd = 11 %) and 16 % (sd = 10 %) and from 2002 to 2006 with 29 % (sd = 5 %), 29 % (sd = 7 %) and 24 % (sd = 5 %) for 4, 8 and 16 species, respectively. The differences

Table 3-2: Contribution of organic carbon storage in the light (LF) and heavy fraction (HF) to carbon and nitrogen storage determined in the bulk values in 0 - 5 cm. Different letters indicate significant differences (p < 0.05) between diversity levels for the same investigation period, respectively. Standard deviation in parentheses.

Variable	Year	Diversity level	Bulk soil (g m^{-2})		Light fraction (g m^{-2})		Heavy fraction (g m^{-2})	
C_{org}	2002 -2004	4 species mixtures	257 (146)	a	57 (31)	a	200 (135)	a
		8 species mixtures	243 (123)	a	60 (28)	a	183 (107)	a
		16 species mixtures	313 (190)	a	55 (40)	a	258 (177)	a
	2004 -2006	4 species mixtures	117 (110)	a	38 (81)	a	80 (106)	a
		8 species mixtures	160 (98)	a	54 (43)	a	106 (109)	a
		16 species mixtures	185 (196)	a	62 (42)	a	123 (176)	a
	2002 -2006	4 species mixtures	374 (124)	a	95 (73)	a	280 (75)	a
		8 species mixtures	403 (128)	a	114 (38)	a	289 (110)	a
		16 species mixtures	497 (47)	b	117 (28)	a	381 (24)	b
N	2002 -2004	4 species mixtures	9.2 (15.8)	a	1.8 (2.2)	a	7.5 (14.4)	a
		8 species mixtures	3.8 (16.5)	a	1.3 (1.8)	a	2.5 (11.4)	a
		16 species mixtures	5.5 (12.8)	a	0.9 (1.8)	a	4.6 (11.9)	a
	2004 -2006	4 species mixtures	32.0 (13.9)	a	3.7 (4.7)	a	28.3 (12.9)	a
		8 species mixtures	43.0 (13.1)	a	4.3 (2.5)	a	38.7 (14.2)	a
		16 species mixtures	39.4 (17.1)	a	4.3 (2.6)	a	35.0 (15.0)	a
	2002 -2006	4 species mixtures	43.5 (10.8)	a	5.3 (3.8)	a	38.1 (8.3)	a
		8 species mixtures	43.0 (10.7)	a	5.6 (2.8)	a	37.4 (10.7)	a
		16 species mixtures	44.9 (7.5)	a	5.2 (1.8)	a	39.7 (6.0)	a

between the species richness levels were not significant ($0.138 \leq p \leq 0.842$). Organic carbon of the light fraction and standing root biomass were not correlated (2004: $R = 0.26$, $p = 0.213$; 2006: $R = 0.17$, $p = 0.522$).

The stock changes of nitrogen of the light fraction were neither between 2002 and 2004 nor between 2002 and 2006 significantly impacted by species richness (2002 - 2004: $p = 0.666$; 2002 - 2006: $p = 0.816$) or standing root biomass (2004: $R = 0.17$, $p = 0.422$; 2006: $R = 0.05$, $p = 0.844$). Between 2002 and 2004 nitrogen of the light fraction increased by 1.8 g m^{-2} (sd = 2.2 g m^{-2}), 1.3 g m^{-2} (sd = 1.8 g m^{-2}) and 0.9 g m^{-2} (sd = 1.8 g m^{-2}) for 4, 8 and 16 species mixtures, respectively. This corresponded to a proportion to storage of 14 % (sd = 14 %), 12 % (sd = 15 %) and 6 % (sd = 12 %). Between 2002 and 2006 the stock changes of nitrogen of the light fraction were 5.3 g m^{-2} (sd = 3.8 g m^{-2}), 5.6 g m^{-2} (sd = 2.8 g m^{-2}) and 5.2 g m^{-2} (sd = 1.8 g m^{-2}) for 4, 8 and 16 species mixtures respectively. Nitrogen of the light fraction contributed with 12 % (sd = 7 %) to bulk storage.

In 2006, stocks of nitrogen of the light fraction were higher on plots containing legumes (10.8 g m^{-2} sd = 2.6 g m^{-2}) compared to plots without legumes (8.2 g m^{-2} sd = 3.0 g m^{-2}) ($p = 0.046$) and resulted in an increased stock changes of nitrogen of the light fraction between 2002 and 2006 ($p = 0.050$) (Figure 3-3). With 14 % (sd = 4 %), the light fraction of legume plots contributed more to the bulk nitrogen storage than the light fraction of no legume plots (8 % sd = 5 %) ($p = 0.012$). In contrast to legumes, the presence of grasses decreased the stock changes of nitrogen of the light fraction between 2002 and 2006 ($p = 0.054$) (Figure 3-3). While tall herbs had no effect, small herbs were found to affect the stock changes of organic carbon and nitrogen of the light fraction. Between 2002 and 2006 plots without small herbs stored less organic carbon and nitrogen in the light fraction than plots where small herbs were present (Figure 3-3).

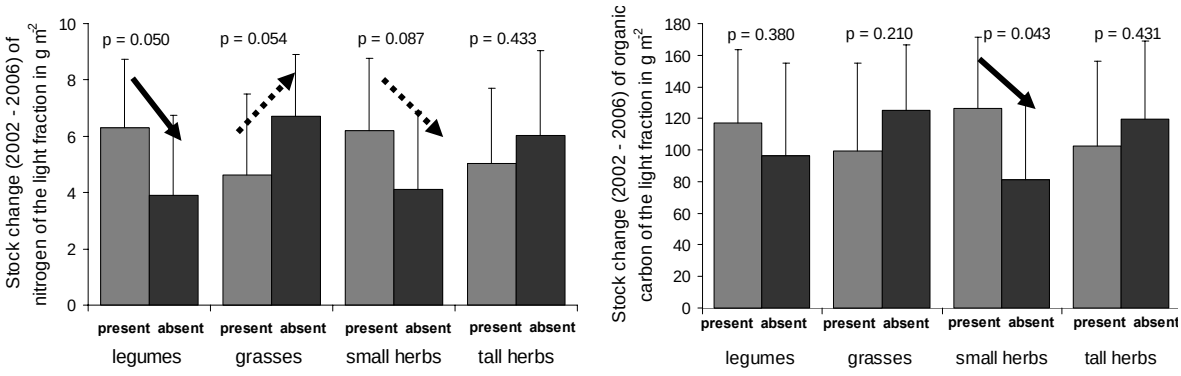

Figure 3-3: Impact of presence and absence of plant functional groups on stock changes (2002 - 2006) of organic carbon and nitrogen of the light fraction in 0 - 5 cm depth

Heavy fraction: Similar to the bulk values, stock changes of organic carbon of the heavy fraction (2002 - 2004) were not impacted by species richness ($p = 0.691$). Over the whole investigation period (2002 - 2006) stock changes were positively altered by species richness ($p = 0.032$). While 4 species mixtures stored 280 g m^{-2} (sd = 75 g m^{-2}) within four years, 16-species mixtures stored 381 g m^{-2} (sd = 24 g m^{-2}) organic carbon in the heavy fraction. Over the whole investigation period (2002 - 2006) organic carbon of the heavy fraction contributed with 71 % (sd = 5 %), 71 % (sd = 7 %) and 84 % (sd = 5 %) for 4, 8 and 16 species mixtures to bulk storage (Table 3-2).

The contribution of nitrogen of the heavy fraction to storage was comparably high. Between 2002 and 2006, 4, 8, and 16 species mixtures contributed with 89 % (sd = 8 %), 87 % (sd = 6 %) and 89 % (sd = 3 %) to bulk stock changes of nitrogen.

Neither stock changes of organic carbon nor nitrogen of the heavy fraction were impacted by the presence or absence of particular functional groups.

3.3.4 Radiocarbon measurements

Radiocarbon measurements showed that the light fraction from the experimental site in 2002 had significantly lower $\Delta^{14}C$ values (-188 ‰ sd = 96 ‰) compared to the light fraction of the experimental site in 2006 (-90 ‰ sd = 34 ‰) ($p = 0.006$) (Figure 3-4). The $\Delta^{14}C$ mass balance for the light fraction of The Jena Experiment revealed that the increase of $\Delta^{14}C$ values was solely driven by the input of recent material ($\Delta^{14}C \approx 54$ ‰). From 2002 to 2006, the $\Delta^{14}C$ values of the heavy fraction organic carbon also significantly shifted towards recent values (2002: -51 ‰ sd = 15 ‰; 2006: -29 ‰ sd = 11 ‰, $p = 0.003$). A $\Delta^{14}C$ mass balance was applied to the heavy fraction and a comparison between the calculated $\Delta^{14}C$ value (-15 ‰ sd = 9 ‰) and the measured $\Delta^{14}C$ value (-28 ‰ sd = 11 ‰) showed that 94 % of the mass increase between 2002 and 2006 were from recent origin.

In 2006, species richness (light fraction $p = 0.917$; heavy fraction $p = 0.232$) had no impact on the $\Delta^{14}C$ values of both density fractions. The presence of grasses seemed to decrease the $\Delta^{14}C$ values of the heavy fraction compared plots without grasses (grasses: -31 ‰ sd = 10 ‰; no grasses: -18 ‰ sd = 7 ‰) ($p = 0.058$).

The $\Delta^{14}C$ value of the light and heavy fraction of the arable land were -210 ‰ (sd = 28 ‰) and -48 ‰ (sd = 26 ‰), respectively. The $\Delta^{14}C$ value of organic carbon of the light and heavy fraction of the meadow site were 3 ‰ (sd = 4 ‰) and 26 ‰ (sd = 6 ‰), respectively

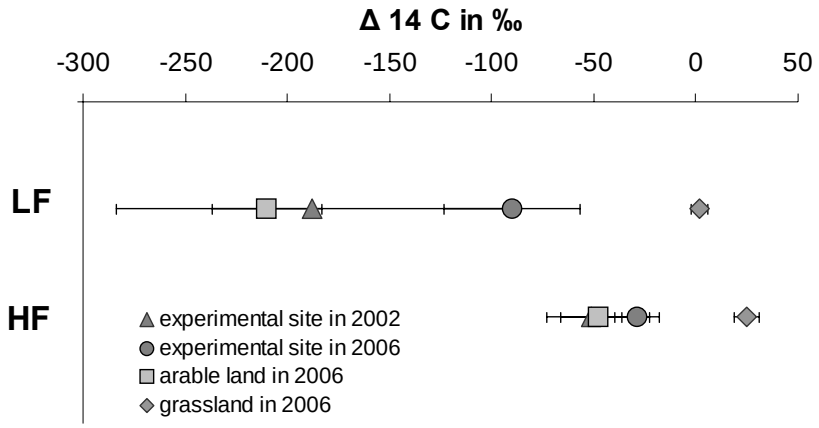

Figure 3-4: $\Delta^{14}C$ values of the experimental site in 2002 and 2006 and arable land and grassland in 2006 in 0 - 5 cm for light (LF) and heavy fraction (HF).

and included the highest proportion of recent material. For the $\Delta^{14}C$ mass balance it was assumed that the light fraction of the meadows contained a similar proportion of "old" ($\Delta^{14}C \approx -188$ ‰) light fraction as The Jena Experiment in 2002 and that the remaining light fraction was of recent origin ($\Delta^{14}C \approx 54$ ‰). The calculated $\Delta^{14}C$ value (6 ‰ sd = 9 ‰) of the meadows and the measured $\Delta^{14}C$ value (3 ‰ sd = 4 ‰) were similar and indicated that the assumptions were right.

3.4 Discussion

The main changes to be expected by conversion from arable land to grassland take place in the upper centimetres of the soil (Conant et al., 2001). The sown perennial grassland species of The Jena Experiment originated an input of dead and living roots, plant litter and microbial debris (Magid et al., 2001; Poirier et al., 2005) to the light fraction. Consequently, the proportion of carbon of the light fraction within the bulk soil increased with time, indicating that the light fraction of arable land was depleted and is now built up again after conversion (Carter et al., 1998). Thus, the amount of the light fraction of The Jena Experiment shifted towards the amount of light fraction, which was found on the meadow sites.

Interestingly, organic carbon and nitrogen did not simultaneously respond to conversion: organic carbon of the light fraction responded faster than nitrogen of the light fraction. While the storage rates of organic carbon of the light fraction were constant over the investigation period, the nitrogen storage rates increased with time. An increase of storage rates over time was also observed for nitrogen of the heavy fraction.

Within four years, 109 g m^{-2} (sd = 51 g m^{-2}) of organic carbon and 5.4 g m^{-2} (sd = 2.8 g m^{-2}) of nitrogen were stored in the light fraction. But major parts of the light fraction were rapidly decomposed by macro-, meso- and microorganisms and consequently transferred to the heavy fraction. Organic carbon of the heavy fraction increased by 311 g m^{-2} (sd = 101 g m^{-2}) within four years and nitrogen of the heavy fraction increased by 38.4 g m^{-2} (sd = 8.2 g m^{-2}). Therefore, we can conclude that not only the light fraction, as often described (Biederbeck et al., 1994; Bolinder et al., 1999; Conant et al., 2004), but also the heavy fraction was sensitive to land use change (Leifeld et al., 2005). The direct input of recent plant material in the light fraction and input of transformed plant material into the heavy fraction could be confirmed by increased $\Delta^{14}C$ values on both fractions at the end of the investigation period. They indicate a rapid decomposition of recent plant inputs by microorganisms and a sequestration of microbial transformation products in the heavy fraction (Gleixner et al., 2002; Leifeld et al., 2005; Swanston et al., 2005; McLauchlan et al., 2006). The heavy fraction is build up more or less in parallel to the light fraction after conversion from arable land to grassland. The transfer of carbon and nitrogen into the heavy fraction within years was also confirmed by other studies (Trumbore et al., 1989; Swanston et al., 2005; McLauchlan et al., 2006). McLauchlan et al. (2006) studied a chronosequence of former agricultural fields in the Midwestern United States that spanned 40-years after conversion to grassland. They found that across all 31 studied sites carbon originating from the heavy fraction increased more rapidly than expected and that new organic carbon inputs appeared in the heavy fraction within years.

To explain the finding that the light fraction had decreased $\Delta^{14}C$ values compared to the heavy fraction thermo gravimetric analyses were made (data not shown). The $\Delta^{14}C$ values were not as first expected decreased by charcoal. Instead, the light fraction with the lowest $\Delta^{14}C$ values contained more plant derived highly stable compounds compared to light fraction with higher $\Delta^{14}C$ values like from the experimental site in 2006 or from the meadows.

Studies of the bulk soil from all 82 plots of The Jena Experiment revealed a positive correlation between carbon storage, root input and plant diversity in the top soil in 2004 and 2006 (Steinbeiss et al., 2008). A positive relationship between species richness and bulk organic carbon storage was also found for the plots used for density fractionation. The standard deviation of density fractions was relatively high compared to the small expected differences between the plant diversity levels and hampered the explanatory power of our

results. High variation of density fractions were also reported from Russel et al. (2004) and Leifeld et al. (2005). Beside methodical obstacles, a positive relationship between species richness and heavy fraction organic carbon was found. The storage within 4 years and transformation of organic carbon from the light fraction into the heavy fraction were higher at the 16 species level compared to the 4 and 8 species level. From this it can be deduced that the higher bulk organic carbon storage at increased diversity is not an effect of increased amounts of light fraction but an effect of increased storage in the heavy fraction. Since the transformation is mainly driven by soil microbial communities, the differences between the species richness levels might become more evident with time (Bartelt-Ryser et al., 2005; Habekost et al., 2008), when the microbial communities better adapted to the different conditions found at plots with different species richness levels. It can be assumed that over long-term the highest proportions of heavy fraction organic carbon and nitrogen will be found on plots with high diversity, because on these plots the microbial community might be most diverse and have the highest resource use efficiency (Eisenhauer et al., 2008).

Plant functional groups deliver input of different quantity and quality (Spehn et al., 2002). Thus, the presence and absence of distinct functional groups (small herbs, legumes and grasses) were found to have an impact on light fraction organic carbon and nitrogen storage. Mixtures containing small herbs had a higher standing root biomass, which resulted in an increased amount of organic carbon and nitrogen in the light fraction compared to mixtures without small herbs. Further, plots containing grasses were found to have lower stocks of nitrogen of the light fraction as a consequence of the wide C/N ratio of the input material, whereas the nitrogen of the light fraction of plots containing legumes was higher due to the ability of legumes to fix nitrogen. This corroborated the finding that the light fraction is influenced by recent vegetation but that the heavy fraction is controlled by soil microbial transformations of plant material input.

3.5 Conclusion

We found that conversion from arable land to grassland led to sensitive changes of organic carbon and nitrogen in the light and heavy soil fraction. Higher bulk organic carbon storage at higher plant diversity was not caused by increased storage in the light fraction but by increased transformation of plant derived input into the heavy fraction. For nitrogen no relationship between storage in different density fractions and plant diversity was

found. Distinct plant functional groups had an impact on the storage of nitrogen and organic carbon in the light but not in the heavy fraction. The integration of recent material in both density soil fractions within only four years after the establishment of The Jena Experiment indicated that only parts of the input were stored in the light fraction. The major part of the plant material input was directly microbially transformed and sustainable stored in the heavy fraction.

3.6 Acknowledgements

The project (GL 262-6) is funded by the Deutsche Forschungsgemeinschaft as part of the special research group (FOR 456) with support of the Friedrich Schiller University Jena and the Max Planck Society. We gratefully acknowledge all the people who were involved in the planning and set-up process of the experiment, especially E.-D. Schulze, W.W. Weisser, J. Schumacher, and C. Roscher. Thank also goes to the gardeners who maintained the field site with weeding and all the other things to do on a regular basis.

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4 Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment

Chapter source: Habekost *et al.*, 2008. Seasonal changes in the microbial community in a grassland plant diversity gradient four years after establishment. Soil Biology & Biochemistry 40: 2588-2595

Abstract

Aboveground plant diversity is known to influence belowground diversity and ecosystem processes. However, the knowledge on soil microbial succession from an agricultural field to grassland varying in plant diversity is scarce. Therefore, we investigated the effects of vegetation cover, varying plant biodiversity and season on soil microbial parameters in a temperate grassland ecosystem. In May and October 2006 mixed soil samples were taken from the field site from The Jena Experiment; a large biodiversity experiment in Germany which was established in 2002 on a former agricultural field. Sampled plots differed in plant species richness (0, 4, 8, 16), number of plant functional groups (0, 1, 2, 3, 4), and plant functional group composition. We measured basal respiration (BR) and microbial biomass ($C_{mic/CFE}$; chloroform fumigation extraction method), phospholipid fatty acids (PLFA), and substrate induced respiration (SIR). We found distinct seasonal variations in the microbial community structure; BR and amount of PLFAs were higher at the end of the vegetation period than in spring, which was primarily due to increased biomass of fungi and Gram-negative bacteria. Furthermore, BR and the amount of PLFAs were higher on vegetated plots than on bare ground plots. Although the number of plant functional groups had no effect on microbial parameters, plant species richness affected the amount of PLFAs at the end of the vegetation period with higher biomass in 4 than in 8 and 16 species mixtures. Moreover, the proportion of Gram-negative bacteria was increased whereas the proportion of fungi was decreased in presence of legumes. The present study showed distinct seasonal changes in the soil microbial community composition, which is probably driven by the availability and quality of organic resources. Further, our results highlight the time-lag of belowground responses to aboveground vegetation manipulations with only few significant changes four years after the establishment of the experiment.

Keywords: Biodiversity, Basal respiration (BR), Chloroform fumigation extraction method (CFE), Phospholipid fatty acids (PLFA), Substrate induced respiration (SIR), The Jena Experiment

4.1 Introduction

Biodiversity is known to impact ecosystem processes such as net primary productivity due to complementarity and sampling effects (Hector et al., 1999; Tilman et al., 2001). It is increasingly recognized that changes in plant diversity also affect the belowground system (Wardle et al., 2004). Above- and belowground components of terrestrial ecosystems essentially depend on each other since plants provide carbon sources for the soil fauna and microflora. On the other hand, microorganisms and detritivore animals decompose organic matter, thereby increasing the availability of nutrients for plants and enhancing plant growth (Porazinska et al., 2003). The impact of aboveground biodiversity on soil biota may alter the functioning of microorganisms and therefore result in changes in the decomposition of organic matter (Orwin et al., 2006). Increasing plant species richness may beneficially affect the diversity of soil biota by including plants differing in root morphology, root chemical composition, and temporal variability of resource inputs. The increased morphological, chemical and temporal variability of belowground structures and resources is likely to result in increased diversity of niches supporting more diverse assemblages of soil biota (Lavelle et al., 1995; Hooper et al., 2000). We hypothesized that changes in plant diversity modify resource availability for heterotrophic microbial communities in soil, and thus modify their activity, biomass and composition.

Studies investigating the effects of plant diversity on soil microbial communities are scarce and mostly restricted to gross parameters such as microbial biomass, culturable microogranisms or single functional groups of microorganisms (Spehn et al., 2000; Stephan et al., 2000; Porazinska et al., 2003; Zak et al., 2003). Additionally, there is the need to follow changes in microbial community composition and functioning with time after establishment of biodiversity experiments (Maly et al., 2000; Balser and Firestone, 2005).

Using The Jena Experiment field site (Roscher et al. 2004) we assessed changes in microbial community composition and functioning four years after establishment of a plant diversity gradient. We hypothesized that food supply over the season is more variable in communities with low plant species richness due to a decreased overlap of different phenologies in time. A reduction in plant species richness can therefore have considerable effects on soil microbial communities via differences in the chemical composition of plant residues or via the timing of residue availability.

In order to test the proposed hypotheses we asked whether (1) season and aboveground plant biodiversity influence the amount of soil microorganisms four years after the establishment of the experiment; (2) soil microbial communities are more diverse on vegetated plots than under bare ground conditions; and (3) soil microbial community composition differs in presence of plants from certain functional groups.

4.2 Materials and Methods

4.2.1 Site description, soil and biomass sampling

Soil samples were collected from the field site of The Jena Experiment in the north of the city of Jena, Germany. This long-term experiment containing species, which are common to Central European Molinio-Arrhenatheretum grasslands, was established in 2002 to investigate the role of biodiversity for element cycling and trophic interactions (Roscher et al., 2004). Before experiment establishment, the site was used as an arable field for the last 40 years and ploughed and fertilized regularly. The soil of the field site is classified as Eutric Fluvisol (FAO, 1998) developed from up to 2 m-thick loamy fluvial sediments (Roscher et al., 2004). The texture ranges from sandy loam to silty clay. Due to the varying soil texture the field site was subdivided into four blocks with homogenous soil properties. After sowing plots varying in plant diversity no fertilizer was applied. The chosen grassland species were classified into four functional groups (small herbs, tall herbs, grasses and legumes) according to their physiological, phenological and above- and belowground morphological traits. The plant diversity gradient established in The Jena Experiment ranges from 1 (2, 4, 8, 16) to 60 plant species and 1 (2, 3) to 4 plant functional groups, respectively. Species number and number of functional groups were varied as independently as possible when compiling the plot mixtures. Thus, the experimental design allows to test between sampling and complementarity effects and to attribute processes to species or functional group richness. In addition to the vegetated plots bare ground plots were kept free of vegetation. Further information on the experimental set-up including the plant species and soil characteristics is given in Roscher et al. (2004) and Steinbeiss et al. (2008), respectively. For analysis of microbial parameters, 27 plots were chosen representing a gradient in plant species diversity (from 0 to 16) and in the number of plant functional groups (from 0 to 4). Accounting for the block design of the experiment, two replicates with 4, 8 and 16 plant species were sampled from each of the four blocks. However, bare ground plots (no plant species sown) could only be sampled in three blocks.

In May and October 2006, eight soil samples per plot were taken with a core cutter (inner diameter: 5.6 cm) to a depth of 5 cm and pooled separately for the two sampling dates. Mixed samples were placed in cooling boxes immediately after sampling and stored at 4°C. Soil samples were sieved (2 mm) within 48 h after sampling and shared for the different analyses. Sub-samples for PLFA analysis were frozen at -20°C until analyses. Sub-samples for the other analysis were analyzed within 4 weeks from the stored material.

Soil samples for organic carbon and total nitrogen measurements were taken in April 2002 and 2006 as a paired sampling using a split tube sampler (inner diameter 4.8 cm). Samples were dried at 40°C, sieved to 1 mm, ground and analyzed for soil organic carbon and nitrogen using an elemental analyzer at 1150°C (Elemetaranalysator vario Max CN, Elementar Analysensysteme GmbH, Hanau, Germany).

Aboveground biomass was harvested in May and August 2006 on three randomized sites (20 x 50 cm) per plot by cutting standing biomass 3 cm above ground. The biomass of the sown species was dried at 70°C to constant weight. Plant material was chaffed and ground to fine powder. Carbon and nitrogen were analyzed at 20 mg sub-samples with an elemental analyzer (see above) and C/N ratio was determined.

4.2.2 PLFA analysis

PLFAs were extracted according to Bligh and Dyer (1959) and modified by Kramer and Gleixner (2006). Briefly, soil lipids were extracted by a mixture of chloroform, methanol and 0.05 M phosphate buffer (pH 7.4) and split up into phospholipids by eluting with chloroform, acetone and methanol from a silica-filled solid phase extraction column. Subsequently, the phospholipids were hydrolyzed and methylated by a methanolic KOH solution and the PLFA-methyl esters were identified and quantified by GC-AED (Agilent, Böblingen, Germany) and GC/MS (Thermo Electron, Dreieich, Germany). PLFA 19:0 was used as internal standard. PLFA concentration was calculated as mg PLFA per g soil dry weight. Among 27 recorded PLFAs the following represented bacterial biomass: 14:0, 15:0, 15:0br iso, 15:0br anteiso, 16:0, 17:0, 18:0 and 20:0. PLFA 18:2ω6 was a marker for saprophytic fungal biomass according to Zelles (1997). Gram-negative bacteria were represented by 16:1, 17:1, 17:1(2), 18:1, 18:1(2), 18:1n9, 18:1n11, 19:1, 18:0 cyclo, and 20:1 and Gram-positive bacteria were represented by 16:0br, 17:0br, 17:0br(2), 17:0br(3) and 17:0br(4). PLFA 14:0 could not be assigned to a bacterial group (Zelles, 1997).

4.2.3 Microbial Biomass Carbon ($C_{mic/CFE}$)

Microbial biomass carbon was determined using the Chloroform Fumigation Extraction (CFE) method as described by Vance et al. (1987) using 10 g fresh soil samples. Non-fumigated samples were extracted with 50 ml 0.5 M K_2SO_4 for 1 h at 130 rev min^{-1} and filtered subsequently. Extracted samples were kept frozen until analysis. Samples for fumigation were placed in a vacuum desiccator and fumigated with ethanol-free chloroform for 24 h. Fumigated samples were extracted with 0.5 M K_2SO_4 under the same conditions as non-fumigated samples. The C content of the K_2SO_4 extracts was measured on a high TOC elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Microbial biomass carbon was calculated as $C_{mic/CFE} = E_c/k_{ec}$, with E_c the difference between organic C extracted from fumigated soils and organic C extracted from nonfumigated soils, and k_{ec} the efficiency constant 0.45 (Jörgensen, 1996).

4.2.4 Basal respiration (BR) and substrate induced respiration (SIR)

BR and SIR were measured using an O_2 microcompensation apparatus (Scheu, 1992). The microbial respiratory response was measured at hourly intervals for 24 h at 22°C. BR (µl O_2 g^{-1} soil dry weight h^{-1}) was determined without addition of substrate and measured as mean of the O_2 consumption rates of hours 12 to 22 after the start of the measurements. SIR was calculated from the respiratory response to D-glucose (Heal et al., 1997). Glucose was added in appropriate amounts (according to preliminary studies) to saturate the catabolic enzymes of the microorganisms (4 mg g^{-1} dry soil solved in 400 µl deionized water). The mean of the lowest three readings within the first 10 h was taken as maximum initial respiratory response (MIRR; µl O_2 g^{-1} soil dw h^{-1}).

4.2.5 Statistical analysis

Statistical analyses and model calculations were carried out with SPSS Version 15.0 (SPSS Inc., Chicago, USA). The relation between PLFAs, SIR, BR and $C_{mic/CFE}$ was tested with correlation analyses. Accounting for differences in soil properties between the blocks, the block effect was included as random factor fitted first in all analyses of variance (ANOVAs, type I SS). Plant species diversity and number of plant functional groups, bare ground plots and vegetated plots or presence of legumes were included as fixed factors. Aboveground plant biomass and C/N ratio of plant biomass were set as covariates. Differences between spring and autumn were analysed with a repeated measures ANOVA

using sampling date as fixed factor. In addition, comparisons among PLFA profiles were analysed by Principal Components Analysis (PCA). Data used in the PCA were standardized to the maximum peak per plot. The results of the PCA along axis 1 and 2 were compared by ANOVAs (type I SS). The Shannon diversity index, commonly used to characterise species diversity in a community, was used as measure of PLFA diversity as $H`=-\Sigma[Pi*\log Pi]$, with Pi representing the proportional abundance of a given PLFA. The Shannon index was compared by ANOVAs as described above. Additionally, bare ground plots and vegetated plots were compared by PCA including both sampling times. All references to statistical differences are based upon a significance level of 0.05.

4.3 Results

4.3.1 Soil and plant parameters

Soil organic carbon increased significantly from 19.1 g kg^{-1} (sd = 2.6 g kg^{-1}, sd = standard deviation) in 2002 to 22.3 g kg^{-1} (sd = 2.8 g kg^{-1}) in 2006 ($p < 0.001$) in vegetation covered plots, while the concentration in the bare ground plots stayed constant within the first four years of the experiment. Moreover, the nitrogen concentration increased significantly from 2002 (2.1 g kg^{-1} sd = 0.2 g kg^{-1}) to 2006 (2.3 g kg^{-1} sd = 0.3 g kg^{-1}) ($p < 0.001$). Soil organic carbon and nitrogen concentrations were not significantly affected by plant species richness, number of functional groups or the presence or absences of legumes.

The C/N ratio of plant shoot biomass decreased significantly from 26.2 sd = 8.3 in May to 18.3 sd = 3.6 in August ($p < 0.001$) (Table 4-1). At both sampling dates, differences between plots varying in species richness were not significant. In May, C/N ratio of plant shoot biomass from plots containing legumes was half the amount of plants from plots without legumes ($p = 0.001$). In August, the lower C/N ratio on plots with legumes was caused by higher nitrogen concentrations. Furthermore, in May and August aboveground plant biomass in legume plots exceeded that of non-legume plots (May: 653 g dw m^{-2} sd = 340 g dw m^{-2} and 150 g dw m^{-2} sd = 50 g dw m^{-2}, for legumes and non-legumes plots respectively; October: 202 g dw m^{-2} sd = 70 g dw m^{-2} and 92 g dw m^{-2} sd = 34 g dw m^{-2}, for legumes and non-legumes plots respectively).

Table 4-1: Aboveground plant biomass and C/N ratio of plant biomass in presence and absence of legumes and at different sown species richness in May and August 2006. Standard deviation in parentheses.

Diversity level	Aboveground plant biomass (g dw m^{-2}) May 2006	Aboveground plant biomass (g dw m^{-2}) August 2006	C/N ratio biomass May 2006	C/N ratio biomass August 2006
all plots	464 (365)	160 (80)	26.2 (8.3)	18.3 (3.6)
plots containing legumes (n = 15)	653 (340)	202 (70)	21.9 (5.9)	16.6 (2.6)
plots without legumes (n = 12)	150 (50)	92 (34)	33.3 (6.7)	21.1 (3.3)
4 species mixtures (n = 8)	310 (210)	144 (60)	26.5 (9.0)	17.2 (3.3)
8 species mixtures (n = 8)	547 (517)	152 (74)	25.7 (7.3)	19.3 (4.7)
16 species mixtures (n = 8)	535 (291)	185 (104)	26.3 (9.5)	18.3 (2.8)

4.3.2 Microbial biomass

The amount of PLFAs, SIR and BR were lower in spring (PLFA: 4.4 µg g^{-1} dw sd = 1.1 µg g^{-1} dw; SIR: 7.5 O_2 h^{-1} g^{-1} dw sd = 1.2 O_2 h^{-1} g^{-1} dw; BR: 2.2 O_2 h^{-1} g^{-1} dw sd = 0.4 O_2 h^{-1} g^{-1} dw) than at the end of the growing season (PLFA: 7.2 µg g^{-1} dw sd = 1.4 µg g^{-1} dw, $p < 0.001$; SIR: 32.5 O_2 h^{-1} g^{-1} dw sd = 6.6 O_2 h^{-1} g^{-1} dw, $p < 0.001$; BR: 3.7 O_2 h^{-1} g^{-1} dw sd = 0.7 O_2 h^{-1} g^{-1} dw, $p < 0.001$). Further, the amount of PLFAs, SIR and BR were positively correlated with each other in October (SIR and BR: $R = 0.79$, $p < 0.001$; SIR and PLFA: $R = 0.58$, $p = 0.002$). $C_{mic/CFE}$ was positively correlated with the amount of PLFAs ($R = 0.48$, $p = 0.012$).

Soil microbial biomass differed considerably between bare ground plots and vegetated plots (Table 4-2). In May, bare ground plots had slightly higher amounts of PLFAs compared to plots with vegetation cover. During the vegetation period microbial biomass increased on the vegetated plots in contrast to bare ground plots. Consequently, the amount

Table 4-2: Means of the amount of phospholipid fatty acids (PLFA), microbial carbon measured using the Chloroform Fumigation Extraction method ($C_{mic/CFE}$), substrate induced respiration (SIR) and basal respiration at bare ground plots (n = 3) and vegetated plots (n = 24) in May and October. Different letters indicate significant differences ($p < 0.05$) between plots with and without vegetation in May and October, respectively.

ND = not determined; standard deviation in parentheses.

Season	Vegetation	PLFA (µg g^{-1} dw)		$C_{mic/CFE}$ (mg g^{-1} dw)		SIR (O_2 h^{-1} g^{-1} dw)		Basal respiration (O_2 h^{-1} g^{-1} dw)	
May	mean of vegetated plots	4.4 (1.2)	a	0.22 (0.04)	a	7.5 (1.3)	a	2.3 (0.3)	a
	bare ground plots	4.8 (0.4)	a	0.19 (0.02)	a	7.6 (0.7)	a	1.6 (0.4)	b
October	mean of vegetated plots	7.3 (1.4)	a	ND		33.7 (6.0)	a	3.9 (0.7)	a
	bare ground plots	6.3 (1.7)	a	ND		23.1 (2.1)	b	2.5 (0.1)	b

Table 4-3: Means of the amount of phospholipid fatty acids (PLFA), microbial carbon measured as chloroform fumigation extraction ($C_{mic/CFE}$), substrate induced respiration (SIR) and basal respiration at different sown plant species diversity. Different letters indicate significant differences ($p < 0.05$) between the 4 to 16 species mixtures in May and October, respectively.

ND = not determined; standard deviation in parentheses.

Season	Diversity level	PLFA ($\mu g\ g^{-1}$ dw)		$C_{mic/CFE}$ ($mg\ g^{-1}$ dw)	SIR ($O_2\ h^{-1}\ g^{-1}$ dw)		Basal respiration ($O_2\ h^{-1}\ g^{-1}$ dw)	
May	4 species mixtures (n = 8)	4.7 (1.6)	a	0.24 (0.04)	7.7 (0.8)	a	2.2 (0.3)	a
	8 species mixtures (n = 8)	4.1 (0.8)	a	0.22 (0.05)	7.1 (1.2)	a	2.3 (0.3)	a
	16 species mixtures (n = 8)	4.4 (1.2)	a	0.21 (0.02)	7.8 (1.8)	a	2.3 (0.4)	a
October	4 species mixtures (n = 8)	8.1 (1.4)	a	ND	34.2 (5.8)	a	3.6 (0.1)	a
	8 species mixtures (n = 8)	6.7 (1.5)	b	ND	31.6 (6.0)	a	3.9 (0.7)	a
	16 species mixtures (n = 8)	7.1 (1.0)	b	ND	35.2 (6.4)	a	4.1 (1.7)	a

of PLFAs, SIR and BR were higher on vegetated plots than on bare ground plots in October (PLFA: $p = 0.138$; SIR: $p = 0.001$; BR: $p < 0.001$). However, $C_{mic/CFE}$ was not affected by vegetation cover or diversity (vegetation cover: $p = 0.178$; diversity: $p = 0.263$).

Beside the differences between bare ground plots and those with vegetation cover, the amount of PLFAs was also affected by sown plant species diversity (Table 4-3). Although the amount of PLFAs was not affected in May ($p = 0.681$), it was higher in the 4 species than in the 8 and 16 species mixtures in October (4 vs. 8 species: $p = 0.036$; 4 vs. 16 species: $p = 0.035$). SIR and BR were not affected by sown plant species or plant functional group diversity. However, when including bare ground plots in the analysis, SIR was significantly affected in October ($p = 0.016$) and the gradient between bare ground plots and 16 species plots was significantly steeper ($p = 0.020$). Further, the presence of specific plant functional groups had no significant effect on microbial parameters.

4.3.3 Microbial community composition

In May, principal component 1 explained 30.7 % of the variation in PLFA composition, and principal component 2 explained further 12.7 % (Table 4-4). Bare ground plots were clearly separated from vegetation covered plots ($p < 0.001$). The PCA loading of component 1 did not significantly differ between the different diversity levels when bare ground plots were excluded ($p = 0.097$). However, plots containing legumes significantly differed from plots without legumes ($p = 0.001$). PLFAs 19:1, 15:0 branch, 16:1, 17:0 branch, 18:1n9 were the main drivers of principal component 1.

Table 4-4: Summary of the PCA for different plant diversity levels (4, 8 and 16 species plots) and bare ground plots for May and October. Eigenvalues, proportional variance (PrVar) and cumulative variance (Cum Var) for principal component 1 and 2 (PC1 and PC2) including eigenvectors that each plant diversity level contributes to that PC.

		Eigenvalues			Eigenvectors			
	PC	Value	PrVar	Cum Var	bare ground plots	4 species	8 species	16 species
May	1	8.00	30.77	30.77	2.01	-0.55	0.00	-0.14
	2	3.31	12.72	43.49	0.27	0.38	-0.16	-0.37
October	1	11.26	43.33	43.33	1.47	-0.26	-0.31	0.05
	2	3.41	13.12	56.45	1.38	0.15	-0.05	-0.64

In October, principal component 1 and principal component 2 accounted for 43.3 % and 13.1 % of the variation, respectively (Table 4-4). The results were generally similar to those in May except that the presence of legumes had no significant effect along principal component 1 ($p = 0.193$) but along principal component 2 ($p = 0.012$). Further, bare ground plots and covered plots differed significantly ($p = 0.004$) but analysing only covered plots showed no effect on plant species diversity and the number of plant functional groups on PLFA patterns. PLFAs 17:0 branch, 15:0 branch, 17:1, 17:0, 19:1, 16:1, 16:0, 15:0, 18:1 and 16:0 branch were the main drivers of principal component 1.

By comparing both sampling dates principal component 1 explained 34.0 % and principal component 2 explained 10.9 % of the variation in the microbial community (Figure 4-1, Table 4-5). In May and October, bare ground plots were not significantly different along principal component 1 ($p = 0.334$). The same was true for vegetated plots ($p = 0.291$). Bare

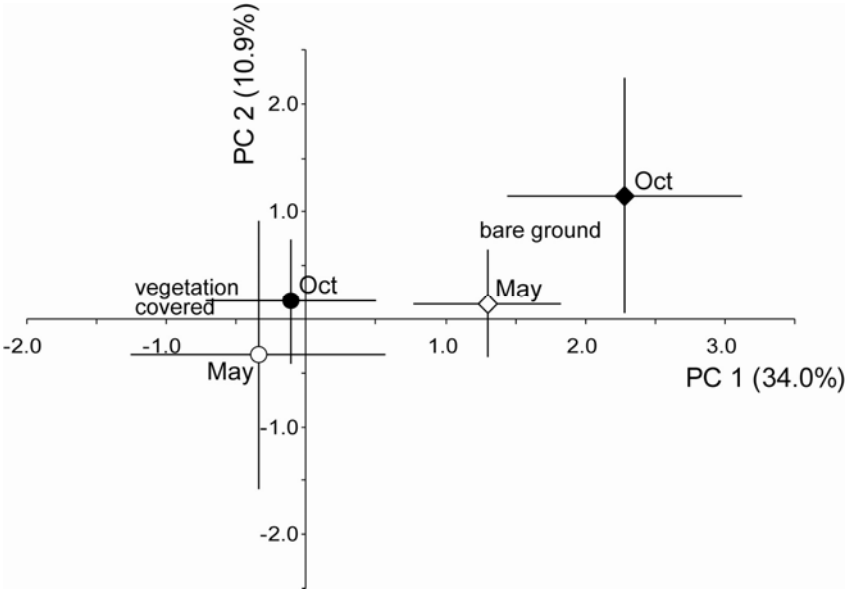

Figure 4-1: Principal components analysis (PCA) of the PLFA patterns of plots with and without vegetation (rhomboids: bare ground = no plant species present; circles: vegetation covered plots containing 4, 8, or 16 plant species) in May (unfilled symbols) and October (filled symbols) 2006.

Table 4-5: Summary of the PCA for different plots (bare ground = no plant species present; vegetated plots containing 4, 8, or 16 plant species) combined for May and October 2006. Eigenvalues, proportional variance (PrVar) and cumulative variance (Cum Var) for principal component 1 and 2 (PC1 and PC2) including eigenvectors that each plant diversity level contributes to that PC.

		Eigenvalues			Eigenvectors			
	PC	Value	PrVar	Cum Var	bare ground plots May	bare ground plots October	vegetated plots May	vegetated plots October
May and October	1	8.85	34.0	34.0	1.30	2.28	-0.34	-0.11
	2	2.82	10.9	44.9	0.15	1.14	-0.33	0.17

ground and vegetated plots significantly differed in May ($p = 0.011$) and October ($p < 0.001$). However, bare ground plots and plots with vegetation cover were more similar in May than in October.

Averaged over all plots, PLFAs of Gram-negative bacteria dominated the microbial communities (Table 4-6). Their relative abundance (as percentages of total) increased from May (54.7 %) to October (55.2 %) ($p = 0.016$). PLFAs of Gram-positive bacteria decreased significantly from 16.4 % to 14.9 % during the vegetation period ($p < 0.001$) while PLFAs of fungi comprised 4.8 % of the total PLFAs and did not change. None of the tested microbial functional groups was affected by plant species diversity (Table 4-7). However, at both sampling dates, the proportion of Gram-negative bacteria was significantly higher in presence of legumes (May: $p = 0.012$; October: $p = 0.014$). In contrast to this, the proportion of fungi was decreased in the presence of legumes (May: $p = 0.015$; October: $p = 0.003$). The other plant functional groups (grasses, small herbs and tall herbs) had no effect on the composition of the microbial community. C/N ratio of plant biomass significantly affected the proportion of bacterial groups and fungi in October. While Gram positive bacteria ($R = 0.49$, $p = 0.015$) and fungi ($R = 0.49$, $p = 0.016$) increased with increasing C/N ratios the proportion of Gram-negative bacteria ($R = -0.37$, $p = 0.074$) seemed to decrease. The Shannon index was significantly lower in May than in October ($p < 0.001$) but was not affected by plant species diversity, number of plant functional groups and the presence of single plant functional groups.

Table 4-6: Properties of different microbial groups on bare ground plots (n = 3) and vegetated plots (n = 24) in May and October. Different letters indicate significant differences (p < 0.05) between May and October for the mean of all vegetated plots and the bare ground plots, respectively. Standard deviation in parentheses.

Season	Vegetation	Gram - %		Gram + %		fungi %		bacteria %	not identified %	Shannon index	
May	mean vegetated plots	54.7 (1.4)	a	16.4 (1.4)	a	4.8 (0.8)	a	22.5 (0.9)	1.6 (0.5)	2.80 (0.04)	a
October		55.2 (1.6)	b	14.9 (0.8)	b	4.9 (1.0)	b	23.0 (0.8)	2.0 (0.4)	2.87 (0.02)	b
May	bare ground plots	54.8 (1.7)	a	16.6 (1.4)	a	3.7 (0.7)	a	23.6 (1.0)	1.2 (0.7)	2.81 (0.00)	a
October		57.1 (2.4)	b	14.2 (3.0)	a	2.7 (0.5)	a	24.2 (0.4)	1.7 (0.3)	2.88 (0.03)	a

Table 4-7: Properties of different microbial groups in presence and absence of legumes and at different sown species richness in May and October. Different letters indicate significant differences (p < 0.05) between plots with and without legumes and the 4 to 16 species mixtures for the same sampling date, respectively. Standard deviation in parentheses.

Season	Diversity level	Gram - %		Gram + %		fungi %		bacteria %	not identified %	Shannon index	
May	plots containing legumes (n = 15)	55.1 (1.0)	a	16.3 (1.4)	a	4.5 (0.5)	a	22.4 (1.0)	1.8 (0.4)	2.80 (0.05)	a
	plots without legumes (n = 12)	54.1 (1.7)	b	16.6 (1.3)	a	4.9 (1.1)	b	23.0 (0.7)	1.4 (0.6)	2.81 (0.02)	a
	4 species mixtures (n = 8)	54.4 (2.0)	a	16.6 (1.6)	a	4.9 (0.7)	a	22.5 (1.0)	1.6 (0.7)	2.80 (0.03)	a
	8 species mixtures (n = 8)	54.9 (1.2)	a	16.4 (1.3)	a	4.8 (1.0)	a	22.3 (0.9)	1.6 (0.6)	2.82 (0.01)	a
	16 species mixtures (n = 8)	54.7 (0.9)	a	16.2 (1.4)	a	4.7 (0.7)	a	22.7 (0.7)	1.7 (0.7)	2.80 (0.06)	a
October	plots containing legumes (n = 15)	55.9 (1.6)	a	14.7 (0.9)	a	4.5 (0.8)	a	22.9 (0.8)	2.0 (0.4)	2.87 (0.02)	a
	plots without legumes (n = 12)	54.9 (2.0)	a	15.0 (1.5)	a	4.9 (1.5)	a	23.3 (0.9)	1.8 (0.3)	2.88 (0.02)	a
	4 species mixtures (n = 8)	54.9 (1.7)	a	15.0 (0.8)	a	5.2 (1.2)	a	22.9 (1.0)	2.0 (0.4)	2.87 (0.02)	a
	8 species mixtures (n = 8)	55.7 (1.2)	a	14.8 (0.7)	a	4.7 (1.2)	a	22.9 (0.7)	1.9 (0.4)	2.87 (0.01)	a
	16 species mixtures (n = 8)	55.2 (2.1)	a	15.0 (1.0)	a	4.8 (0.6)	a	23.1 (0.7)	1.9 (0.3)	2.88 (0.03)	a

4.4 Discussion

4.4.1 Land use change and soil microbial community

Converting arable systems into grasslands result in shifts in microbial biomass and community composition (Potthoff et al., 2006). The permanent vegetation cover alters abiotic and biotic soil properties and belowground ecosystem functions such as decomposition and nutrient cycling (Orwin et al., 2006). In the present study, bare ground plots and plots with vegetation cover differed significantly in several microbial parameters. While bare ground plots had slightly increased microbial biomass in October, the increase was more pronounced on vegetated plots. Beside biotic factors, the vegetation drives microclimatic conditions like soil moisture and temperature, which are prominent factors in determining soil microbial community composition (Zogg et al., 1997; Fierer et al., 2003). The amount of PLFAs, SIR and BR were higher in October than in May. While the whole bacterial community can be investigated by measuring PLFAs, SIR represents the active part of the microflora (e.g. r-strategists like zymogenous species) that prefers easily degradable organic compounds (Dilly and Munch, 1998). Consequently, PLFA and SIR are not necessarily correlated (Merila et al., 2002). Higher PLFA, SIR and BR in October than in May indicate that the active biomass as well as the total biomass has changed during the vegetation period. After "starving" in winter the microogranisms can re-establish during the vegetation period parallel to plant growth. The establishment is accompanied by changing C/N ratios of the input material during the vegetation period and might be correlated with an increased growth of fine roots during spring and summer. This might explain our observation of higher amounts of Gram-negative bacteria in October compared to spring. Generally, we found that the importance of the input source of carbon substrates on the proportion of microbial groups is more distinct in autumn than in spring.

Apart from the differences in total microbial biomass on vegetated plots, differences among the sown plant diversity levels were also examined. In the present study, $C_{mic/CFE}$ was highest in the 4 species mixtures. Only a few studies have examined the relationship between aboveground plant diversity and the soil microbial community and inconsistent results are common (Porazinska et al., 2003; Zak et al., 2003; Carney and Matson, 2005). For instance, an increase in plant diversity from one to two species had a positive effect on soil microbial biomass, microbial respiration and the decomposition rate (Wardle and Nicholson, 1996) but this may not always be the case (Wardle and Barker, 1997). Spehn et

al. (2000) found that soil microbial biomass but not respiration or decomposition rate decreased as plant richness decreased from 32 to 1 species, while Zak et al. (2003) found that microbial biomass as well as microbial respiration increased with increasing plant species richness. In the present study $C_{mic/CFE}$ was only affected by plant species diversity in autumn supporting observations by Spehn et al. (2000) who also found no relationship between these two factors in June but in October. Presumably, differences between diversity levels are becoming more distinct during the vegetation period because of the different quality and quantity of inputs entering the belowground system under varying plant species diversity. During winter these differences might abate.

The microbial community composition strongly depended on the presence of vegetation as indicated by PCA. Reasons for the differences between the microbial communities on vegetated plots compared to bare ground plots are likely the same as discussed for the microbial biomass. The presence of plants probably changed environmental conditions and concomitantly soil conditions as indicated by increased organic carbon and nitrogen concentrations on plots with vegetation cover (Steinbeiss et al., 2008). Soil bacteria likely responded to these changes. Other studies did not find significant changes in microbial community or biomass two years after vegetation change (Maly et al., 2000; Balser et al., 2005). The results of pot experiments on carry-over effects of previous cultivation systems on microbial community development suggest that “soil memory” is an important factor in ecosystem development leading to time-lags before effects of the new land use can be detected (Bartelt-Ryser et al., 2005). The present study showed that four years after establishing experimental grassland systems first effects of the aboveground plant community on $C_{mic/CFE}$ and microbial community composition are detectable and presumably these differences will increase within the next years.

4.4.2 Plant species diversity and soil microbial community composition

The effect of key plant species on microbial communities is important to consider because the abundance, activity, and composition of decomposer communities have been shown to vary considerably with different plant species (Eom et al., 2000; Stephan et al., 2000). Effects of the plant community composition on soil microbial communities are due to plant species-specific variations in the quality and quantity of plant material that enters the soil modifying microbial community structure and activity. Soil fungi showed the most pronounced responses to plant presence and C/N ratio of plant biomass. Fungi are known

to be the first colonizers of litter with wide C/N ratios (Bowen and Harper, 1990). However, they are sensitive to dry conditions (Frey et al., 1999) which are often occurring on arable fields and similarly on the bare ground plots of The Jena Experiment. In vegetable cropping systems Schutter et al. (2001) found higher fungal biomass in spring compared to later in the year. On the contrary, we could not support these findings on soil fungi but for other groups of microorganisms like Gram-negative bacteria. Zymogenous microorganisms (i.e. Gram-negative bacteria) that strongly depend on the input of fresh organic material create hot spots of decomposition (Griffiths et al., 1999). Presumably, in October most of the "old" litter is already decomposed and new input like root exudates can be used by Gram-negative bacteria becoming more important at the end of the vegetation period. In October, Gram-negative bacteria are negatively and Gram-positive bacteria are positively correlated with increasing C/N ratios of plant biomass probably indicating the use of different food sources. While Gram-positive bacteria are found to use more soil organic matter derived carbon sources, Gram-negative bacteria prefer plant-derived carbon as carbon source (Kramer and Gleixner, 2008).

Beside the number of species, the presence of single plant functional groups can have a distinct impact on ecosystem processes (Bezemer et al., 2006; Lanta and Leps, 2007). Especially legumes are often reported to influence above- and belowground processes by providing nitrogen rich organic matter (Mulder et al., 2002; Spehn et al., 2002). Fresh organic matter inputs control microbial activity and SOM mineralization. In line with these findings, the present study underlines the observation that legumes support larger amounts of bacterial PLFAs, in particular Gram-negative bacteria, as indicated previously (Hedlund, 2002). This suggests that improved resource quality, indicated by lower C/N ratios (higher nitrogen concentrations), on plots containing legumes likely enhanced bacterial growth in the rhizosphere at the expense of fungi.

Diversity indices provide more information on community composition than simple species richness; they also take the relative abundances of different species into account (evenness). The increase in the Shannon index from May to October probably indicates a shift in the microbial PLFA abundance from a small number of PLFAs with high abundances in spring to a more balanced microbial community at the end of the vegetation period.

4.5 Conclusion

Our results showed that four years after conversion of arable land to experimental grassland of The Jena Experiment first effects of plant community composition on soil microbial community composition became detectable. The present study showed distinct seasonal changes in the soil microbial community structure, which were probably driven by the availability and quality of organic resources. Further, our results highlighted the time-lag of belowground responses to aboveground vegetation manipulations with only few significant effects four years after the establishment of the experiment. We hypothesise that these effects will increase with time.

4.6 Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft within the scope of the biodiversity research group with support from the Friedrich Schiller University Jena and the Max Planck Society. We thank Theodora Volovei (Darmstadt University of Technology) for the support during the measurements of soil respiration, J. Kirschstein assisting in the soil sampling campaigns and Janine Seyfferth for her help during the PLFA extraction. We gratefully acknowledge all the people involved in planning set up and maintenance of the experiment, especially E.-D. Schulze, W. W. Weisser, B. Schmid, J. Schumacher, C. Middelhoff and C. Roscher.

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4.8 Appendix

Species mixtures of the investigated plots

4 species mixtures:

- *Arrenatherum elatius, Campanula patula, Luzula campestris, Prunus vulgaris*
- *Achillea millefolium, Crepis biennis, Festuca pratensis, Luzula campestris*
- *Lathyrus pratensis, Medicago lupulina, Plantago lanceolata, Taraxacum officinale*
- *Knautia arvensis, Leontodon autumnalis, Plantago media, Vicia cracca*
- *Phleum pratense, Plantago media, Trifolium hybridum, Vicia cracca*
- *Bromus erectus, Plantago lanceolata, Poa trivialis, Prunella vulgaris*
- *Cardamine pratensis, Crepis biennis, Medicago lupulina, Trifolium repens*
- *Heracleum sphondylium, Medicago x varia, Tragopogon pratensis, Trisetum flavescens*

8 species mixtures:

- *Cynosurus cristatus, Glechoma hederacea, Lotus corniculatus, Medicago lupulina, Phleum pratense, Primula veris, Trifolium flavescens, Veronica chamaedrys*
- *Lathyrus pratensis, Medicago lupulina, Medicago x varia, Onobrychis viciifolia, Trifolium campestre, Trifolium dubium, Trifolium hybridum, Trifolium pratense*
- *Anthriscus sylvestris, Galium mollugo, Geranium pratense, Heracleum sphondylium, Knautia arvensis, Leucanthemum vulgare, Ranunculus acris, Sanguisorba officinalis*
- *Crepis biennis, Galium mollugo, Leontodon hispidus, Lotus corniculatus, Medicago lupulina, Onobrychis viciifolia, Plantago media, Sanguisorba officinalis*
- *Alopecurus pratensis, Arrhenatherum elatius, Cynosurus cristatus, Dactylis glomerata, Festuca rubra, Holcus lanatus, Poa trivialis, Trisetum flavescens*
- *Anthoxanthum odoratum, Anthriscus sylvestris, Bromus erectus, Leucanthemum vulgare, Lotus corniculatus, Onobrychis viciifolia, Poa trivialis, Trifolium hybridum*
- *Ajuga reptans, Bellis perennis, Glechoma hederacea, Leontodon autumnalis, Primula veris, Prunella vulgaris, Taraxacum officinale, Veronica chamaedrys*
- *Achillea millefolium, Ajuga reptans, Bromus erectus, Cardamine pratensis, Festuca pratensis, Pimpinella major, Plantago media, Primula veris*

16 species mixtures:

- *Anthoxanthum odoratum, Anthriscus sylvestris, Ajuga repens, Avenula pubescens, Bromus erectus, Carum carvi, Geranium pratense, Lathyrus pratensis, Lotus corniculatus, Plantago lanceolata, Poa pratensis, Ranunculus repens, Taraxacum officinale, Tragopogon pratensis, Trifolium campetsre, Vicia cracca*
- *Achillea millefolium, Alopecurus pratensis, Anthoxanthum odoratum, Anthricus sylvestris, Avenula pubescens, Bromus hordeaceus, Campanula patula, Centaurea jacea, Geranium pratense, Heracleum sphondylium, Holcus lanatus, Leucanthemum vulgare, Pimpinella major, Poa pratense, Poa trivialis, Trisetum flavescens*
- *Alopecurus pratensis, Anthoxanthum odoratum, Arrhenatherum elatius, Bellis perennis, Bromus erectus, Festuca pratensis, Holcus lanatus, Leontodon autumnalis, Leontodon hispidus, Phleum pratense, Plantago lanceolata, Poa*

pratensis, Primula veris, Prunella vulgaris, Ranunculus repens, Veronica chamaedrys

- *Ajuga reptans, Alopecurus pratensis, Anthriscus sylvestris, Bromus hordeaceus, Campanula patula, Cardamine pratensis, Cynusurus cristatus, Geranium pratense, Medicago lupulina, Plantago media, Poa pratensis, Primula veris, Ranunculus repens, Trifolium campestre, Trifolium dubium, Trifolium repens*
- *Ajuga reptans, Glechoma hederacea, Lathyrus pratensis, Leontodon hispidus, Medicago lupulina, Onobrychis viciifolia, Plantago media, Prunella vulgaris, Ranunculus reptans, Taraxacum officinale, Trifolium campestre, Trifolium fragiferum, Trifolium hybridum, Trifolium repens, Veronica chamaedrys, Vicia cracca*
- *Ajuga reptans, Anthoxanthum odoratum, Bellis perennis, Bromus erectus, Crepis biennis, Festuca rubra, Galium mollugo, Geranium pratense, Phleum pratense, Onobrychis viciifolia, Ranunculus repens, Rumex acetosa, Trifolium dubium, Trifolium fragiferum, Veronica chamaedrys, Vicia cracca*
- *Alopecurus pratensis, Bromus hordeaceus, Carum carvi, Crepis biennis, Cynusurus cristatus, Heracleum sphondylium, Lathyrus pratensis, Leontodon autumnalis, Luzula campestris, Onobrychis viciifolia, Pimpinella major, Plantago media, Taraxacum officinale, Trifolium campestre, Trifolium hybridum, Veronica chamaedrys*
- *Anthriscus sylvestris, Campanula patula, Cardamine pratensis, Centaurea jacea, Cirsium oleraceum, Geranium pratense, Medicago x varia, Rumex acetosa, Tragopogon pratensis, Trifolium campestre, Trifolium hybridum, Trifolium dubium, Trifolium pratense, Trifolium repens, Trifolium fragiferum, Vicia cracca*

5 Linking plant diversity and soil microbial community characteristics in an experimental grassland approach

Chapter source: Maike Habekost et al. 2008. Linking plant diversity and soil microbial community characteristics in an experimental grassland approach. Submitted to Journal of Ecology

Abstract

The link between soil microbial communities and plant diversity (with special reference to the number of plant species and their functional groups) is not well understood. Therefore, we investigated the impact of plant diversity on soil microbial biomass and soil microbial community composition in experimental grassland ecosystems. The Jena Experiment, established in 2002 on a former arable land site, included different mixtures of 60 species each typically occurring in the Central European mesophilic grasslands. The diversity gradient covers 1, 2, 4, 8, 16 and 60 species and 1, 2, 3 and 4 functional groups (grasses, legumes, small herbs and tall herbs), which were sown in a factorial design to exclude interfering effects between sown species and functional group richness. In May 2007, soil samples were taken from all 82 plots of The Jena Experiment and from two adjacent arable land sites and meadows. Composition and amount of the microbial biomass were determined by the phospholipid fatty acids (PLFA) analyses and the chloroform fumigation extraction (CFE).

We found that microbial biomass significantly increased with increasing plant diversity. This was probably due to a higher and more diverse food supply leading to a higher niche complementarity and facilitation at higher diversity levels. In addition, the microbial community composition was altered by plant diversity, whereas the eveness remained unaffected. While the number of plant functional groups was most important for microbial biomass, the composition of the soil microbial community changed in response to both, sown species richness and number of functional groups. The presence of certain functional groups (grasses and legumes) changed the proportion of fungi and Gram-negative bacteria in the whole microbial community. Five years after the establishment of The Jena Experiment, we found clear evidence that plant diversity had a major effect on the soil microbial community.

Keywords: chloroform fumigation extraction (CFE), phospholipid fatty acids (PLFA), plant functional groups, species number, The Jena Experiment

5.1 Introduction

Life above and below ground is linked by complex interactions (Hooper et al., 2000). Apart from the impact of season and specific soil properties like temperature, texture and moisture (Papatheodorou et al., 2004; Marhan et al., 2007; Steenwerth et al., 2008), mainly vegetation cover influences soil microbial communities (Hedlund, 2002; Potthoff et al., 2006; Habekost et al., 2008). It affects the soil microbial community by virtue of the biomass production, the litter quality, seasonal variations of litter production, plant carbon allocation and root exudates (Angers et al., 1998; Bais et al., 2006; Bezemer et al., 2006). In turn, soil microbial communities decompose organic matter, thereby increasing the availability of nutrients for plants and enhancing plant growth (Porazinska et al., 2003; van der Heijden et al., 2008) and consequently the transfer of organic matter from above ground to belowground systems. The productivity of aboveground can easily be determined and is well-established. In contrast to this, the quantification of belowground biomass and their inputs is very critical because direct root sampling has its limitations partly due to soil disturbance and the inability to account for coincidental growth and death between the sampling dates (Hendrick et al., 1993). Aboveground biomass has been found to be a useful and reliable measure for estimating the belowground biomass and is therefore widely used as a proxy for root input (Gill et al., 2002).

Plant diversity changes a wide range of ecosystem processes (Hooper et al., 2000; Loreau et al., 2001) but the mechanisms of their impact are not well understood. The link between plant diversity and belowground processes for example remains still open. With increasing plant diversity ressource availability for microbial communities in soil is modified (Zak et al., 2003) and might lead to higher niche complementarity including niche differentiation and facilitation of the soil microbial community. Beside species richness, the number of plant functional groups, containing species with similar morphological, phenological and physiological traits, might have an impact on soil microogranisms. Plant functional groups like legumes or grasses clearly differ in their litter quality and carbon and nitrogen release to soil and were found to strongly influence microbial decomposition (Scherer-Lorenzen, 2008). Plant species and functional groups not only provide microorganisms with a wide range of nutrition, microorganisms also preferentially use different food sources (Schutter et al., 2001; Paterson et al., 2008). Fungi colonize litter with wide C/N ratios (Bowen et al., 1990), Gram-negative bacteria are mostly root associated and thus decompose small organic molecules (Griffiths et al., 1999) whereas Gram-positive bacteria are also able to

decompose more complex material like soil organic matter or litter (Kramer et al., 2006). The presence of distinct plant functional groups promotes distinct microbial groups. Therefore higher plant diversity, as number of species or number of functional groups, might increase the diversity of the soil microbial community, which can be estimated with proxies like the Shannon index (Hill et al., 2003). In addition to the Shannon diversity index the Smith and Wilson's index of evenness (Smith et al., 1996) was used to further characterise the microbial community.

So far, studies investigating the impact of plant diversity on soil microbial biomass and the community structure under field conditions are scarce and mostly restricted to culturable bacteria or very specific ecosystems (Spehn et al., 2000; Stephan et al., 2000; Zak et al., 2003; Gruter et al., 2006). Further, most studies investigating the effect of plant diversity on soil microbial communities studied either the effect of plant species richness or plant functional richness. These studies have a limited explanatory value because both parameter may interact and play a role for soil microbial community. We studied the effect of plant diversity on the soil microbial community. The soil microorganisms were determined by chloroform fumigation extraction and phospolipid fatty acid analysis, at the field site of The Jena Experiment, which is a grassland diversity experiment that was establishment in 2002 on a former arable land. In The Jena Experiment, the sown species richness (1 - 60) and the number functional groups (1 - 4) were varied as independent as possible to reach a factorial design (Roscher et al., 2004). Additionally, long-term meadows, ongoing arable plots and fallows were included in the analyses and the microbial communities found at these sites were used as reference microbial communities.

We hypothesize that plant diversity affects belowground microbial community biomass and composition because quality, quantity, variety and timing of carbon and nitrogen release, which are prerequisites for microbial nutrition, are different at different biodiversity levels. Since plant species differ in their carbon and nutrient release into the soil these differences might be even larger among plant species with different functional characteristics. We assume sown species richness (1) as well as the number of plant functional groups (2) and the presence species belonging to particular functional groups (3) to have an impact on soil microbial biomass and their community composition.

5.2 Materials and Methods

5.2.1 Site description and experimental design

The field site of The Jena Experiment is located close to the city of Jena (Germany) in the floodplains of the river Saale. The soil (Eutric Fluvisol) has developed from up to 2-m-thick fluvial sediments presenting a systematic variation of soil texture. The sand content decreased with distance from the river from 40 % to 7 %, while the silt content increased from 44 % to 69 %. The clay content showed almost no spatial pattern and varied between 16 % and 24 % (Steinbeiss et al., 2008). Consequently, the experimental plots were arranged in four blocks parallel to the river to account for these differences in soil characteristics. Before the establishment of The Jena Experiment in 2002, the site was used as arable land since the early 1960s and ploughed and fertilized regularly. The Jena Experiment comprises 82 plots (each 20 m by 20 m) which were sown in May 2002. The species mixtures were chosen from a species pool of 60 being typical for Central European mesophilic grasslands, by the random replacement method. Species were grouped into four functional groups according to their morphological, phenological and physiological traits (Roscher et al., 2004). The diversity gradient ranged from 1 to 60 species (1, 2, 4, 8, 16 and 60) and from 1 to 4 functional groups (1, 2, 3 and 4). Each level of sown species richness was represented with four replicates per block resulting in 16 replicates per species richness level in total except for 16 species mixtures which were represented by 14 replicates. Bare ground plots with no vegetation cover and 60-species mixtures were represented four times with one plot per block. The levels of numbers of functional groups were represented with 34, 20, 12 and 16 plots for 1, 2, 3 and 4 functional groups, respectively. The diversity gradient was maintained by weeding twice a year. All plots were mown every June and September in accordance to the management of extensive meadows used for hay production. Additionally to the main experiment, two types of fallows with two respective replicates were established. The aim was to allow succession on a mowed replicate and on one without any disturbance. Further, soil microbial characteristics were determined on two adjacent meadows and two arable plots on the experimental site. The arable plots were continuously managed according to conventional agricultural procedures and the meadows were mown twice a year similar to the plots from The Jena Experiment.

5.2.2 Soil and biomass sampling

In early May 2007, 6 soil samples per plot were taken with a core cutter (inner diameter: 5.6 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) to a depth of 5 cm, pooled and placed immediately in cooling boxes. During the first 48 hours after sampling the soil was kept at 4°C and sieved to 2 mm mineral size fraction. The sieved samples for phospholipid fatty acid analyses and chloroform fumigation extraction analyses were stored at -20°C. Sub-samples from the sieved soil cores were dried to constant weight at 40°C, ground and analyzed for organic carbon and nitrogen (Elemental Analyser vario Max C/N, Elementar Analysensysteme GmbH, Hanau, Germany). The relative standard deviation of repeated mesurements of an certified reference soil was < 2 %. To calculate soil organic carbon and nitrogen stock changes between 2002 and 2007 stock data from 2002 were used from the literature (Steinbeiss et al., 2008).

In late May 2007 shortly before mowing, the aboveground biomass was harvested on three randomized quadrates (20 cm x 50 cm) per plot by cutting standing biomass 3 cm above ground. The biomass was dried at 70°C to constant weight, chaffed and ground to fine powder. Carbon and nitrogen were measured from 20 mg sub-samples with an elemental analyzer (Elemental Analyser vario EL II, Elementar Analysensysteme GmbH, Hanau, Germany) and C/N ratios were calculated. The repeated measurements of certified reference plant biomass resulted in an excellent relative standard deviation for organic carbon and nitrogen concentrations of < 1 %.

5.2.3 Phospholipid fatty acids (PLFA) analysis

For PLFA analysis soil samples were shaken with a mixture of chloroform, methanol and 0.05 M phosphate buffer (pH 7.4) to extract soil lipids (Bligh et al., 1959; Kramer et al., 2006). Soil lipids were split up into neutral lipids, glycollipids and phospholipids by eluting with chloroform, acetone and methanol from a silica-filled solid phase extraction column. Subsequently, the phospholipids were hydrolyzed and methylated by a methanolic KOH solution and the PLFA-methyl esters were identified and quantified by GC-AED (Agilent, Böblingen, Germany) and GC/MS (Thermo Electron, Dreieich, Germany). Peak areas and the resulting PLFA amount per mg dry weight were calculated relative to the internal standard PLFA 19:0. The following PLFA were used as a marker for bacterial biomass 14:0, 15:0, 15:0br iso, 15:0br anteiso, 16:0, 17:0, 18:0 and 20:0. PLFA 18:2ω6 was used as a fungal biomarker (Zelles, 1997). Gram-negative bacteria were represented

by 16:1, 16:1(2), 17:1, 17:1(2), 18:1, 18:1n9, 18:1n11, 18:0 cyclo, 20:1 and 20:1(2) and Gram-positive bacteria were represented by 16:0br, 16:0br(2), 17:0br, 17:0br(2), 17:0br(3), 18:0br and 18:0br(2). PLFA 14:0br could not be assigned to a bacterial group (Zelles, 1997).

5.2.4 Microbial Biomass Carbon ($C_{mic/CFE}$)

We applied the chloroform fumigation extraction (CFE) method to determine the amount of microbial biomass carbon ($C_{mic/CFE}$) (Vance et al., 1987). For the non-fumigated samples 10 g of fresh soil were extracted with 50 ml 0.5 M K_2SO_4 for 1 h at 130 rev min^{-1}, filtered subsequently and kept frozen until the analysis. The fumigated samples were fumigated with ethanol-free chloroform for 24 h in a vacuum desiccator and extracted afterwards in the same way as the non-fumigated samples. The difference of carbon contents of fumigated and non-fumigated samples were measured on a high TOC elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany; rel standard deviation of reference material ≤ 5 %). The efficiency constant k_{ec} = 0.45 (Jörgensen, 1996) was used to calculate the microbial biomass.

5.2.5 Statistical analysis

Statistical analyses were carried out with SPSS Version 16.0 (SPSS Inc., Chicago, USA). Correlation analysis was used to test the relation between PLFAs, $C_{mic/CFE}$, soil and plant variables and diversity. The Jena Experiment is based on a factorial design with different combinations of the sown species richness and the number of functional groups. As the two diversity parameter are positively correlated and result in a not completely balanced design an analyses of variance (ANOVA) with sequential sum of squares (type I SS) was applied (Shaw et al., 1993). The block effect, describing systematic differences in soil properties between the blocks, was included as a random factor and fitted first. Aboveground biomass, C/N ratio of the aboveground biomass and the soil organic carbon stock changes between 2002 and 2007 were set as covariates and tested before sown species richness and the number of functional groups, which were included as fixed factors. The order of the experimental factor sown species richness and number of functional groups were changed to assess their relative importance. As a last factor the presence and absence of small herbs, tall herbs, grasses and legumes was respectively tested in the model. To reach equidistance between the diversity levels the sown species

richness term was log-transformed. To include bare ground plots 1 was added to the sown species richness before taking the logarithm (Steinbeiss et al., 2008). The presence and absence of functional groups were tested with ANOVAs including the block effect. The Shannon diversity index was used as a measure of PLFA diversity (Eq. 1).

$$H' = -\sum \left[P_i * \log P_i \right] \quad \text{(Eq. 1)}$$

Pi = proportional abundance of a given PLFA

For calculation of the evenness of PLFAs the Smith and Wilson`s index of evenness (E_{var}) (Eq. 2) (Smith et al., 1996) was used.

$$E_{var} = 1 - \left[\frac{2}{\pi \arctan \left\{ \sum_{i=1}^{s} \left(\log_e (n_i) - \sum_{j=1}^{s} \log_e \left(n_j \right) / s \right)^2 / s \right\}} \right] \quad \text{(Eq. 2)}$$

n_i = number of individuals PLFA i in the sample

n_j = number of individuals of PLFA j in the sample

s = number of species in the entire sample

This evenness index, which is based on the abundance of PLFAs, is independent of number of PLFAs and sensitive towards rare and common PLFAs. Principal Components Analysis (PCA) was used to compare patterns of PLFA profiles. The data used in the PCA was normalized to the peak area of the highest peak per plot being 100 %. All references to statistical differences are based upon a significance level of 0.05.

5.3 Results

5.3.1 Soil and plant parameters

The soil moisture at the sampling date was in the mean 11.6 % with a small standard deviation (sd) of 3.2 %. It was significantly correlated to the soil texture as it decreased with increasing sand content (R = -0.33, p = 0.002) and increased with increasing silt (R = 0.33, p = 0.002) and clay content (R = 0.30, p = 0.005). Soil organic carbon stocks increased from 1093 g m^{-2} (sd = 116 g m^{-2}) in 2002 to 1426 g m^{-2} (sd = 167 g m^{-2}) in 2007. The changes in soil organic carbon stocks between 2002 and 2007 were positively

correlated to plant diversity (sown species richness R = 0.31, p = 0.003; number of functional groups: R = 0.27, p = 0.013).

The amount of biomass, harvested in May 2007, ranged between 188 g m^{-2} (sd = 162 g m^{-2}) for monocultures and 941 g m^{-2} (sd = 110 g m^{-2}) for 60 species mixtures and from 296 g m^{-2} (sd = 303 g m^{-2}) to 637 g m^{-2} (sd = 324 g m^{-2}) for plant communities containing 1 to 4 functional groups, respectively (Appendix). Aboveground biomass was positively correlated to sown species richness and the number of functional groups (species richness: R = 0.48, p < 0.001; number of functional groups: R = 0.42, p < 0.001). Plots containing legumes produced more than twice the amount of biomass compared to plots without legumes. The C/N ratio of aboveground biomass was not correlated to the sown species richness (R = 0.01, p = 0.902) but decreased with increasing number of functional groups (R = -0.21, p = 0.056). Further, the C/N ratio was affected by the absence and presence of legumes and grasses. Plots containing grasses had a C/N ratio of 30.0 (sd = 8.9) which was significantly higher than that of plots without grasses (C/N ratio: 22.0 sd = 6.5 p < 0.001). On plots containing legumes the C/N ratio was significantly lower (20.4 sd = 4.4) compared to plots without this functional group (32.8 sd = 7.8 p < 0.001).

5.3.2 Microbial biomass

Higher plant diversity led to higher amounts of PLFAs and $C_{mic/CFE}$ (Figure 5-1). The amount of PLFAs ranged from 3.8 µg g^{-1} dw (sd = 1.3 µg g^{-1} dw) on plots with monocultures up to 4.7 µg g^{-1} dw (sd = 0.5 µg g^{-1} dw) on plots with 60 species mixtures, while the $C_{mic/CFE}$ ranged from 0.33 mg g^{-1} dw (sd = 0.12 mg g^{-1} dw) to 0.48 mg g^{-1} dw (sd = 0.05 mg g^{-1} dw). The amount of PLFA and $C_{mic/CFE}$ increased with increasing number of functional groups and was highest at plots with 3 functional groups. The presence of

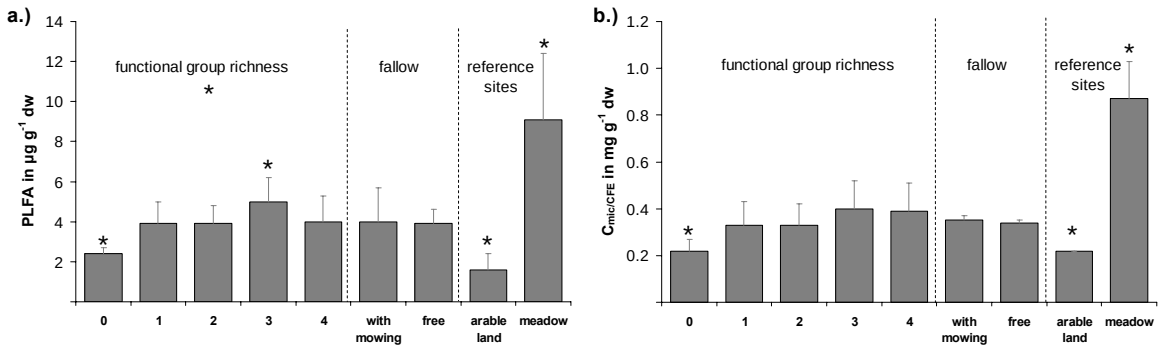

Figure 5-1: Amount of phospholipid fatty acids (PLFA) a.) and microbial carbon ($C_{mic/CFE}$) b.) Asterisks mark significant (p < 0.05) differences between different numbers of functional groups, fallows and reference sites analysed by t-tests. The asterisks below the headline of the functional group richness refers to results from an ANOVA and indicates significance at the 0.05 level (see Table 5-2).

grasses significantly increased the amount of PLFAs from 4.1 µg g^{-1} dw (sd = 0.9 µg g^{-1} dw) to 4.6 µg g^{-1} dw (sd = 1.0 µg g^{-1} dw) ($p = 0.020$). In contrast to grasses all other functional groups did not alter the amount of PLFAs significantly ($0.087 \leq p \leq 0.485$). The amount of $C_{mic/CFE}$ was not significantly influenced by any of the tested functional groups. Bare ground plots contained significantly less PLFAs (2.4 µg g^{-1} dw sd = 0.3 µg g^{-1} dw, $p = 0.001$) and $C_{mic/CFE}$ (0.22 mg g^{-1} dw sd = 0.05 mg g^{-1} dw, $p = 0.009$) than vegetation covered plots from the diversity experiment.

At the sites used as regular ploughed arable land, 1.6 µg g^{-1} dw (sd = 0.8 µg g^{-1} dw) of PLFA and of 0.22 mg g^{-1} dw (sd < 0.01 mg g^{-1} dw) of $C_{mic/CFE}$ were found, which was significantly lower compared to the vegetation covered plots from the main experiment ($p < 0.001$ for PLFA and $C_{mic/CFE}$) (Figure 5-1). Established seminatural meadows had approximately twice the amount of PLFAs and $C_{mic/CFE}$ (PLFA: 0.22 mg g^{-1} dw sd = 0.05 mg g^{-1} dw; $C_{mic/CFE}$: 0.87 mg g^{-1} dw sd = 0.16 mg g^{-1} dw) compared to plots of the main experiment ($p < 0.001$ for PLFA and $C_{mic/CFE}$). The amount of PLFAs and $C_{mic/CFE}$ of two types of fallows (free succession and succession with mowing), which were additionally analyzed, ranged between the amounts measured for 4 to 16 species mixtures and 2 to 3 functional groups (Figure 5-1).

Both variables to assess microbial biomass, $C_{mic/CFE}$ and the amount of PLFAs, were significantly correlated ($p < 0.001$) (Table 5-1). While soil texture highly influenced the amount of PLFAs, it had no impact on $C_{mic/CFE}$. The relationship between variables characterising microbial biomass, soil as well as aboveground vegetation were tested with a correlation analyses. The amount of PLFAs was negatively correlated to sand content ($p = 0.002$) and positively correlated to the silt ($p = 0.004$) and clay ($p = 0.002$) content. The amount of PLFAs and $C_{mic/CFE}$ were significantly positively correlated to soil moisture (PLFA: $p < 0.001$; $C_{mic/CFE}$: $p < 0.001$). Further, the amount of PLFAs and $C_{mic/CFE}$ and the soil organic carbon stock changes were significantly correlated (PLFA: $p < 0.001$; $C_{mic/CFE}$: $p < 0.001$). While the amount of PLFAs and the C/N ratio of aboveground biomass were not significantly correlated ($p = 0.385$), the amount of PLFAs was significantly positively correlated to aboveground biomass ($p = 0.024$). Additionally, we found that microbial biomass was positively correlated to plant diversity (PLFA: sown species richness: $p = 0.021$; number of functional groups: $p = 0.002$; $C_{mic/CFE}$: sown species richness: $p < 0.001$; number of functional groups: $p = 0.004$).

Table 5-1: Correlation between plant- or soil-related parameters and the amount of PLFAs and microbial biomass $C_{mic/CFE}$

variable	parameter	significance	pearson correlation
PLFA	sand content	0.002	-0.33
	silt content	0.004	0.32
	clay content	0.002	0.31
	soil moisture	< 0.001	0.61
	soil C_{org} stock change 2002-2007	< 0.001	0.54
	aboveground biomass	0.024	0.25
	C/N ratio of aboveground biomass	0.385	0.10
	ln sown species richness	0.021	0.25
	number of functional groups	0.002	0.34
$C_{mic/CFE}$	sand content	0.796	-0.03
	silt content	0.947	0.01
	clay content	0.428	0.09
	soil moisture	< 0.001	0.49
	soil C_{org} stock change 2002-2007	< 0.001	0.38
	aboveground biomass	0.386	0.10
	C/N ratio of aboveground biomass	0.957	0.01
	ln sown species richness	< 0.001	0.38
	number of functional groups	0.004	0.31

The variables, which were correlated to microbial biomass, were included in the ANOVA and fitted before the experimental factors to assess whether diversity has significant effects on soil microbial biomass after correcting for abiotic and plant related effects. Systematic differences between blocks, i.e. soil texture, were included as a block factor. In the ANOVA a high proportion of the variance was explained by soil organic carbon stock changes (12 %) and plant diversity as a sum of sown species richness and number of functional groups (12 %) (Table 5-2). The number of functional groups explained 5 % to 9 % of the variance ($0.010 \leq p \leq 0.031$) and was more important for the amount of PLFAs, than sown species richness, which was not significant irrespective of whether it was tested before or after the number of functional groups ($0.146 \leq p \leq 0.401$). The importance of functional groups was not related to the presence of a distinct plant functional group ($0.293 \leq p \leq 0.880$). The ANOVA for the $C_{mic/CFE}$ showed that soil organic carbon stock change had significant effects ($p = 0.001$) and explained most of the variance (Table 5-2). Sown species richness and the number of functional groups were not significant (sown species richness: $0.211 \leq p \leq 0.421$; number of functional groups: $0.245 \leq p \leq 0.537$) but still explained 9 % of the variance. To test whether the microbial biomass was influenced by the presence of particular functional groups, the microbial biomass on plots with and without single functional groups was compared.

Table 5-2: Summary of sequential analysis of variance (ANOVA with type I sum of squares) of the amount of phospholipid fatty acids (PLFA) and microbial carbon ($C_{mic/CFE}$). The final column (% of SS) contains the proportion of the sum of squares explained by a particular parameter. Different order of fitting of biodiversity parameters is shown and a bold line within PLFA and $C_{mic/CFE}$ denotes a reversed fitting of diversity parameters. Asterisks mark significance at the 0.05 (*), 0.01 (**) or 0.001 (***) level.

variable	parameter	significance	F-value	% of SS
PLFA	block	(***) < 0.001	10.55	23.7
	aboveground biomass	(*) 0.018	5.90	4.4
	C/N ratio of aboveground biomass	0.210	1.60	1.2
	soil C_{org} stock change 2002-2007	(***) < 0.001	16.12	12.0
	ln sown species richness	0.146	1.77	5.3
	number of functional groups	(*) 0.031	3.16	7.1
	number of functional groups	(**) 0.010	4.15	9.3
	ln sown species richness	0.401	1.03	3.1
$C_{mic/CFE}$	block	(*) 0.019	3.55	11.1
	aboveground biomass	0.466	0.54	0.5
	C/N ratio of aboveground biomass	0.527	0.40	0.4
	soil C_{org} stock change 2002-2007	(***) 0.001	12.83	13.4
	ln sown species richness	0.211	1.51	6.3
	number of functional groups	0.537	0.73	2.3
	number of functional groups	0.245	1.42	4.5
	ln sown species richness	0.421	0.99	4.1

5.3.3 Microbial community composition

Principal component 1 (PC1) explained 29.2 % of the variance and PLFAs of Gram-positive bacteria (17:0br(2), 18:0br(1) and (3)) Gram-negative bacteria (18:1, 20:1(1) and (2)) and unspecified bacteria (15:0, 17:0 and 20:0) had the highest loadings on this axis.

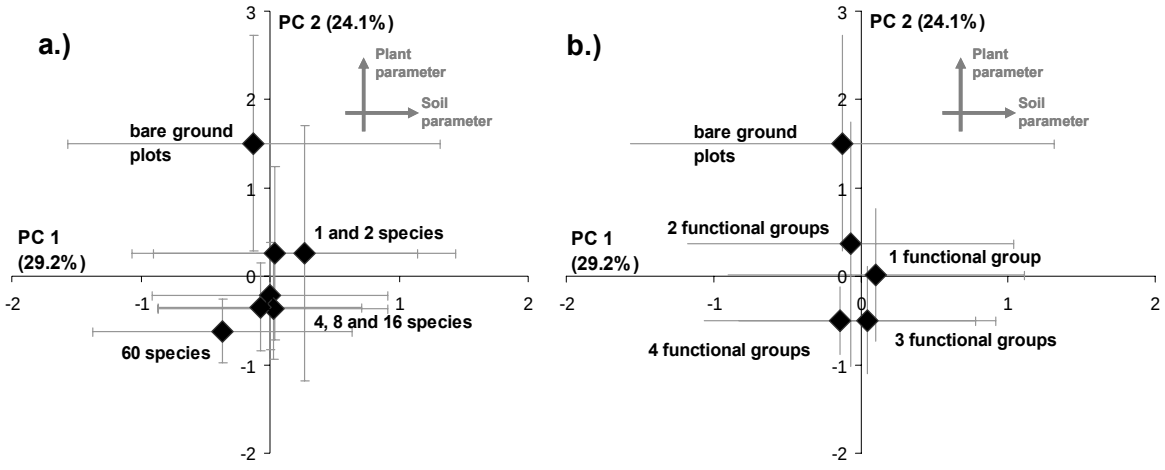

Figure 5-2: Summary of the PCA for bare ground plots and different plant diversity levels: a.) 1 to 60 sown species and b.) 1 to 4 functional groups. Along PC1 loadings were correlated to the experimental block and concomitantly to soil texture variables and to soil organic carbon stock changes between 2002 and 2007. The loadings of PC2 were mainly driven by plant parameters (abobeground biomass, sown species richness and number of functional groups).

The PCA loadings of component 1 per plot did not differ significantly between the different diversity levels (sown species richness: $p = 0.717$; number of functional groups: $p = 0.744$) (Figure 5-2). But the loadings of PC1 were significantly negatively correlated to soil variables (block: $R = -0.82$, $p < 0.001$; organic carbon stock change: $R = -0.22$, $p = 0.042$). Principal component 2 (PC2) explained 24.1 % of the variance and the main drivers of the principal component were PLFAs from Gram-positive bacteria (17:0br(3)), Gram-negative (16:1) and unspecific bacteria (15:0br iso and 18:0). The loadings of PC2 were correlated to aboveground biomass and plant diversity (aboveground biomass: $R = -0.23$, $p = 0.043$; sown species richness: $R = -0.37$, $p = 0.001$; number of functional groups: $R = -0.33$, $p = 0.002$). Along PC2 the loadings of plots with different sown species richness levels and functional groups differed significantly, respectively (sown species richness: $p = 0.001$; number of functional groups: $p = 0.001$). Nevertheless, sown species richness had a higher explanatory power (86 %) compared to number of functional groups (77 %). The amount of the PLFAs explaining variance along principal component 2 increased significantly with increasing biodiversity ($p = 0.008$).

Table 5-3: Proportions of different microbial groups at different levels of sown species richness, functional group richness, fallows and reference sites. Standard deviation in parentheses.

diversity level	Gram - %	Gram + %	fungi %	bacteria %	unidentified %
mean all plots	54.4 (2.6)	13.3 (4.3)	4.2 (1.1)	26.0 (2.5)	2.0 (0.4)
bare ground plots	55.5 (1.0)	11.6 (4.0)	2.8 (0.4)	28.4 (3.1)	1.8 (0.2)
1 specie mixtures	54.7 (3.0)	13.8 (5.2)	3.7 (1.0)	25.8 (7.0)	2.0 (0.6)
2 species mixtures	53.8 (2.6)	13.6 (4.4)	4.3 (1.0)	26.4 (3.2)	1.9 (2.1)
4 species mixtures	54.3 (2.3)	13.3 (4.2)	4.4 (1.2)	25.8 (2.2)	2.1 (0.5)
8 species mixtures	53.4 (2.8)	14.3 (4.7)	5.0 (1.0)	25.3 (2.2)	2.0 (0.7)
16 species mixtures	54.7 (2.5)	13.0 (4.2)	4.4 (1.2)	25.8 (1.9)	2.1 (0.3)
60 species mixtures	55.2 (1.5)	12.4 (4.2)	3.9 (0.5)	26.1 (2.5)	2.3 (0.3)
1 functional group	54.5 (2.4)	13.9 (4.1)	4.0 (1.1)	25.7 (2.5)	2.0 (0.3)
2 functional groups	54.4 (2.4)	12.9 (4.2)	4.4 (1.0)	26.4 (3.0)	1.9 (0.6)
3 functional groups	53.0 (3.8)	14.8 (5.3)	4.7 (1.2)	25.5 (2.1)	2.1 (0.4)
4 functional groups	54.7 (1.8)	12.7 (3.8)	4.6 (1.2)	25.8 (1.9)	2.2 (0.4)
fallow with mowing	57.1 (4.9)	11.4 (6.7)	4.4 (1.1)	25.0 (1.2)	2.1 (0.5)
free fallow	57.0 (1.3)	8.5 (0.8)	4.1 (0.3)	28.8 (0.7)	1.7 (0.1)
arable land	52.6 (0.2)	14.8 (1.6)	3.3 (0.3)	27.1 (1.1)	2.2 (0.4)
meadow	52.3 (0.8)	18.8 (3.1)	3.8 (1.2)	21.9 (0.9)	3.9 (0.3)

Averaged over all plots, Gram-negative bacteria dominated the microbial community with 54.4 %, while Gram-positive bacteria and fungi comprised 13.3 % and 4.2 % of total PLFA (Table 5-3). The amount of PLFA related to unspecific bacteria was 26.0 %. The proportion of Gram-negative bacteria of the arable land and meadow plots was 52.6 % (sd = 0.2 %) and 52.3 % (sd = 0.8 %). Arable land and meadow sites differed in the proportion of general bacteria, which was significantly lower at the meadows ($p = 0.025$). The finding that meadows had a higher proportion of Gram-positive bacteria was not significant ($p = 0.245$).

Similar to analyses of microbial biomass, the explanatory power of soil and aboveground plant variables as well as plant diversity for variation in the proportion of microbial groups was estimated by an ANOVA (Table 5-4). Plant diversity, tested as sown species richness and number of functional groups, had no statistical significant effect on the proportion of Gram-positive, Gram-negative and unspecific bacteria ($0.093 \leq p \leq 0.662$). Instead of plant diversity, C/N ratio of aboveground biomass and soil organic carbon stock changes were more important for the contribution of these microbial groups to total microbial biomass. The proportion of Gram-negative bacteria and unspecific bacteria were significantly driven by the quality of input materials, measured as C/N ratio of aboveground biomass (Gram-negative bacteria: $p = 0.036$; unspecific bacteria: $p = 0.002$). The proportion of Gram-positive bacteria was significantly altered by the C/N ratio of aboveground biomass and the soil organic carbon stock changes (C/N ratio of aboveground biomass: $p = 0.026$; soil organic carbon stock changes: $p = 0.026$). In contrast to the other microbial groups, the proportion of fungi was significantly affected by sown species richness ($p < 0.001$) and the number of functional groups ($p = 0.010$). The presence of grasses were only significant when fitted before diversity ($p < 0.001$). While, except for fungi, the number of functional groups was of minor importance for the proportion of microbial groups, the impact of presence and absences of plant functional groups was strong for all microbial groups.

Table 5-4: Summary of sequential analysis of variance (ANOVA with type I sum of squares) of the proportions of different microbial groups. The proportion of the sum of squares explained by a particular parameter is given in the final column (% of SS). Different order of fitting of biodiversity parameters is shown and a bold line within microbial groups denotes a reversed fitting of diversity parameters. Asterisks mark significance at the 0.05 (*). 0.01 (**) or 0.001 (***) level.

variable	parameter	significance	F-value	% of SS
Gram -	block	(***) < 0.001	10.89	28.1
	aboveground biomass	0.521	0.42	0.4
	C/N ratio of aboveground biomass	(*) 0.036	4.61	4.0
	soil C_{org} stock change 2002-2007	0.313	1.04	0.9
	ln sown species richness	0.327	1.18	5.1
	number of functional groups	0.098	2.19	5.7
	number of functional groups	0.093	2.23	5.7
	ln sown species richness	0.337	1.16	5.0
Gram +	block	(***) < 0.001	55.28	66.0
	aboveground biomass	0.689	0.16	0.1
	C/N ratio of aboveground biomass	(*) 0.026	5.16	2.1
	soil C_{org} stock change 2002-2007	(*) 0.026	5.22	2.1
	ln sown species richness	0.388	1.06	2.1
	number of functional groups	0.216	1.53	1.8
	number of functional groups	0.110	2.09	2.5
	ln sown species richness	0.606	0.727	1.4
fungi	block	(***) < 0.001	6.73	13.9
	aboveground biomass	(*) 0.029	4.98	3.4
	C/N ratio of aboveground biomass	(***) < 0.001	18.03	12.4
	soil C_{org} stock change 2002-2007	0.159	2.04	1.4
	ln sown species richness	(***) 0.001	4.67	16.0
	number of functional groups	(*) 0.011	4.03	8.3
	number of functional groups	(***) < 0.001	7.20	14.8
	ln sown species richness	(*) 0.025	2.77	9.5
bacteria	block	(***) < 0.001	45.62	62.0
	aboveground biomass	0.576	0.32	0.1
	C/N ratio of aboveground biomass	(**) 0.002	10.67	4.8
	soil C_{org} stock change 2002-2007	0.402	0.71	0.3
	ln sown species richness	0.447	0.96	2.2
	number of functional groups	0.504	0.79	1.1
	number of functional groups	0.279	1.31	1.8
	ln sown species richness	0.662	0.65	1.5

Furthermore, it was tested whether the presence or absence of particular functional groups had an impact on the proportion of microbial groups. Plots containing grasses had significantly more fungal biomass ($p < 0.001$), whereas the proportion of Gram-negative bacteria was significantly reduced ($p = 0.018$) (Table 5-5). On plots containing legumes the proportion of fungi was significantly reduced ($p = 0.009$) and the proportion of Gram-negative bacteria tended to be higher ($p = 0.070$) compared to plots without legumes. The proportion of fungi was significantly higher on plots with mixtures containing small herbs ($p = 0.009$), while it was reduced on plots containing tall herbs ($p = 0.035$). Gram-positive bacteria had a lower proportion of the microbial community on plots containing small herbs compared to plots without this functional group ($p = 0.045$).

Table 5-5: Proportions of different microbial groups in mixtures in which distinct functional groups (grasses. legumes. small herbs and tall herbs) were absent or present. Standard deviation in parentheses; Asterisks mark significance between plots with and without the distinct functional group at the 0.05 (*). 0.01 (**) or 0.001 (***) level.

Functional group	Presence/ Absence	Gram -	Gram +	fungi	bacteria	not identified
		%	%	%	%	%
grasses	absent	54.9 (2.5) ***	13.2 (4.3)	3.8 (1.0) *	26.1 (2.9)	1.9 (0.5)
	present	53.7 (2.5)	14.1 (4.6)	4.7 (1.0)	25.8 (2.0)	2.1 (0.4)
legumes	absent	53.9 (2.0)	14.1 (4.4)	4.6 (1.0) **	25.8 (2.3)	2.1 (0.4)
	present	54.6 (3.0)	13.3 (4.5)	4.0 (1.1)	26.1 (2.6)	2.0 (0.5)
small herbs	absent	54.3 (3.0)	14.3 (4.7) *	4.0 (1.0) **	25.8 (2.4)	2.0 (0.3)
	present	54.2 (2.2)	13.1 (4.2)	4.6 (1.1)	26.0 (2.5)	2.1 (0.5)
tall herbs	absent	54.2 (2.4)	14.0 (4.7)	4.1 (1.1) *	26.1 (2.8)	2.0 (0.5)
	present	54.3 (2.7)	13.4 (4.3)	4.5 (1.1)	25.7 (2.2)	2.1 (0.4)

The Shannon index was used to characterise the soil microbial diversity. The Shannon index was not significantly different between the arable land and the experimental site ($p = 0.702$) but it was significantly higher on meadow sites than on the experimental field site ($p = 0.013$) (Table 5-6). The Shannon index was not affected by sown species richness, the number of plant functional groups or the presence of single plant functional groups. The Smith and Wilson index of evenness (E_{var}) of the experimental plots was similar to that of the arable land plots ($p = 0.837$) and is significantly higher than the E_{var} of the meadows (main experiment: $E_{var} = 0.473$ sd = 0.063; meadow: $E_{var} = 0.379$ sd = 0.008; $p = 0.041$). Sown species richness, number of functional groups and presence or absences of functional groups had no impact on E_{var}.

Table 5-6: Shannon index and Smith and Wilson`s indexof eveness (E_{var}) for different levels sown species richness, functional group richness, fallows and reference sites. Standard deviation in parentheses.

diversity level		Shannon index	E_{var}
mean of all plots		2.79 (0.07)	0.480 (0.070)
bare ground plots		2.77 (0.12)	0.575 (0.106)
1 species mixtures		2.80 (0.07)	0.494 (0.070)
2 species mixtures		2.81 (0.08)	0.466 (0.073)
4 species mixtures		2.80 (0.06)	0.464 (0.057)
8 species mixtures		2.80 (0.06)	0.462 (0.061)
16 species mixtures		2.79 (0.06)	0465 (0.043)
60 species mixtures		2.78 (0.05)	0.468 (0.043)
1 functional group		2.81 (0.06)	0.478 (0.061)
2 functional groups		2.79 (0.07)	0.481 (0.077)
3 functional groups		2.80 (0.06)	0.452 (0.062)
4 functional groups		2.79 (0.06)	0.468 (0.054)
fallow with mowing		2.72 (0.16)	0.493 (0.118)
free fallow		2.72 (0.04)	0.568 (0.013)
arable land	*	2.78 (0.04)	0.463 (0.008)
meadow		2.83 (0.01)	0.379 (0.084)

5.4 Discussion

5.4.1 Impact of plant diversity on soil microbial biomass

Soil microbial biomass can be reliably estimated by extracting PLFAs and using the chloroform fumigation extraction method ($C_{mic/CFE}$) (Beck et al., 1997; Zelles, 1999). While PLFA are found in cell membranes of living cells and are rapidly decomposed after cell death (White et al., 1979), $C_{mic/CFE}$ comprises the whole soil microbial biomass (Jenkinson, 1976). Nevertheless both estimates of microbial biomass were well correlated (Dilly et al., 1998; Bailey et al., 2002; Leckie et al., 2004).

We found that microbial biomass was correlated with soil texture, soil moisture and also interestingly with soil organic carbon stock changes. Microbial biomass increased with decreasing particle size and increasing soil moisture and soil organic carbon stock changes. Higher microbial biomass might be found in smaller size fractions since finer particles provide a bigger surface area on which the microorganisms can be attached, a protective habitat through the exclusion of predators and a higher degree of nutrients (Sessitsch et al., 2001; Marhan et al., 2007).

The importance of environmental variables, like soil type and aboveground biomass, can be that strong as to hide the effect of plant diversity (Bossio et al., 1998; Patra et al., 2008). To disentangle the impact of plant diversity, soil and vegetation characteristics we applied a sequential ANOVA, in which the effects of soil texture and aboveground plant biomass and quality were tested first. Despite the impact of soil variables on microbial biomass, a significant effect of plant diversity on the soil microbial community was found. Microbial biomass significantly increased with an increasing number of up to three functional groups. Although plant productivity, measured as aboveground biomass, significantly increased microbial biomass, plant diversity was also important and explained most of the variation. Therefore, additional processes to productivity driven relationships as proposed by Zak et al. (2003) have to be considered. Not only the amount of input material was important for soil microbial biomass but also the diversity of inputs. On plots with higher sown species richness and a higher number of functional groups both was found: a higher input amount and a wider spectrum of inputs material. The food supply for microorganisms was presumably more evenly distributed over the season by virtue of an overlap of life cycles. This in turn may contribute towards constant environmental conditions, regarding soil moisture. Furthermore, more diverse mixtures were shown to store more organic carbon and therefore deliver an additional food source.

Generally, higher plant diversity is assumed to increase the number of available niches for soil microorganisms. The increasing niche complementarity with increasing diversity is supposed to be the reason for higher microbial biomass at higher plant diversity levels.

Five years after conversion from arable land to managed grassland, increased soil microbial biomass clearly indicated that the microbial community had developed compared to the arable land reference site. The amount of soil microbial biomass of arable land plots was lower than the amount measured on bare ground plots and monocultures. Reduced microbial biomass on arable land is probably due to a soil disturbance by tillage and the tillage-induced changes of soil properties (Cookson et al., 2008). In addition to the general negative impact of disturbance on the soil microbial community, which was observed in a wide range of soils and under different types of disturbances (Ravit et al., 2006), a higher cropping intensity negatively affects soil microbial biomass (Hamel et al., 2006). The soil microbial community was also modified by vegetation cover. Monocultures, which were not disturbed and were covered with perennial plant species, were found to have higher

microbial biomass than bare ground plots, which were undisturbed but had no vegetation cover.

The result that plots with 60 species and 4 functional groups had lower microbial biomass than the adjacent meadows indicated that our field site is still developing towards the meadow sites but needs more time to reach this stage. Nevertheless, five years after establishment, we already found an impact of plant diversity on soil microbial biomass, which affirmed the trends seen four years after establishment (Habekost et al., 2008).

In addition to the established diversity gradient, the soil microbial biomass on fallow plots was measured. On the fallow plots, between 3 and 4 functional groups including shrubs (only on the free fallows) were observed in May 2007. The soil microbial biomass corresponded well with the diversity gradient.

In The Jena Experiment, the number of functional groups was found to influence microbial biomass more than sown species richness. The explanatory power was higher in all tested cases. Even though the number of functional groups played a major role for the amount of microbial biomass the presence or absence of certain functional groups was less important. Only plant mixtures including grasses were found to have a higher microbial biomass than mixtures without grasses which was accompanied by a larger proportion of fungal biomass.

5.4.2 Impact of plant diversity on the soil microbial composition

We found that five years with different plant diversity were sufficient to change the soil microbial community composition significantly as shown by the results of the PCA. Soil texture and soil organic carbon stock changes, which were important drivers along PC1, had a general effect on the soil microbial community whereas the changes in these parameters did not change the community composition. The driving PLFAs of PC1 encompassed all important microbial groups (Gram-negative bacteria, Gram-positive bacteria and bacteria in general) except for fungi indicating that soil characteristics, if they were at least in the range reported for our study site, have a general effect on microbial composition. Apart from soil characteristics, plant communities were also important for soil microbial community composition. Different plant communities lead to different organic matter input thus modifying quantity, quality and timing of the input (Angers et al., 1998; Hooper et al., 2000). These differences in input caused the changes of microbial diversity as shown in the PC2. Further, root exudates were often reported to strongly

influence microbial populations (Baudoin et al., 2003; Bais et al., 2006; Weisskopf et al., 2008). In more diverse plant mixtures the food supply for microorganisms was assumed to be higher and more diverse, while food supply in monocultures is more one-sided and temporally limited. The microbial community probably adapted to these different food conditions and amounts (Schutter et al., 2001; Orwin et al., 2006). There is evidence that a higher number and amount of substrates may lead to higher niche diversity for soil microogranisms and that in turn soil microbial communities at higher diversity levels were capable to degrade a wider range and higher amount of substrates compared to microbial communities in monocultures. We assume that shifts in microbial diversity were caused by changes in the abundance of dominant PLFAs because the Shannon index, which is known to be more sensitive to changes of abundance of rare microbial PLAFs (Hill et al., 2003), did not reflect the changes caused by plant diversity. A comparison between the experimental site, the arable land and meadow sites reveals differences in the structure of microbial communities. While arable plots are colonized by rather unspecialized and homogenously distributed microbial communities, the more uneven distribution of PLFAs on meadow sites reflect the higher degree of adaptation towards more heterogeneous inputs. The microbial community of the experimental site is still in transition towards meadows but still needs more time for development.

The adaptation of different micoroorganisms to different food sources was corroborated from the interactions between microbial and plant functional groups. The proportion of Gram-negative bacteria, which are more root associated and promoted by N additions (Sessitsch et al., 2001; Treonis et al., 2004; Billings et al., 2008), were higher on plots containing legumes. Further, C/N ratio of input material was found to be important for Gram-negative bacteria. Soil organic carbon was the major predictor for the proportion of Gram-positive bacteria, which occur more widly spread in the soil and can also degrade soil organic matter (Kramer et al., 2006). The proportion of fungi was explained by the C/N ratios and amount of input material. Furthermore, we found an increasing proportion of fungi with increasing plant diversity, measured as number of functional groups and sown species richness.

5.5 Conclusion

Five years after establishment of The Jena Experiment, we found that plant diversity clearly impacted the soil microbial community. Furthermore, biotic factors, i.e. plant diversity (sown species richness, number of functional groups as well as the presence and absence of functional groups), was found to be more important for soil microbial community than abiotic controls. The amount of soil microbial biomass increased with an increasing number of functional groups. Sown species richness was of minor importance for microbial biomass. On the contrary the microbial composition was influenced by both diversity measures: sown species number and number of functional groups. Additionally, the amount and quality of input material altered microbial composition. This highlights the importance and utilisation of different food sources for microogranisms. Overall, increasing plant diversity is of crucial importance for ecosystem functioning since changes of microbial community encompassed the amount and composition resulting in larger and probably more stable microbial communities at higher plant diversity levels.

5.6 Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft within the scope of the biodiversity research group (FOR 456) with support from the Friedrich Schiller University Jena and the Max Planck Society. We thank Janine Seyfferth, for her help during the PLFA extraction. We gratefully acknowledge all the people that were involved in planning, set up and maintaining of the experiment.

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6 Synthesis

We found strong links between plant diversity and belowground ecosystem functioning such as carbon and nitrogen storage and transformation of inputs. An improved understanding of belowground dynamics as a function of plant diversity can facilitate the assessment of consequences of the predicted global changes and the resultant species loss. Our results showed that soil properties are important for storage, but furthermore revealed the huge impact of plant diversity on the microbial transformation of inputs resulting in a sustainable sequestration of soil organic carbon and nitrogen in the top and sub soil.

Processes like interactions with mineral surfaces had an impact on storage in the top soil. Apart from these processes plant diversity positively increased the storage within the top and sub soil. The process was less driven as first expected by a high biomass production and concomitantly increased inputs at higher plant diversity levels but rather by microbial transformation of inputs. Microbial activity is most essential for storage, as long as the system is not input limited. Thus input availability is also an important precondition for storage. Through microbial transformation, input will result in sustainable sequestration, since around 80 % of the storage occurred in the heavy fraction, representing a more stable soil organic matter pool. Consequently increased plant diversity might mitigate climate warming through enhanced sequestration of anthropogenic produced carbon dioxide emissions in soil. Increased storage due to increased plant diversity will help to sustain or even improve ecosystem functioning since a higher organic matter content of soils is widely known to have positive influences on soil fertility, soil structure and soil stability and therefore also contributes towards a higher resistance against erosion processes. However, diversity and its subsequent carbon storage can help to diminish climate warming. In regards to climate warming the question evolves whether the impact of diversity on storage stays constant with time. General research on the time dependency of storage rates of grasslands indicated that storage increased linearly after conversion but the impact of biodiversity on carbon storage over a longer period remains uncertain and gives way to further investigations.

Similar to the mechanisms of storage, abiotic conditions were found to be a prerequisite but not a main driver for soil microbial diversity. From the investigated abiotic parameters only soil organic carbon stocks were related to microbial abundance. This reveals the key role of microorganisms, which are dependent on organic carbon as a food source and in addition enhance the sustainable storage through transformation of plant input. Plant

diversity is of major importance for microbial abundance and composition by virtue of increasing niche complementarity. In contrary to the redundancy hypothesis, which predicts that beyond a critical and most probably low diversity level most species are functional redundant, we found that the microbial community shifted along the entire plant diversity gradient as well as that aboveground diversity is the main driver for these shifts. Within the studied diversity gradient of up to 60 species and 4 functional groups no threshold was found above that species would be redundant. Instead, higher plant diversity promotes a higher stability and effective resource consumption within microbial communities and supports the strong link between above- and belowground communities.

All measured values of diversity (sown species richness, number of functional groups and presence of distinct functional groups) were found to be of different importance, when special processes or parameters were separately considered. Nonetheless, the overall process of sustainable storage requires an interaction of sown species richness, a certain number of plant functional groups within plants and the presence of distinct functional groups. For the investigated ecosystem functions and properties neither a special plant species nor a special plant functional group was found to be of extraordinary importance compared to others. Instead, a high diversity encompassing all investigated diversity measures is essential. The fact that the vulnerability of e.g. carbon storage would be much higher if only one functional group or plant species would be the driver for storage should not be an argument to reduce conservation efforts because high diversity might be important for maintaining ecosystem functioning under changing conditions. Furthermore, the amplitude of ecosystem functioning is regulated by diversity and therefore high plant diversity can function as an insurance against future global changes.

As the work was carried out under specific experimental conditions, care has to be taken when results are transferred to the “real” world. In The Jena Experiment species composition was randomised from a definite species pool. Furthermore, it should be emphasised that The Jena Experiment was not intended to reproduce a particular sequence of species loss. Species identity was not a criterion for arrangement of mixtures. Instead, the number of species and the belonging to functional groups were most important. In the “real” word, plant diversity changes would not be randomised as it was carried out in the experimental design, but driven by other constraints e.g. fertiliser input. Nevertheless in order to study plant diversity per se, manipulation experiments on diversity are indispensable. An additional advantage of diversity experiments like The Jena Experiment

is that the experimental design account for gradual changes in soil properties like texture. Thereby, even slight changes between diversity levels became detectable.

Changes in ecosystem functioning caused by changed diversity can only be predicted at larger scales, if not only unidirectional causality approaches, in which diversity is either cause or effect, are applied. Feedbacks among diversity changes, ecosystem functioning and environmental factors must be addressed. Impact of aboveground diversity on belowground ecosystem functioning can only be the first step towards a greater understanding between diversity and ecosystem functioning. In the second step the feedback from belowground to aboveground should be investigated. Soil microbial communities, for example, were shown to be changed by aboveground diversity. As soil microbial communities represent a mechanistic link between plant diversity and ecosystem function, changes of soil microbial communities in turn might have an impact on aboveground productivity, diversity and composition, which should also be taken into account. The feedback of soil microogranisms an aboveground can be of special importance in nutrient poor ecosystems and should be investigated in the future.

The link between aboveground diversity and belowground diversity was corroborated and the outstanding importance of all three diversity measures (species richness, the number of functional groups as well as the presence of certain key functional groups) for ecosystem functioning was underlined. In order to mitigate climate change and ensure proper ecosystem functioning, efforts should be taken to reduce species extinction.

7 Summary

Soils are the most important terrestrial carbon sinks and have an enormous storage potential. Increased storage can be achieved by land use change e.g. from arable land to grassland. In addition to this, increasing plant diversity is known to be important for ecosystem functioning and an increase in soil organic carbon storage. The link between aboveground plant diversity and below ground diversity and the link to belowground processes are currently under discussion. Furthermore, the mechanisms by which aboveground diversity impacts storage as well as the sustainability of storage are still not well understood. Additional effort was taken to reveal the impact of plant diversity on the abundance and structure of soil microbial communities because soil organic carbon and nitrogen storage is assumed to be mainly mediated by soil microogranisms. Addressing these issues the thesis contributes to a better understanding of the impact of plant diversity on belowground ecosystem functioning.

For investigations the field site of The Jena Experiment was used. The Jena Experiment is a grassland diversity experiment, which was established in 2002 on former agricultural land, and aimed to reveal the impact of plant diversity on ecosystem functioning. The species pool comprises 60 species common to the Central European Molinio-Arrhenatheretum grasslands. The species were grouped into the following four functional groups: small herbs, tall herbs, grasses and legumes. The diversity gradient spanned from 1 (2, 4, 8, 16) to 60 species and from 1 (2, 3) to 4 functional groups. In addition, two arable land and meadow plots were integrated into the sampling design and used as a reference sites.

The relationship between plant diversity and soil organic carbon and nitrogen storage in soil profiles up to 100 cm depth was investigated. Between 2002 and 2007 organic carbon and nitrogen were stored in the top (0 - 20 cm depth) and sub (60 - 90 cm depth) soil. In the top soil the main drivers for storage were soil texture and plant diversity. Plant diversity probably changed soil microbial communities and concomitantly increased organic matter transformation and storage at higher diversity levels. While the root input was not important for storage in the top soil, it was found to significantly affect storage in the sub soil, where the system seemed to be input-limited. Due to the increased substrate use efficiency of microorganisms in the sub soil, the major part of the input was transformed and stored. Furthermore, the increased storage at higher plant diversity levels indicated that the soil microbial community was shifted towards one which was more

efficient in the use of ressources. It can be concluded that the amount of storage is depth dependent but that plant diversity increases storage independent from soil depth. Furthermore, abiotic factors were important for soil organic carbon and nitrogen storage but biotic factors controlled storage beyond these.

Density fractionation was used to separate soil into a light and a heavy fraction, representing different soil organic carbon and nitrogen pools. Generally, it is assumed that input is first stored in the labile light fraction and than decomposed and stored associated with minerals for long-term in the heavy fraction. At The Jena Experiment, both density fractions were sensitive to land use change and increased within two and four years after conversion. Furthermore, main organic carbon and nitrogen storage was found in the heavy fraction and there organic carbon storage was positively affected by plant diversity. Therefore, it can be concluded that increasing diversity increased sustainable sequestration of organic carbon. In addition, $\Delta^{14}C$ values of both density fractions increased with time and indicated that recent input was not exclusivly stored in the light fraction and from there transferred into the heavy fraction but mainly directly microbially transformed and stored into the heavy fraction.

In order to directly measure and characterise the soil microbial community of The Jena Experiment phospholipid fatty acids (PLFA), chloroform fumigation and physiological measures (substrate induced and basal respiration; only for samples of 2006) were used. In 2006, a sub-set of experimental sites was sampled to investigate the general impact of vegetation (bare ground plots vs. vegetation covered plots) and season (May vs. October). Vegetation as well as increasing duration of the vegetation period had a positive impact on the amount and physiological activity of soil microorganisms. The fact that the vegetation had a stronger impact on the soil microbial community composition than seasonal changes was probably due to the availability and quality of organic resources. Based on the result that vegetation had a strong impact on soil microbial community the effect of plant diversity (as number of species, number of functional groups and the presence of distinct plant functional groups) on the microbial community was analysed in 2007. Although the soil texture and moisture were found to have an impact on microbial abundance, increased plant diversity, namely the number of functional groups, lead to increased amounts of soil microbial biomass. For soil microbial biomass not only the amount of plant biomass input but also the quality and heterogeneity of input was important. The same result was found for the microbial composition and indicates that increasing diversity increases niche

complementarity of soil microorganisms. The soil microbial community composition was not altered by abiotic factors but it was found to be driven by biotic paramaters. Microbial community composition changed along the plant diversity gradient.

The results newly establish the strong impact of plant diversity on the soil organic carbon and nitrogen storage beyond abiotic controls. Soil microorganisms were found to play the central role for transformation of organic input and were the main drivers for a sustainable storage at higher diversity levels. As specific interactions between the aboveground and belowground compartments are vital for the ecosystem functioning, this should strengthen our efforts to reduce species extinction.

8 Zusammenfassung

Böden gehören zu den bedeutendsten terrestrischen Kohlenstoffsenken und verfügen über ein sehr großes Speicherpotential. Erhöhte Kohlenstoffspeicherung im Boden kann unter anderem durch einen Landnutzungswechsel von Acker- zu Grünland erreicht werden. Außerdem wird angenommen, dass höhere pflanzliche Diversität die Speicherung von Kohlenstoff im Boden erhöht und positiv auf das Funktionieren von Ökosystemen wirkt. Über die Verbindung zwischen oberirdischer und unterirdischer Diversität und den im Boden ablaufenden Prozesse wird derzeit in der Wissenschaft diskutiert. Auch die genauen Mechanismen, aufgrund derer erhöhte Diversität zu erhöhter Speicherung führt und die Frage der Nachhaltigkeit der Speicherung sind noch unklar. Es wird vermutet, dass vor allem Bodenmikroorganismen sehr wichtig für eine nachhaltige Speicherung des Eintrages im Boden sind. Deshalb wurde zusätzlich zur Kohlenstoff- und Stickstoffspeicherung im Boden der Einfluss von pflanzlicher Diversität auf die Menge von Bodenmikroorganismen und die Struktur der Mikroorganismengemeinschaften untersucht. Die vorliegende Arbeit soll dazu beitragen, den übergeordneten Einfluss von pflanzlicher Diversität auf Prozesse im Boden besser zu verstehen.

Als Untersuchungsraum wurde die Fläche des Jena Experimentes genutzt. Das Jena Experiment ist ein Grünlanddiversitätsexperiment, das 2002 auf einer ehemals ackerbaulich genutzten Fläche angelegt wurde und als Ziel hat, den Einfluss von pflanzlicher Diversität auf das Funktionieren von Ökosystemen zu untersuchen. Der Pflanzenartenpool beinhaltet 60 Arten des zentral europäischen Molinio-Arrhenatheretums. Die Pflanzenarten wurden in vier funktionelle Gruppen (kleine Kräuter, große Kräuter, Gräser und Leguminosen) unterteilt. Der Diversitätsgradient umfasst 1 (2, 4, 8, 16) bis 60 Arten und 1 (2, 3) bis 4 funktionelle Gruppen. Zusätzlich zum Jena Experiment wurden je zwei Äcker und Grünlandflächen beprobt und als Referenzflächen genutzt.

Der Einfluss der Artenzahl auf die Speicherung von Kohlenstoff und Stickstoff in Bodenprofilen bis zu 1 m Tiefe wurde untersucht. Von 2002 bis 2007 wurden Kohlenstoff und Stickstoff sowohl im Oberboden (0 - 20 cm) als auch im Unterboden (60 - 90 cm) gespeichert. Die wichtigsten Parameter für die Speicherung im Oberboden waren die Bodentextur und die pflanzliche Diversität. Dabei wird angenommen, dass die oberirdische Diversität die bodenbürtige Mikroorganismengemeinschaft beeinflusst und somit Auswirkungen auf die Umsetzung von organischem Material hat. Während Wurzeleintrag im Oberboden die Speicherung nicht wesentlich beeinflusst, waren Wurzeln im

eintraglimitierten Unterboden ein wichtiger Parameter. Aufgrund der im Unterboden erhöhten mikrobiellen Effizienz des Umsatzes, wurde der größte Teil des Eintrags gespeichert. Die tiefenunabhängig ansteigende Speicherung bei steigender Diversität lässt sich durch eine Verschiebung der Mikroorganismengemeinschaft hin zu Mikroorganismengemeinschaften mit höherer Effizienz erklären.

Dichtefraktionierungen wurden genutzt, um den Boden physikalisch in zwei Fraktionen (leichte Fraktion < 1.6 g cm^{-3} und schwere Fraktion > 1.6 g cm^{-3}) aufzutrennen, die Kohlenstoff- und Stickstoffpools mit unterschiedlichen Eigenschaften repräsentieren. Allgemein wird angenommen, dass der Eintrag zunächst als leichte Fraktion gespeichert wird und anschließend mikrobiell umgesetzt, langfristig in der schweren Fraktion gespeichert wird. Dort liegen Kohlenstoff und Stickstoff mineral-assoziiert vor. Beim Jena Experiment reagierten beide Dichtefraktionen empfindlich auf die Umstellung von Acker- zu Grünland und die Kohlenstoff und Stickstoffgehalte nahmen jeweils innerhalb von zwei und vier Jahren nach Landnutzungswechsel zu. Den größten Anteil an der Speicherung hat die schwere Fraktion. Dort wurde auch ein positiver Zusammenhang zwischen pflanzlicher Diversität und Kohlenstoffspeicherung gefunden. Es kann daher davon ausgegangen werden, dass die erhöhte Speicherung bei höherer Diversität langfristig ist. Die in beiden Fraktionen ansteigenden $\Delta^{14}C$ Werte deuten darauf hin, dass nicht nur in die leichte Fraktion rezentes Material eingetragen wurde, sondern dass ein großer Anteil des rezenten Eintrages direkt mikrobiell umgesetzt und in der schweren Fraktion gespeichert wurde.

Die Bodenmikroorganismen wurden mittels Phospholipidfettsäuren (PLFA) und Chloroformfumigation (CFE) sowie über physiologische Untersuchungen (Substratinduzierte- und Basalrespiration; nur in 2006) näher charakterisiert. In 2006 wurde auf einem Teil der Versuchsflächen der Einfluss von Vegetation (Vegetationsbedeckung vorhanden vs. nicht vorhanden) und Jahreszeit (Mai vs. Oktober) auf die Bodenmikroorganismen untersucht. Vegetationsbedeckung und zunehmende Länge der Vegetationsperiode wirken positiv auf die Menge an Mikroorganismen und auf deren physiologische Aktivität. Ausgehend von dem Ergebnis, dass die Vegetationsbedeckung aufgrund der erhöhten und verbesserten Futterversorgung einen größeren Einfluss auf die Bodenmikroorganismen hat als die Jahreszeit, wurden in 2007 sämtliche Flächen beprobt um den Einfluss von pflanzlicher Diversität (Artenzahl, Anzahl funktioneller Gruppen und An- bzw. Abwesenheit von bestimmten funktionellen Gruppen) zu untersuchen. Obwohl

Bodenart und -feuchte bestimmende Parameter für die Menge an Mikroorganismen waren, wurde darüber hinaus ein positiver Zusammenhang zwischen pflanzlichen Diversität, vor allem der Anzahl funktioneller Gruppen, und der Menge an Bodenmikroorganismen gefunden. Für die Menge und Zusammensetzung der Bodenmikroorganismengemeinschaft waren nicht nur der erhöhte pflanzliche Eintrag bei höherer Diversität, sondern vor allem auch die bessere Qualität und größere Heterogenität des Eintrages wichtig. Diese Ergebnisse deuten auf eine erhöhte Nischenkomplementarität bei höherer pflanzlicher Diversität hin. Abiotische Parameter hatten im Gegensatz zu biotischen Parametern keinen Einfluss auf die Zusammensetzung der Mikroorganismengemeinschaft.

Die Ergebnisse der Arbeit zeigen deutlich den großen Einfluss von pflanzlicher Diversität auf die Kohlenstoff und Stickstoffspeicherung im Boden, wobei der biotische Einfluss deutlich über den abiotischen hinaus reicht. Bodenmikroorganismen haben eine zentrale Rolle bei der Umsetzung und bei der nachhaltigen Speicherung von pflanzlichem Eintrag; vor allem bei erhöhter Diversität. Da die Interaktionen zwischen oberirdischer und unterirdischer Diversität essentiell sind für das Funktionieren von Ökosystem, sollte pflanzliche Diversität so weit es geht erhalten oder erhöht werden.

Author Contributions to Manuscripts from the Dissertation of Maike Habekost

Manuscript 1: Organic carbon and nitrogen storage in soil depth profiles of experimental grasslands with varying plant diversity. Submitted to Biogeochemistry

Authors: Maike Habekost, Sibylle Steinbeiss, Gerd Gleixner

- **Maike Habekost** is the first author and is responsible for writing this paper. She sampled the soil cores in 2007 and organised the samples preparation and measurements. She carried out statistical analyses and data interpretation.
- **Dr. Sibylle Steinbeiss** provided the soil data of 2002 and contributed with suggestions for data analysis. She made several suggestions to improve the manuscript.
- **PD Dr. Gerd Gleixner** contributed with suggestions for data interpretation and reviewed the manuscript.

Manuscript 2: Partitioning of organic carbon and nitrogen in soil density fractions of experimental grassland with varying plant diversity. Submitted to Soil Biology & Biochemistry

Authors: Maike Habekost, Sibylle Steinbeiss, Gerd Gleixner

- **Maike Habekost** is the first author and is responsible for writing this paper. She sampled the soil cores in 2006 and organised the samples preparation and measurements. She did the density fractionation of soil samples from 2002, 204 and 2006 and did the preparation of density fractions for chemical analysis. She carried out statistical analyses and data interpretation.
- **Dr. Sibylle Steinbeiss** provided the bulk soil data of 2002 and 2004 and contributed with suggestions for data interpretation. She reviewed the manuscript and made suggestions for improvement.
- **Holger Beßler** provided the standing root biomass data from 2004 and 2006. He further critically reviewed the manuscript.
- **Prof. Christof Engels** critically reviewed the manuscrip
- **PD Dr. Gerd Gleixner** is responsible for initiating this study and reviewed the manuscript.

Manuscript 3: Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biology & Biochemistry 40: 2588-2595

Authors: Maike Habekost, Nico Eisenhauer, Stefan Scheu, Sibylle Steinbeiss, Alexandra Weigelt, Gerd Gleixner

- **Maike Habekost** is the first author and is responsible for writing this paper. She did the soil sampling in May 2006. She performed the PLFA and CFE extraction and the AED-measurements. She formulated the hypothesis and analyzed and interpreted the data.
- **Dr. Nico Eisenhauer** measured substrate use efficiency and basal respiration. He actively contributed to improve the manuscript.
- **Prof. Stefan Scheu** reviewed and made several corrections on manuscript drafts.

- **Dr. Sibylle Steinbeiss** provided the bulk soil data of 2002. She reviewed the manuscript and corrected misspellings.
- **Dr. Alexandra Weigelt** provided aboveground biomass and C/N data. Further, she carried out a critical review of the mansuscript.
- **PD Dr. Gerd Gleixner** reviewed the manuscript and made several corrections of the manuscript.

Manuscript 4: Linking plant diversity and soil microbial community characteristics in an experimental grassland approach. Submitted to Journal of Ecology

Authors: Maike Habekost, Cornelius Middelhoff, Yvonne Oelmann, Christiane Roscher, Ernst-Detlef Schulze, Sibylle Steinbeiss, Alexandra Weigelt, Wolfgang Weisser, Wolfgang Wilcke, Gerd Gleixner

- **Maike Habekost** is the first author and is responsible for writing this paper. She did the soil sampling in May 2007. She performed the PLFA and CFE extraction and the AED-measurements. She carried out the literature review and formulated the hypothesis. Further, she did the statistical analyses and interpretation of the data.
- **Cornelius Middelhoff** provided the aboveground biomass data and reviewed the manuscript.
- **Dr. Yvonne Oelmann** provided the C/N data of aboveground biomass and reviewed the manuscript.
- **Dr. Chritiane Roscher** provided the species richness of succession plots and reviewed and improved the manuscript.
- **Prof. Ernst-Detlef Schulze** provided the aboveground biomass data and reviewed the manuscript.
- **Dr. Sibylle Steinbeiss** provided the bulk soil data of 2002. She reviewed the manuscript and corrected misspellings.
- **Dr. Alexandra Weigelt** provided aboveground biomass and C/N data. Further, she carried out a critical review of the mansuscript.
- **Prof. Dr. Wolfgang Weisser** provided the aboveground biomass data and reviewed the manuscript.
- **Prof. Dr. Wolfgang Wilcke** provided the C/N data of aboveground biomass and reviewed the manuscript.
- **PD Dr. Gerd Gleixner** reviewed the manuscript and made several corrections of the manuscript.

Contributions of Maike Habekost to manuscripts written by other authors

Plant biodiversity positively affects short-term soil carbon storage in experimental grasslands. Global change Biology, 2008, doi: 10.1111/J1365-2486.2008.01637.x
Authors: Sibylle Steinbeiss, Holger Beßler, Christof Engels, Vicky M. Temperton, Nina Buchmann, Christiane Roscher, Yvonne Kreutziger, Jussi Baade, Maike Habekost, Gerd Gleixner
Contributions of Maike Habekost: She did the soil sampling in 2006 and organized the sample preparation and measurements. Further, she reviewed the manscript.

First direct indications of a diversity effect on plant source water. Submitted to Ecology
Authors: Romain Barnard, Francesco de Bello, Anna Gilgen, Maike Habekost, Gerd Gleixner, Alexandra Weigelt, Rolf Siegwolf, Michael Scherer-Lorenzen, Nina Buchmann
Contributions of Maike Habekost: Maike Habekost organicsed the soil water sampling during May and June 2006. She organized measuremnts of $\delta^{18}O$ and δD analysis and wrote the according part in the material and methods section of the manuscript. She critically reviewed the masnucript.

Plant functional diversity rather than species richness drives soil functions in experimental grasslands. Submitted to Ecology Letters
Authors: Xavier Le Roux, Bernhard Schmid, Romain Barnard, Annie Clays-Josserand, Joana Salles, Nadine Guillaumaud, Maike Habekost, Pascal Niklaus, Yvonne Oelmann, Stephan Rosenkranz, Michael Scherer-Lorenzen, Sibylle Steinbeiss, Alexandra Weigelt, Franck Poly
Contributions of Maike Habekost: Maike Habekost sampled soil water in 2006 and provided the according data of dissolved organic carbon content. She reviewed the manuscript and made suggestions for improvement.

Plant diversity effects on soil micororganisms support the single hypothesis. Submitted to Ecology
Authors: Nico Eisenhauer, Alexandru Milcu, Maike Habekost, Sibylle Steinbeiß, Holger Beßler, Christoph Scherber, Alexandra Weigelt, Alexander Sabais, Stephan Partsch, Christof Engels, Gerd Gleixner, Wolfgang W. Weisser, Stefan Scheu
Contributions of Maike Habekost: Maike Habekost organized the sampling in May 2007 and prepared the samples for analyses. She provided soil water data (dissolved organic carbon and inorganic carbon) from spring 2007. Further, she critically reviewed the manuscript and made suggestions for improvement.

Curriculum vitae

Name:	Maike Habekost
Date of birth:	11.10.1979
Place of birth:	Hannover, Germany
Marital status:	unmarried

School education

1986 – 1990	Primary school in Lauenau/Lower Saxony
1990 – 1992	Middle school in Rodenberg/ Lower Saxony
1992 – 1999	Grammar school in Bad Nenndorf/Lower Saxony
1999	General qualification for university entrance (*Abitur*)

Studies

1999 – 2004	Studies of geoecology with focus on soil science and landscape ecology at the Technical University of Karlsruhe/Baden-Wuerttemberg Diploma thesis "Anordnung eins gemischten Pflanzenbestandes aus Wildpflanzen zur Auswertung von Herbizideffekten"
2004 – 2005	Further studies (*Aufbaustudiengang*) „Fluid mechanisms in hydraulic engineering and environmental protection" at the Technical University of Karlsruhe/Baden-Wuerttemberg
Since 2005	PhD student at the Max Planck Institute for Biogeoscience in Jena/Thuringia, funding by the Max-Planck-Gesellschaft and the Deutsche Forschungsgemeinschaft within the framework of The Jena Experiment "Influence of plant diversity on soil organic carbon storage and microbial transformation of organic carbon in soils"

Other activities

08/2001 – 09/2001	Chemical analysis of offshore seawater, Terra mare, Wilhelmshaven
08/2003 – 09/2003	Renaturation of intra-urban streams, Regiowasser and University of Freiburg/Baden-Wuerttemberg
07/2004 – 08/2004	Soil mechanics and ground water, Pelagonija ADG-DGR Geotehnika, Skopje/Mazedonia
09/2004 – 06/2005	Student assistant at the Federal Waterways Engineering and Research Institute, Karlsruhe/Baden-Wuerttemberg
11/2004 – 06/2005	Student assistant at the Institute for Water and River Basin Management, Technical University of Karlsruhe/Baden-Wuerttemberg

Jena, 06.11.2008 Maike Habekost

Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Jena, den 06.11.2008 Maike Habekost

Acknowledgments

This work could not have been done without the help of numerous people.
Many thanks go to:

- the Max Planck Society and the Deutsche Forschungsgemeinschaft for funding my thesis in the framework of The Jena Experiment.
- **PD Dr. Gerd Gleixner** at the Max Planck Institute for Biogeochemistry, who greatly supported my work. He gave me freedom to find my own scientific way, inspired me with his innovative ideas and found always time for helpful and motivating discussions.
- **Prof. Dr. Roland Mäusbacher** at the Friedrich Schiller University Jena and **Prof. Dr. Stefan Scheu** at the Georg August University Göttingen for taking the part of the co-supervisor of my thesis and their ongoing interest in my work.
- **Sibylle Steinbeiss** for helpful and promptly comments on further versions of the manuscript and support in statistical analysis.
- **Jens Kirschstein** and numerous student helpers for their aid during serveral soil and water sampling campaigns and sample preparation.
- the "RoMa" team (**Ines Hilke, Birgit Fröhlich and Sandra Matthäi**) of the Max Planck Institute for Biogeochemistry for the performance of chemical analysis and a fruitful collaboration and **Heike Geilmann** for patient isotope measurements of hundreds of samples.
- **Steffen Rühlow** for help and advice during the measurements of my PLFA samples and **Iris Kuhlmann** for motivation during the density fractionation of my soil samples.
- **Agnes Fastnacht, Dr. Alexandra Weigelt** and the gardener of The Jena Experiment for help and advice in all questions concerning sowing, planting, weeding and harvesting of plants.
- my collegues and friends (**Tanja Reinhold, Anne-Gret Seifert, Elena Hettmann and Antje Gude**) who made our institute a happy place to work in.
- all the other **PhDs from the Jena Experiment** for fruitfull discussions and assistance at the weeding campaigns
- **Christian Jones** and **Veronika Jäger** for critically reading the manuscript and finding of a lot of spelling mistakes
- **Daniel**, my twin sister, **Astrid**, and my **parents**.