PLANT AND SOIL RELATED EFFECTS ON THE SOIL MICROBIAL COMMUNITY COMPOSITION AND SOIL MICROBIAL CARBON DYNAMICS

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FIGURES

3.1. Mean (n=6 ± standard error) total phospholipid fatty acids (PLFA) concentration and neutral lipid fatty acid (NLFA) concentration in soils at two different soil depths (0-5 cm and 5-10 cm) as affected by low and high plant diversity. Stars in the first row (closer to bars) indicate significant differences between plant diversity levels; stars in the second row indicate significant differences between soil depths (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$). **29**

3.2. Mean (n=6 ± standard error) $\delta^{13}C$ values (‰) of individual phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) in top soils (0-5 cm depth) with low and high plant diversity. Fatty acids are grouped into Gram positive (G+) bacteria, actinobacteria, saprotrophic fungi, Gram negative (G-) bacteria (cyclo), Gram negative (G-) bacteria and arbuscular mycorrhizal fungi (AMF). Arrows pointing up represent an increase of $\delta^{13}C$ values with plant diversity; whereas arrows pointing down indicate an opposite trend. Stars and plus signs indicate significant and marginally significant differences of $\delta^{13}C$ values between plant diversity levels (*: $p \leq 0.05$; +: $p \leq 0.1$), respectively. **30**

3.3. Average δ13C values (n=6 ± standard error) of total phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) in top (0-5 cm) and deeper soils (5-10 cm) from communities with low and high plant diversity. **31**

3.4. Linear correlations between: a) $\delta^{13}C$ values of Gram negative (G-) bacterial markers and root biomass; b) $\delta^{13}C$ values Gram positive (G+) bacterial markers and $\delta^{13}C$ values of soil organic carbon ($\delta^{13}C$ -SOC). Filled and empty circles represent high and low plant diversity, respectively. Significant differences are indicated by p values lower than 0.05. **32**

3.5. Mean (n=6 ± standard error) $\delta^{13}C$ values (‰) of root sugars (sucrose and raffinose-family) and root material in top soils (0-5 cm) with low and high plant diversity. Stars indicate significant differences of $\delta^{13}C$ values between plant diversity levels (*: $p \leq 0.05$). **39**

4.1. Microbial biomass in (a) soils with a gradient in plant diversity (plant species richness) and (b) soils with a gradient of plant functional groups. **47**

4.2. Microbial biomass in soils with gradient different plant functional groups: (a) number of grasses; (b) number of small herbs; (c) number of tall herbs and (d) number of legumes. **47**

4.3. a) Bacterial diversity measured from Simpson index of T-RFLP and b) PLFA evenness measured from Simpson's index of diversity. Both a) and b) correspond to soils with a gradient in plant diversity. **48**

5.1. Mean (n=3 ± standard error) microbial biomass in sandy and clayey soils sown with C3 and C4 plants in different seasons. Stars indicate significant differences in mean total microbial biomass between seasons and soil type (*: p≤ 0.05; ***: p≤ 0.001). **55**

5.2. Redundancy analysis (RDA) of the mean relative proportions (mol%) of individual phospholipid fatty acids (PLFAs) in sandy and clayey soils sown with C3 and C4 plants. Root biomass (RB) and soil organic carbon (SOC) were set as explanatory variables to explain the variance of individual PLFAs. Stars indicate significant differences between soil types (*: p≤ 0.05; **: p≤ 0.01). **56**

5.3. Redundancy analysis (RDA) of mean (n= 3 ± standard error) plant-derived C in individual phospholipid fatty acids (PLFAs) in sandy and clayey soils. Root biomass (RB) was set as explanatory variables to explain the variance of plant-derived C in individual PLFAs. Stars indicate significant differences between soil types (*: p≤ 0.05). **58**

5.4. Mean (n= 3 ± standard error) plant-derived C in different microbial groups (Gram positive bacteria (G+), actinobacteria (Actino), cyclic Gram negative bacteria (G- (cy)), Gram negative bacteria (G-) and saprotrophic fungi (SF)) in a) non-growing and b) growing season, in sandy (white bars) and clayey (gray bars) soils. Stars indicate significant differences between seasons (*: p≤ 0.05). Letters indicate significant differences (p≤ 0.05) between plant-derived C in different microbial groups. **59**

TABLES

2.1. Mean (n= 3 ± standard deviation) values of soil characteristics and root biomass in sandy and clayey soils from the C3-C4 vegetation change experiment. **19**

3.1. ANOVA (analyses of variance) results from the differences in a) total PLFA concentration; b) NLFA concentration; c) weighted means (δ^{13}C values weighted by concentration) of total PLFA and d) weighted means of NLFA related to plant diversity. Stars indicate significant differences of δ^{13}C values between plant diversity levels and soil layers (**:p≤ 0.01; *: p≤ 0.05; $^{+}$: p≤ 0.1). **27**

3.2. Results of automated selection of three best models ('dredge' function in 'MuMIn' package). Possible explanatory variables included were: soil moisture, soil organic carbon (SOC), canopy leaf nitrogen (N.area), root biomass, root length per volume (root length), specific root length, plant diversity (Div), δ^{13}C values of soil organic carbon (δ^{13}C-SOC), root material (δ^{13}C-Root) and root sugars (δ^{13}C-Sugars). **33**

3.3. Results of the best ANOVA model (see Table 2) for each microbial group. Stars indicate significant differences of δ^{13}C values between plant diversity levels (***: p≤ 0.001; **: p≤ 0.01; *: p≤ 0.05; $^{+}$: p≤ 0.1) **35**

4.1. ANOVA (Analysis of variance) and ANCOVA (analysis of covariance) results from the changes in microbial biomass, root-associated and soil-related microorganisms, bacterial diversity and PLFA evenness related to plant diversity measures: plant species richness (PSR); functional group (FG); grasses, small and tall herbs and legumes. Root biomass, leaf area index (LAI), soil moisture and soil organic carbon (SOC) were set as covariates to test for their influence on the changes in microbial biomass, root-associated and soil-related microorganisms, bacterial diversity and PLFA evenness. Bold numbers represent significant differences (P values ≤ 0.05). **45**

5.1 Results of the ANOVA (analysis of variance) from the (a) total microbial biomass and PERMANOVA (Permutational Multivariate Analysis of Variance Using Distance Matrices) from (b) microbial community composition and (c) the δ^{13}C values of individual PLFA obtained from both the sandy and clayey soils of the C3-C4 vegetation change experiment. Stars indicate significant differences between season, vegetation type or soil type (*: p≤ 0.05; **: p≤ 0.01; ***: p≤ 0.001). **57**

CONTENTS

Acknowledgements **I**

Figures **III**

Tables **V**

I. Introduction **1**

1.1 The role of soil microbial communities in the carbon cycle in soils 1

1.2 Plant-microbe interactions and their influence on soil carbon dynamics 2

1.3 Functions and classification of soil microorganisms 2

1.3.1 Factors affecting the functions of microbial communities in soils 3

1.3.1.1 Biotic factors - Implications of plant diversity on the microbial C cycling 3

1.3.1.2 Abiotic factors 4

1.4 Analysis of soil microorganisms - Lipid biomarkers 5

1.4.1 Tracing C sources through isotopic compound specific analyses 6

1.5 Objectives 7

1.6 Thesis organization 7

II. Materials and Methods **9**

2.1 The Jena-Ecotron Experiment 9

2.1.1 Site description and experimental design 9

2.1.1.1 Plant communities originating from the Jena experiment 9

2.1.1.2 Setting up the Jena-Ecotron experiment 9

2.1.2 Soil sampling and preparation 10

2.1.3 PLFA and NLFA extraction and analyses 10

2.1.3.1 PLFA and NLFA compound specific δ^{13}C measurements 11

2.1.4 Explanatory variables 12

2.1.4.1 Leaf related covariates: canopy leaf nitrogen, shoot biomass and specific leaf area 12

2.1.4.2 Root related covariates: root biomass, root length and δ^{13}C values from roots and root sugars 13

2.1.4.3 Soil related covariates: soil moisture, SOC and δ^{13}C-SOC 14

2.1.5 Statistical analyses 14

2.2 The Jena Experiment 15

2.2.1 Site description and experimental design 15

2.2.2 Soil sampling and preparation 16
2.2.3 PLFA extraction and analysis 16
2.2.4 Genetic diversity of soil bacteria 17
2.2.5 Simpson's diversity index and Simpson's evenness 17
2.2.6 Explanatory variables 17
2.2.6.1 Root biomass and leaf area index (LAI) 17
2.2.6.2 Soil moisture, SOC 18
2.2.7 Statistical analyses 18
2.3 C3-C4 vegetation change experiment 18
2.3.1 Site description and experimental design 18
2.3.2 Soil sampling and preparation 19
2.3.3 PLFA extraction, analysis and compound specific measurement 20
2.3.3.1 Assessing the C origin in PLFA 21
2.3.4 Explanatory variables 21
2.3.5 Statistical analysis 22
III. Plant diversity generates enhanced soil microbial access to recently photosynthesized carbon in the rhizosphere 23
Abstract 23
3.1 Introduction 24
3.2 Results 26
3.2.1 Total concentration of PLFA and NLFA 26
3.2.2 Compound specific δ^{13}C of PLFA and NLFA 29
3.2.3 Response of plant and soil related covariates to plant diversity 31
3.2.4 Plant and soil related impacts on microbial group specific δ^{13}C values 31
3.3 Discussion 35
3.3.1 Effects of plant diversity and soil depth on the PLFA and NLFA concentrations 36
3.3.2 Response of plant and soil related covariates to plant diversity 36
3.3.3 Plant and soil related impacts on ^{13}C uptake into soil microbial groups 37
3.4 Conclusion 40
IV. The positive plant diversity effect on soil microbial community composition and soil bacterial diversity is driven by plant- and soil-related factors 41
Abstract 41
4.1 Introduction 42

4.2 Results 44
4.2.1 Influence of plant diversity on plant and soil-related covariates 44
4.2.2 Soil microbial biomass 44
4.2.3 Plant diversity effect on different soil microbial groups 44
4.2.4 Bacterial diversity and PLFA evenness 48
4.3 Discussion 48
4.3.1 Plant diversity effect on plant and soil-related covariates 48
4.3.2 Soil microbial biomass 49
4.3.3 Plant diversity effect soil microbial groups 49
4.3.4 Plant diversity effect on bacterial diversity and PLFA evenness 50
4.4 Conclusion 51
V. The soil microbial community and its carbon uptake are more affected by soil type and season than by vegetation type in a vegetation change experiment (C3 and C4 plants) 52
Abstract 52
5.1 Introduction 53
5.2 Results 54
5.2.1 Microbial biomass and microbial community composition 54
5.2.2 Isotopic signature of microbial markers and uptake of plant-derived C 56
5.3 Discussion 59
5.3.1 The effect of the vegetation change 59
5.3.2 Effects of soil type on the soil microbial community 60
5.3.3 Seasonal differences of the soil microbial community 60
5.3.4 Plant-derived C in different soil microbial groups 61
5.4 Conclusions 61
VI. Synthesis 63
6.1 Plant diversity effects on soil microorganisms 63
6.2 The effects of soil characteristics and vegetation properties on soil microbial communities 64
6.3 General conclusions 65
6.4 Perspectives for future research 66
Summary 68
Zusammenfassung 71
References 75

Selbständigkeitserklärung **92**
Curriculum vitae **93**
Scientific publications **94**
Scientific presentations **95**
Appendix **96**

CHAPTER I

INTRODUCTION

1.1 The role of soil microbial communities in the carbon cycle in soils

Carbon (C) is the structural component of all forms of life on our planet; it is present from the smallest organism to the widest ecosystem. The amount of C on Earth is ca. 50000 Pg (Lal, 2004), however, the importance of this element does not fall solely on its abundance; C is at a pint point position because it stands centrally in processes that are triggering rapid changes globally (i.e. global climate change). Rising atmospheric CO_2 concentrations, among other greenhouse gases are inducing temperature increases that might have devastating consequences for life as we know it (Mitchell et al., 1995; Lal, 2003; IPCC, 2007). Soils are important CO_2 sinks (Batjes, 1998; Schmidt et al., 2011; Batjes, 2014) that help mitigating this "greenhouse effect"; however several human activities (e.g. land-use change for agricultural practices and plant diversity loss) are contributing to change the role of soils in nature from C sinks to C sources (Trumbore et al., 1995; Chapin Iii et al., 2000; Nave et al., 2010; Handa et al., 2014). Currently, the soil C (2500 Pg C) is the third largest C pool on Earth (Batjes, 1996; Falkowski et al., 2000; Pacala and Socolow, 2004), it surpasses more than four times the amount of the biotic C pool (560 Pg (Batjes, 1996; Falkowski et al., 2000; Pacala and Socolow, 2004)), and by over three times that of the atmospheric C pool (760 Pg (Batjes, 1996; Falkowski et al., 2000; Pacala and Socolow, 2004)). Soil C is comprised of soil inorganic C (SIC, 38 %) and soil organic C (SOC, 62 %) (Batjes, 1996). Generally, SOC has a higher reactivity and slower turnover time (Schnitzer, 1991) than SIC; therefore numerous efforts have been directed to understanding SOC dynamics and how anthropogenically-induced changes, such as biodiversity loss might alter the balance of the C cycling in soils and globally (Tilman et al., 2001; Fornara and Tilman, 2008; Steinbeiss et al., 2008a; Marquard et al., 2009; Handa et al., 2014).

Soil microorganisms are pivotal in the cycling of C in the belowground ecosystem (Gleixner, 2013). Most of the C that enters the soil is processed by soil microorganisms, CO_2 is an important byproduct of the microbial C decomposition and is released back to the atmosphere through microbial respiration; another fraction of C is stored by the microbial community as biomass and energy. Following the death of the soil microbes, their remains can be either reprocessed by other members of the soil microbial community or stabilized and stored as soil organic matter (SOM) (Gleixner et al., 2002). The interactions between plants

and soil microorganisms play a very relevant role in controlling the exchange of C between above and belowground, as well as the microbial processing of C belowground.

1.2 Plant-microbe interactions and their influence on soil carbon dynamics

Most soil microorganisms are heterotrophic and therefore depend completely on autotrophic organisms for the supply of resources belowground. Plants are the main providers of C resources to the soil microbial community (Wardle, 2002); they assimilate CO_2 directly from the atmosphere through photosynthesis (Trumbore, 1997) and deliver it to soil microorganisms through rapid rhizodeposition of exudates or through plant litter deposition (Gleixner, 2013). In turn, soil microorganisms mineralize SOM and release limiting nutrients (Porazinska et al., 2003) otherwise non-available for plants. These processes provide soil microorganisms and plants with the needed resources and at the same time fueled the (re)cycling of C and other important elements between and within environmental compartments. There is a general good understanding of the overall interaction between plans and soil microorganisms; however until now it remains uncertain how changes in plant characteristics, such as increase/decrease of plant diversity and changes in vegetation abundance and vegetation type might impact microbial functions that mediate key ecosystem processes in soils.

1.3 Functions and classification of soil microorganisms

Soil microorganisms are commonly classified by the functions they perform, these functions are primarily related to the type of C substrate they are better adapted to assimilate; thus, there are soil microorganisms that have a high affinity for readily available C resources, such as recently photosynthesized rhizodeposits (i.e. root-associated microorganisms (Denef et al., 2009)); another part of the microbial community is more suited to decompose more complex substances, such as those present in stabilized SOM (Kramer and Gleixner, 2008; Bahn et al., 2013), and others feed indistinctively on recently delivered plant C (Treonis et al., 2004) or in already stabilized sources of C (Garcia-Pausas and Paterson, 2011). Additional to the microbial classification by functions, soil microorganisms are often differentiated by their morphology into bacterial and fungal groups. Bacterial groups are sub-classified based on the Gram staining method, depending on the chemical properties of their cell walls different bacterial groups react by retaining one of two different dyes (Gram, 1884). Gram positive (G+) bacteria, which have a single cell membrane reacts to crystal violet dye and Gram negative (G-) bacteria (double cell membrane) reacts to safranin or fuchsine dyes (red-pinkish

coloring (Holt et al., 1994; Madigan et al., 2004). Moreover, soil fungal groups might be classified depending on their route of colonization (Bonfante and Anca, 2009) into ectomycorrhizal and endomycorrhizal (i.e. arbuscular mycorrhizal fungi (AMF)), the latter are very relevant in terms of rapid nutrient cycling between plants and soils, because they colonize most terrestrial plants and actively trade C for nutrients (Drigo et al., 2010). Another way of classifying fungi is by their preferred C substrate into saprotrophic and biotrophic fungi (Crowther et al., 2012). Undoubtedly, soil microbial communities are mediators of very important processes in the belowground system; however soil microorganisms are very sensitive to changes in their habitat. Therefore, in order to understand how changes in the ecosystem might trigger changes in the processes that are controlled by soil microbes it is of key importance to identify factors that potentially influence the performance of microbial communities in soils.

1.3.1 Factors affecting the functions of microbial communities in soils

1.3.1.1 Biotic factors - Implications of plant diversity on the microbial C cycling

Clearly, biotic factors dominate the functions of the microbial communities in soils mainly because they are intrinsically related to the availability, quality and diversity of C resources (Thoms et al., 2010; Thoms and Gleixner, 2013; Lange et al., 2014; Lange et al., 2015; Scheibe et al., 2015). Some of the most relevant biotic factors that have been recognized to induce changes in the microbial community composition and consequently in the processes that microorganisms mediate in soils include plant biomass production (above and belowground), litter quality, seasonal variability of plant abundance (and litter production), root exudation, plant identity and plant diversity (Angers and Caron, 1998; Bais et al., 2006; Bezemer et al., 2006).

Loss of biodiversity is one of the most relevant triggers of changes to the soil C cycle (Tilman et al., 2001; Hooper et al., 2005; Fornara and Tilman, 2008; Steinbeiss et al., 2008a; Marquard et al., 2009); therefore especial emphasis has been put into understanding how plant diversity affects the cycling of C in the belowground system, and the consequences of accelerated plant diversity loss. In terms of soil microbial C cycling, high plant diversity influences directly and indirectly soil microorganisms. High plant diversity increases primary production above and belowground (Cardinale et al., 2006; Ravenek et al., 2014), it has been assumed that greater photosynthetic assimilation of CO_2 in highly diverse ecosystems would induce an increased supply of recently photosynthesized C to the soil system through higher root exudation (Chung et al., 2007; Chung et al., 2009), however until now the evidence to

support this assumption is still scarce, and alternative mechanisms have not yet been described. Moreover, plant diversity influences soil microbial functions through the input of more diverse C resources (Hooper et al., 2000). Interestingly, the increased resource availability and resource heterogeneity in highly diverse plant mixtures results in higher soil microbial biomass, higher soil microbial diversity (Bell et al., 2005) and greater soil microbial activity, which ultimately contribute significantly to the formation and storage of SOC (Lange et al., 2015). A great progress has been made in view to understanding the mechanisms that control the plant diversity effect on soil microbial dynamics; however it is still not completely resolved how different factors potentially affect these processes and how changes to plant diversity might trigger positive or negative responses that could impact the whole functioning of the ecosystem.

1.3.1.2 Abiotic factors

Several abiotic factors (i.e. soil properties) might influence the microbial cycling of elements in soils. Soil moisture, pH, soil texture and seasonal variations of temperature and vegetation cover are among the abiotic factors that affect more strongly the microbial community composition in soils (Grayston et al., 2001; Johnson et al., 2003; Medeiros et al., 2006; Habekost et al., 2008b; Berg and Steinberger, 2010; Thoms et al., 2010; Cao et al., 2011; Epron et al., 2011; de Vries et al., 2012; Lange et al., 2014). Greater soil moisture influences positively the microbial biomass and microbial activity in soils (Lange et al., 2014) mainly through dissolution and motility of C resources (Davidson et al., 2000) belowground. Changes in soil pH have been shown to change the proportions of G+ bacteria, G- bacteria and fungi in several ecosystems (Bååth and Anderson, 2003; Kaur et al., 2005; Thoms et al., 2010; Thoms and Gleixner, 2013), thereby influencing soil microbial functioning. Furthermore, the effects of some abiotic factors are closely related to plant characteristics. The effect of soil texture has been related to root distribution, the finer mineralogy of smaller soil sizes (i.e. clayey soils) might sustain a better root net (Merckx et al., 1985), thus providing better habitat conditions for soil microbes, especially root-associated microorganisms. Seasonal responses have been linked to either temperature variability or to changes in plant abundance among seasons. Intuitively, when environmental conditions are better for plant development (e.g. in summer in temperate regions), the availability of C resources delivered to the soil microbial communities is greater (Grayston et al., 2001; Habekost et al., 2008b; Thoms and Gleixner, 2013) and this in turn activates all microbial functions belowground. The effects of individual factors on soil microbial communities have

been extensively documented. However, little is known about the interactions between different factors and the relative importance of their effects on the microbial community composition, microbial biomass and the processes microorganisms mediate in soils.

1.4 Analysis of soil microorganisms - Lipid biomarkers

Biochemical analyses of soils provide are a useful tool to describe soil microbial community composition and microbial functions in soils. Lipid biomarkers, namely phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) are widely analyzed to discern microbial community composition and to quantify microbial biomass in soils, which provide valuable insights to the soils microbial dynamics (Frostegard et al., 1991; Bossio and Scow, 1998; Bossio et al., 1998; Zelles, 1999a; Potthoff et al., 2006; Thoms et al., 2010; Chowdhury and Dick, 2013; Kramer et al., 2013; Thoms and Gleixner, 2013; Lange et al., 2014; Ng et al., 2014; Scheibe et al., 2015; Watzinger, 2015). PLFA are structural components in all living organisms, they are ideal indicators of living microbial biomass because they rapidly decompose after cell death (White et al., 1979; Zelles et al., 1992). Furthermore, differences in the chemical structure (saturation, chain length, branching) of individual PLFA markers and their relative abundances relate differently to distinct microbial groups (Steer and Harris, 2000; Leckie, 2005); in general saturated compounds are present in all microorganisms; branched saturated PLFA (iso and anteiso saturation) are specific for G+ bacteria (Frostegård and Bååth, 1996); actinobacteria, which are a subgroup of G+ bacteria, have distinctively higher proportions of methylated PLFAs (Kroppenstedt, 1985); monounsaturated compounds are distinctive for G- bacteria (Frostegård and Bååth, 1996; Zelles, 1997) and polyunsaturated PLFA markers represent fungi and higher organisms (Zelles, 1997). Thus, PLFA analysis serves as a fingerprint analysis for determination of soil microbial community composition. However, the specificity of PLFA depends greatly on the type of habitat and environmental conditions (Appendix A1.1), therefore the assignment of PLFA markers to different microbial groups must be done with caution. NLFA are energy storage compounds in fungi (Olsson et al., 1995; Olsson and Johnson, 2005), since most bacteria do not store energy in the form of lipids, NLFA are good representatives of fungal groups in the soil microbial community. Special emphasis has been given to the study of AMF through NLFA analysis due to the key role of AMF in the C cycling belowground (Drigo et al., 2010).

1.4.1 Tracing C sources through isotopic compound specific analyses

In order to discern the sources of C uptake by soil microorganisms and to determine the functions of different microbial groups in soils, labelling experiments involving isotopic analyses of stable C (δ^{13}C) are performed. For instance, labelling of all photosynthetic products through the continuous application of enriched $^{13}CO_2$ allows tracing the C into microbial biomarkers (e.g. PLFA and NLFA) and identifying soil microorganisms that directly metabolize recently photosynthesized C (Butler et al., 2003; Treonis et al., 2004; Denef et al., 2007; Lu et al., 2007; Staddon et al., 2014). Additionally, the correlation of isotopic C values from other compartments, such as SOC with those obtained from PLFA and/or NLFA compound specific analyses might be related to additional C resource preferences of specific parts of the soil microbial community (Elfstrand et al., 2008; Kramer and Gleixner, 2008; Bird et al., 2011).

1.5 Objectives

The general aim of this work was to identify biotic and abiotic factors that potentially alter the dynamics of the microbial communities in soils and consequently the cycling of elements between ecosystem compartments, to that end we focused in accomplishing the following specific objectives:

1. Determine the mechanisms behind the plant diversity effect on the C transfer from plants to soil microorganisms
2. Identify how plant diversity influences the shaping of soil microbial communities and the bacterial diversity in soils
3. Study the effect of vegetation type, seasonal changes and soil type on the soil microbial community composition and the microbial uptake of plant-derived C

1.6 Thesis organization

In order to identify the mechanisms behind the plant diversity effect on the increased C transfer belowground, we investigated changes in plant-derived C uptake in soil microorganisms (Chapter 3). We applied a continuous $^{13}CO_2$ labelling to ecosystem monoliths with two distinct plant diversity levels (4 and 16 plant species mixtures). The C flow form plants to soil microorganisms was determined by analyzing the δ^{13}C enrichment of individual phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) that were designated to different bacterial and fungal groups. Additionally, meaningful plant and soil related covariates were used to identify possible mechanisms explaining the plant diversity effect on the C transfer between above and belowground.

Chapter 4 studied the influence of plant diversity on different microbial groups, bacterial diversity and phospholipid fatty acids (PLFA) evenness. We collected 82 soil samples in the log-term plant diversity experiment "The Jena Experiment". Total microbial biomass and different microbial groups was tested using PLFA analyses. Bacterial diversity was measured using terminal-restriction fragment length polymorphism (T-RFLP). Root biomass, leaf area index (LAI), soil moisture and soil organic carbon (SOC) were used as possible explanatory covariates of the plant diversity effect on soil microbial communities.

In Chapter 5 we investigated the relative importance of the combined effects of soil type, vegetation type and seasonal changes on the soil microbial community composition and the soil microbial C uptake. We analyzed soil samples from a vegetation change experiment (C3 vegetation was replaced by C4 plants) that comprised sandy and clayey soils. Samples were collected in the non-growing and growing seasons in 2012. Phospholipid fatty acids

(PLFA) and compound specific δ^{13}C-PLFA analyses were used to determine microbial community composition and the flow of C from plants to the soil microbial community, respectively. Analysis of variance and redundancy analysis were used to identify the effects of the soil type, vegetation type and seasonal changes in the microbial community composition and the microbial C uptake.

Finally, in Chapter 6 we synthesized the main results and provide suggestions for future research that could further strengthen our knowledge on soil microbial communities and their functions in soils.

CHAPTER II

MATERIALS AND METHODS

This study is comprised by three subprojects that were conducted in distinct sites: The Jena-Ecotron Experiment (Chapter 3), The Jena Experiment (Chapter 4) and the C3-C4 vegetation change experiment (Chapter 5). Detailed site and procedural descriptions are found in the following sections (2.1 – 2.3).

2.1 The Jena-Ecotron Experiment

2.1.1 Site description and experimental design

2.1.1.1 Plant communities originating from the Jena experiment

The Jena Experiment, is a long-term grassland biodiversity experiment located in Jena Germany (50°55' N, 11°35' E, 130 m a.s.l.) established in 2002 on a former agricultural land. The field site comprises 82 plots (20 × 20 m) with a diversity gradient that ranged from one to 60 plant species and from one to four functional groups (grasses, small herbs, tall herbs and legumes). The mean sand content in the field varied from 5.3 to 45%; clay content from 14.4 to 26.3 % and silt content between 40.6 and 73.1 %, pH values were in the range of 7.1 to 8.4 (Roscher et al., 2004). The initial values of SOC and soil N were between 1.5 % and 2.8 % and from 0.2 % to 0.3 %, respectively. For this experiment, 12 plots were selected according to the following criteria: (1) grasses, legumes and herbs were present and (2) realized species numbers were close to sown species. The selected plots (Appendix A2.1) included two sown diversity levels (four and 16 species) with six replicates per diversity level. In December 2011, soil monoliths (2 m depth; 1.6 m dia.) were excavated from the selected plots using steel lysimeters (UMS GMBH, Munich, Germany). The soil monoliths were representative in terms of percentage vegetation cover and standing biomass of the plots from which they originated. All monoliths were stored over winter in the soil at the field site. In spring 2012, they were transported to the Montpellier European Ecotron in France.

2.1.1.2 Setting up the Jena-Ecotron experiment

The soil monoliths were randomly allocated to the 12 controlled environment units of the macrocosm platform in the Ecotron facility in Montpellier, France (Milcu et al., 2014). In

each unit the aboveground compartment of the ecosystems was confined in a transparent dome with a volume of ca. 30 m^3, while the belowground compartment was maintained in a lysimeter. The imposed environmental conditions in the Ecotron simulated the average climatic conditions in the Jena Experiment since 2002. A continuous atmospheric $^{13}CO_2$ labelling was applied inside all units for a period of three weeks (from July 4 to July 24, 2012, during daytime) using an automated system. A cylinder of compressed 99% $^{13}CO_2$ (Eurisotop, France) was connected to a manometer and a high accuracy mass flow controller (F200CV, Bronkhorst, NL). $^{13}CO_2$ was injected in each unit through 30 seconds pulses every 6 minutes. During a pulse, the flow rate of $^{13}CO_2$ was regulated at 4.8 ml min^{-1} and injected along with roughly 0.5 l min^{-1} of CO_2-free air, using a Valco valve (EUTA-SD16MWE dead-end path, VICI, USA). The labelling system was operated with a PXI Chassis (National Instrument, USA) data logger, using Labview programming (National Instrument, USA). The $\delta^{13}C$ value of CO_2 at the outlet of each unit was checked on-line twice every two hours using a Picarro G2101-i isotope analyzer (Picarro, USA), and a homemade automated manifold. This system allowed us to increase the $\delta^{13}C$ signature of the atmospheric CO_2 to +21.2‰ (average on the 12 units, SE=0.67).

Additionally, the surface of all lysimeters was covered by an impermeable cloche at the same time (23:00 h on July 16, 2012). Roughly, the same volume of air was trapped inside. The air was sampled four times at 23:00 h (T0), 00:30 h (T1), 03:30 h (T2) and 5:30 h (T3) in exetainers; two exetainers were sampled per unit. This allowed having measurements of night-time ecosystem respiration as well as $^{13}CO_2$ (Appendix A2.2a and b).

2.1.2 Soil sampling and preparation

Using a split tube (inner Ø= 4.8 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands), three soil cores were collected (from July 16 to 18) from each of the 12 units to a depth of 10 cm. The soil cores were segmented to 0-5 cm depth and 5-10 cm depth and sections from the same depth were pooled together within unit. Soil samples were sieved (2 mm mesh size) and all remaining roots and plant material were carefully removed. Subsequently, samples were stored frozen at -20 ºC until PLFA and NLFA extraction.

2.1.3 PLFA and NLFA extraction and analyses

PLFA and NLFA were extracted according to the method of Bligh & Dyer (1959) as modified by Kramer & Gleixner (2006). Total lipids were extracted from bulk soil (~70 g wet weight) using a mixture of CH_4O, $CHCl_3$ and 0.05 M K_2HPO_4 buffer (2:1:0.8 v/v/v). PLFA

and NLFA were purified by column chromatography (solid phase extraction column, Bond Elut ® SPE, Varian USA) and then hydrolyzed and methylated with methanolic KOH. In order to prevent peak overlapping during quantification and identification of fatty acid methyl esters of PLFA (PLFA-ME), they were separated into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) using an aminopropyl modified SPE column (Bond Elut ® SPE, Düren, Germany) impregnated with $AgNO_3$. Fatty acid methyl esters of NLFA (NLFA-ME) were not separated into SATFA, MUFA and PUFA because we were only interested in the fatty acid 16:1ω5 which did not overlap with other peaks.

The fatty acid 19:0 was added to all fractions (SATFA, MUFA, PUFA and NLFA) as an internal standard, prior to quantification with gas chromatography in a GC-FID system (Agilent Technologies. Palo Alto, USA) using a HP Ultra column (50 m x 0.32 mm internal diameter, 0.52 mm film thickness) and helium as a carrier gas. Following the initial conditions, 140ºC held for 1 min, the temperature increased at a rate of 2ºC min^{-1} until reaching 270ºC (6 min isotherm). Afterwards, the heating rate increased to 30ºC min^{-1} to reach a final temperature of 320ºC that was held during 3 min. Peak identification was done using a GC/MS (Thermo Electron, Dreieich, Germany) and comparing their mass spectra and retention times with known standards and mass spectral data from an in house database (Thoms et al., 2010; Thoms and Gleixner, 2013).

In total 50 PLFA were identified, but only those with a concentration higher than 0.5 µg g^{-1} (soil dry weight) were taken into account for further analysis. The 20 PLFA considered were assigned to G+ bacteria (14:0i, 15:0i, 15:0a, 16:0i, 17:0i and 17:0a (Zelles, 1997)); actinobacteria (16:0(10Me) and 18:0(10Me) (Kroppenstedt, 1985); saprotrophic fungi (18:2ω6,9 (Zelles, 1997)); G- bacteria (cyclo) (17:0cy and 19:0cy (Zelles, 1997)); G- bacteria (16:1ω7, 16:1ω5, 16:1, 17:1, 18:1ω9 and 18:1ω7 (Zelles, 1997)); and non-specific markers (16:0, 18:1ω5, 18:0 (Zelles, 1997)). From NLFA, only the marker for arbuscular mycorrhizal fungi (AMF, 16:1ω5N (Olsson, 1999)) was quantified.

2.1.3.1 PLFA and NLFA compound specific $\delta^{13}C$ measurements

$\delta^{13}C$ values of PLFA and NLFA were measured in triplicate on a GC-IRMS system (HP5890 GC, Agilent Technologies, Palo Alto USA; IRMS: Deltaplus XL, Finnigan MAT, Bremen, Germany) using a HP Ultra column (50 m x 0.32 mm internal diameter, 0.52 mm film thickness) and helium as a carrier gas. The $\delta^{13}C$ values of SATFA and NLFA were analysed under the same conditions as for quantification of FAMEs (see above) with splitless and split (1:10) mode, respectively. For the analysis of $\delta^{13}C$ values of MUFA and PUFA the

splitless mode was used. The oven reached 140ºC and held that temperature for 1 min, the temperature increased at a rate of 2ºC min^{-1} until reaching 252ºC. Followed by a heating rate of 30ºC min^{-1} until a final temperature of 320ºC that was held during 3 min. An external standard (fatty acid 19:0) was injected before and after each triplicate sample measurement to calibrate the isotopic values of every sample.

Isotopic values are expressed as $\delta^{13}C$ value in per mil [‰] relative to the international reference standard V-PDB (Equation 1) using NBS 19 (Werner and Brand, 2001). For data evaluation, the software ISODAT NT 2.0 (SP 2.67, Thermo Fisher, USA) was used.

$$\delta^{13}\mathrm{C\ value}[‰]_{\mathrm{V-PDB}} = \frac{[(^{13}C/^{12}C)_{sa} - (^{13}C/^{12}C)_{std}]}{(^{13}C/^{12}C)_{std}} \times 1000 \qquad \text{(Equation 1)}$$

where $(^{13}C/^{12}C)_{sa}$ is the $^{13}C/^{12}C$ ratio of the sample and $(^{13}C/^{12}C)_{std}$ the $^{13}C/^{12}C$ ratio of the reference standard (0.011202). $\delta^{13}C$ values were also corrected for the methyl C added during methylation (Equation 2) (Kramer and Gleixner, 2006).

$$\delta^{13}\mathrm{C}_{PLFA\&NLFA} = \frac{[N \times \delta^{13}\mathrm{C}_{PLFA-ME\&NLFA-ME} - \delta^{13}\mathrm{C}_{MeOH}]}{(N-1)} \qquad \text{(Equation 2)}$$

where $\delta^{13}C_{PLFA\&NLFA}$ is the isotope ratio of the phospholipid fatty acid or neutral lipid fatty acid, $\delta^{13}C_{PLFA-ME\&NLFA-ME}$ the isotope ratio of the phospholipid fatty acid methyl ester or neutral lipid fatty acid methyl ester, $\delta^{13}C_{MeOH}$ that of methanol used for derivatization and N is the number of C atoms of the PLFA-ME or NLFA-ME.

2.1.4 Explanatory variables

2.1.4.1 Leaf related covariates: canopy leaf nitrogen, shoot biomass and specific leaf area

Canopy leaf nitrogen (g N_{leaf} $m_{soil\ surface}^{-2}$) as a proxy for photosynthetic assimilation was estimated as the sum of species-specific values for leaf area (m^{-2}) × specific leaf mass (g_{leaf} m_{leaf}^{-2}) × leaf nitrogen concentration (g N g_{leaf}^{-2}). Samples of 5-10 fully developed leaves collected from three different individuals (if possible) for each available species per unit were put in moistened filter paper and stored at 4°C for 12 hours. Then, leaf samples were blotted dry with tissue paper to remove any surface water, the leaf area was determined with a leaf area meter (LI3100, LICOR, USA). Samples were dried at 65°C (48 h) and weighed to obtain specific leaf area (as the ratio of leaf area per leaf dry mass). Leaf nitrogen concentrations were measured from ground leaf samples with an elemental analyser (Flash EA 1112; Thermo Italy, Rhodano, Italy). Shoot biomass was harvested on an area of 90 x 65 cm and sorted by species. Subsamples of each species were separated by plant compartments (leaves, stems, inflorescences) and dried at 65°C (48 h). Specific leaf area (reverse of specific leaf mass) and leaf mass were used to get leaf area per m^2 for each species.

2.1.4.2 Root related covariates: root biomass, root length and $\delta^{13}C$ values from roots and root sugars

Different root traits were measured as potential drivers of the plant diversity effect on soil microbial community. Therefore, three cores (Ø= 3.5 cm) were collected in every unit, segmented into 5 cm layers (0-5 cm and 5-10 cm depth) and pooled by layer within unit. Roots were separated immediately from soil with tap water and a sieve of 200 µm mesh size. Root length per volume soil (cm cm^{-3}) was measured in a subsample of fresh roots by image analysis with WinRHIZO (Reagent Instruments, Inc.). Roots were subsequently dried for 48 h at 65°C. Root biomass (mg root cm^{-3} soil) was calculated in a determined volume of soil. Specific root length (m g^{-1}) was calculated dividing root length per volume by root biomass. All three above mentioned measures might impact the plant-microbe-interaction via morphological plant community traits, assuming that differences in root morphology reflect spatial changes in the rhizosphere.

The $\delta^{13}C$ values from roots and root sugars were determined in root samples that were separated from the soil samples collected for PLFA and NLFA analysis. Immediately after sampling, root samples were placed in paper bags, shock-frozen in liquid nitrogen and subsequently freeze-dried. The $\delta^{13}C$ values from roots were measured on 20 mg of freeze-dried and ball-milled root material using an elemental analyzer (NA 1110, CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer IRMS (Delta+XL, Finnigan MAT, Bremen, Germany) (Steinbeiss et al., 2008b).

Total water soluble sugars were extracted using a method adapted from Wild *et al.* (2010). Briefly, freeze-dried root material (ca. 30 mg) was resuspended by adding boiling bi-distilled water and then samples were incubated at 85°C for 10 min on a heating shaker (Thermomixer comfort, Eppendorf AG, Hamburg). Samples were centrifuged (12000 rpm, 5 min) and supernatant was collected. The extraction was repeated twice and supernatants were pooled together. Extracts were filtered through cellulose membrane filters (MULTOCLEAR 0.45 µm RC 13 mm, CS-Chromatographie Service GmbH, Langerwehe) and transferred to anion and cation exchange cartridges (Dionex OnGuard II A and H 1.0 cc cartridges, Thermo Scientific, Sunnyvale, CA, USA) to remove ionic components. The resulting neutral fraction was analyzed by high-performance liquid chromatography-isotope ratio mass spectrometry (HPLC-IRMS, ThermoFinnigan LC-IsoLink system, Thermo Electron, Bremen). Sugars were first separated by HPLC on a NUCLEOGEL SUGAR 810 Ca^{2+} column (Macherey & Nagel, Düren) at 80°C with 0.5 ml min^{-1}, using bi-distilled water as eluent and afterwards $\delta^{13}C$ values were measured in an IRMS (Deltaplus XP, Thermo Electron, Bremen). Data was

evaluated using the software ISODAT NT 2.0 (SP 2.67, Thermo Fisher, USA). The peaks of raffinose, stachyose and verbascose that are all symplastic phloem transport sugars of the raffinose-type oligosaccharides (Turgeon and Medville, 2004) were integrated manually with ISODAT NT 2.0 yielding one peak. Quality control and isotopic offset corrections were evaluated by repeated measurements of sucrose (δ^{13}C= -25.95‰, Merck KGaA, Darmstadt, Germany) with a recovery rate of 95±3%. Identification of sugars was primarily based on comparing retention times of samples and known standards. Additionally, extracts of total water soluble sugars were derivatised with BSTFA + 1% TMCS and analyzed by GC-MS (7890A GC, 220 Ion Trap MS, Agilent Technologies, Palo Alto USA) on a medium polar VF-17ms column (Agilent Technologies), confirming the presence of sucrose, raffinose and stachyose, whereas verbascose derivates were apparently too large for vaporization.

2.1.4.3 Soil related covariates: soil moisture, SOC and δ^{13}C-SOC

Soil moisture was determined gravimetrically (Black, 1965) from 5 g of soil (wet weight) that were collected as subsamples from the soil cores taken for PLFA and NLFA analysis. For SOC measurements, 20 mg of soil were dried at 40ºC to constant weight and homogenized by grinding in a ball mill. SOC was calculated by difference of C_{total} - $C_{carbonates}$ using an elemental analyzer (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany) (Steinbeiss et al., 2008b). The δ^{13}C values of SOC (δ^{13}C-SOC) were determined on 3 mg of air-dried and ball-milled soil. Soil carbonates were removed with 120 µl H_2SO_3 (5–6% SO_2, Merck, Darmstadt, Germany) (Steinbeiss et al., 2008b). Subsequently, the samples were dried at 60ºC and δ^{13}C-SOC values were measured repeatedly (standard deviation < 0.3‰) using EA-IRMS (See above). The system was calibrated versus V-PDB using CO_2 as the reference gas (Werner and Brand, 2001).

2.1.5 Statistical analyses

We performed analyses of variance (two-way-ANOVA) to test for differences in total concentration and δ^{13}C values (weighted by marker specific concentration) of total PLFA (see Table 3.1a, c in section 3.2.1), individual microbial groups from PLFA (Appendix A2.3b) and NLFA (see Table 3.1b, d in section 3.2.1). To analyze the effect of plant diversity on δ^{13}C values of all individual PLFA and NLFA we used linear models (LM) for each soil layer (Appendix A2.3a). The plant diversity effect on individual markers was similar among different soil layers (0-5 cm and 5-10 cm: Appendix A2.3a), but the effect was more pronounced in the top soil (0-5 cm depth), as it is biologically the more active soil layer.

Therefore, we focused in the results and discussion section mainly on the top soil layer. Subsequently, and supported by the results of this linear model (Appendix A2.3a); we grouped the individual $\delta^{13}C$ values for the commonly used microbial groups. For further analyses we used the weighted mean ($\delta^{13}C$ values by concentration) of each microbial group (see Table 2.1c, d in section 3.2.1 and Appendix A2.3b). Although different microbial groups responded differently to plant diversity we used the same approach for each group to identify the most important predictor for explaining variances in the $\delta^{13}C$ values. Out of a global model including all plant and soil related covariates additional to plant diversity (see sections 2.1.4 and Appendix A2.4a and b), we identified for all microbial groups three best models (see Tables 3.2 and 3.3 in section 3.2.4, Appendix 2.5) to explain their response to plant diversity ('dredge' function in MUMln package in R (Barton K., 2014)). In the results section we only referred to the 1st best model, but alternative models are shown in the supporting information. Missing values were replaced by means. All statistical tests were performed with R for windows (R Development Core Team, 2014).

2.2 The Jena Experiment

2.2.1 Site description and experimental design

This work was conducted as part of the Jena Experiment, one of the biggest long-term biodiversity experiments worldwide, which investigates the role of biodiversity on the mechanisms controlling element cycling and ecosystem functioning (Roscher et al., 2004). The experiment was established in 2002 in the northern part of Jena (Germany, 50°55`N, 11°35`E, 130 m a. s. l.) on a former arable land on the floodplain of the Saale River. The main experimental area consists of 82 plots (3 × 3 m each) that were arranged in 4 blocks parallel to the river to account for the textural soil gradient (sand percentage decreased with distance to the river from 39% to 6%; clay and silt content increased, from 16% to 22% and from 44% to 72%, respectively).

Sixty plant species (typical for Central European Molino-Arrhenatheretea grasslands) were selected and grouped into 4 functional groups (according with their physiological and morphological traits, Roscher et al. (2004)): 16 grasses, 12 small herbs, 20 tall herbs and 12 legumes. The plant species diversity gradient consisted of 1, 2, 4, 8, 16 and 60 and the plant functional diversity went from 1 (2, 3) to 4 functional groups.

2.2.2 Soil sampling and preparation

In late April 2012, six soil samples were collected in each plot using a core cutter (5 cm inner diameter; 5 cm depth), samples from the same plot were pooled together in a polyethylene bag and stored at 4º C on site in cooling boxes. All soil samples were sieved (<2 mm) within the following 48 h after sampling and all plant material, stones and animals were removed. Samples were stored at -20ºC until further chemical analysis.

2.2.3 PLFA extraction and analysis

Total lipids were extracted from ca. 70 g of wet soil with a mixture of CH_4O, $CHCl_3$ and 0.05 M K_2HPO_4 buffer (2:1:0.8 v/v/v) (Bligh and Dyer (1959); modified by Kramer and Gleixner (2006)). PLFAs were purified from the total lipid extract by sequential elution of a silica-filled solid phase extraction column (Bond Elut ® SPE, Varian US.). The methanol fraction that contained the PLFA, was hydrolyzed and methylated with a 0.2 M KOH methanolic solution. The fatty acid 19:0 was added to the methylated PLFAs (PLFA-ME) as internal standard, PLFA-ME were then quantified with a GC-FID system (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, United States) using a HP Ultra column (50 m x 0.32 mm internal diameter, 0.52 mm film thickness) and Helium as a carrier gas.

Total PLFA concentration was calculated from the sum of the 39 individual PLFAs identified. Microbial groups were differentiated by specific PLFA markers. G+ bacteria: 14:0i, 15:0i, 15:0a, 16:0i, 17:0i and 17:0a (White et al., 1996; Zelles, 1997); actinobacteria, which are a subgroup of G+ bacteria: 16:0(10Me), 17:0(10Me) and 18:0(10Me) (Kroppenstedt, 1985; Bååth and Anderson, 2003); G- bacteria (cyclo): 17:0cy and 19:0cy (Zelles, 1997, 1999a); G- bacteria: 15:1, 16:1ω11, 16:1ω7, 16:1, 17:1, 18:1ω9 and 18:1ω7 (Zelles, 1997, 1999a); saprotrophic fungi: 18:2ω6,9 (Vestal and White, 1989; Zelles, 1997); arbuscular mycorrhizal fungi: 16:1ω5 (Olsson et al., 1995; Sakamoto et al., 2004). Non-specific markers (14:0, 15:0, 16:0, 17:0, 18:0, 16:0br, 16:1ω12, 17:0br, 18:2, 18:0br, 19:0br, 22:0br, 23:0br, 17:1ω8, 18:1ω5, 19:1, 20:0, 20:1ω9 and 20:5ω3 (Zelles, 1997)) were also accounted for the total microbial biomass. A selected subset of G+ bacterial, actinobacterial and saprotrophic fungal markers were further grouped into soil related microorganisms (Mellado-Vázquez et al., 2016) and a subset of G- bacterial and AMF markers were group into root-associated microorganisms (Mellado-Vázquez et al., 2016).

2.2.4 Genetic diversity of soil bacteria

The genetic diversity of soil bacteria was measured using terminal-restriction fragment length polymorphism (T-RFLP). Briefly, total nucleic acids were extracted from 0.25 g fresh weight of soil using the MoBio Powersoil 96 kit. Bacterial 16S rRNA genes were amplified using forward primer 63F (50-CAGGCCTAACACATGCAAGTC-30 (Marchesi et al., 1998)), fluorescently labelled with 6-FAM at the 50 end, and reverse primer 519r (50-GTAT TACCGCGGCTGCTG-30 (Lane, 1991)). Amplicons were purified by gel filtration using Sephadex G50 (Sigma-Aldrich, Gillingham, UK) before digestion with restriction endonucleases Msp1 (CCGG; Promega, Southampton, UK). A 3730 DNA analyzer (Applied Biosystems, CA, USA) was used for fragment analysis, before binning of individual terminal-restriction fragments using Genemarker software (SoftGenetics, PA, USA). The relative abundance of individual terminal-restriction fragments was calculated by dividing the intensity of each fragment by the total intensity of all fragments before calculation of Simpson's diversity index.

2.2.5 Simpson's diversity index and Simpson's evenness

The Simpson's diversity index from the terminal-restriction fragments (henceforth bacterial diversity) and from the bacterial PLFA markers was calculated as follows (Equations 3 and 4) (Simpson, 1949):

$$\text{Simpson's diversity index} = 1 - D \qquad \text{(Equation 3)}$$

$$D = \Sigma\,(n/N)^2 \qquad \text{(Equation 4)}$$

Where n is the total number of fragments or PLFA markers of a particular species and N is the total number of fragments or PLFA markers of all species. Subsequently, the Simpson's evenness was calculated for the bacterial PLFA markers (hereafter PLFA evenness) as follows (Equation 5) (Hill, 1973):

$$\text{Evenness} = (1/D)/N \qquad \text{(Equation 5)}$$

2.2.6 Explanatory variables

2.2.6.1 Root biomass and leaf area index (LAI)

Mean standing root biomass [g m^{-2}] was calculated in 2011 from three soil cores (3.5 cm in diameter, 40 cm depth) in each plot. Soil cores were segmented into 5 or 10 cm depth layers (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm and 30-40 cm) and same depth sections corresponding to each plot were pooled together. Soil segments were washed with tap water over a 0.5 mm mesh size sieve. Roots were dried at 65±5 °C and subsequently weighed. Soil

segments of 0-5 cm depth were included in the present study (Appendix A2.6). Leaf area index (LAI) was measured in May 2012 using a LAI-2000 plant canopy analyzer (LI-COR) approximately 5 cm above ground level in each plot (Appendix A2.6).

2.2.6.2 Soil moisture, SOC

Soil moisture [%] (Appendix A2.6) was calculated gravimetrically from the differences between wet and dry soil weight (ca. 5 g wet weight) from each plot in 2012. Soil samples were dried, according to standard procedures, for 24 h at 100ºC (Black, 1965). Total soil C [%] and inorganic soil C [%] (Appendix A2.6) concentrations were quantified in 2011 by elemental analysis at 1150ºC (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany)(Steinbeiss et al., 2008b; Tefs and Gleixner, 2012). Inorganic C was measured after removal of organic C for 16 h at 450ºC in a muffle furnace. SOC [%] (Appendix A2.6) was then calculated as the difference between total soil C and inorganic soil C.

2.2.7 Statistical analyses

Analyses of variance (ANOVAs) were conducted to test for the influence of plant species richness, plant functional group richness and abundance of specific plant functional groups on total microbial biomass, root-associated and soil-related microbial biomass, bacterial diversity, PLFA evenness (see Table 4.1 in section 4.2.3). Subsequently, analyses of covariance (ANCOVAs) were performed to identify the drivers of significant plant diversity effects on total microbial biomass, root-associated and soil-related microbial biomass, bacterial diversity, PLFA evenness (see Table 4.1 in section 4.2.3). In the ANCOVAs, root biomass, LAI, soil moisture and SOC were set as considered explanatory variables to explain the variance of the soil microbial factors related to plant diversity. Furthermore, the linear relation among all possible explanatory variables was tested with Pearson's correlation analyses (Appendix A2.7). All statistical tests were conducted with R for windows (R Development Core Team, 2014).

2.3 C3-C4 vegetation change experiment

2.3.1 Site description and experimental design

The C3-C4 vegetation change experiment was established at the Max Planck Institute for Biogeochemistry in Jena, Germany in 2002. Two large lysimeters (2 m deep, approx. 100 m^3 each) were filled with homogenized soil. The soils between the lysimeters differed in their texture (Table 2.1), in one lysimeter the soil had higher sand content and higher SOC

concentration, henceforth termed as "sandy soil". In the other lysimeter the soil had a higher proportion of silt and clay but lower SOC concentration, henceforth termed as "clayey soil". The origin of both soil types was from C3 vegetation. Until 2006 the entire experiment was continuously cropped with C3 vegetation. In November 2006 each lysimeter was split into two plots with different vegetation types (C3 vs. C4 plants), resulting in four plots, each 24 m^2. Scorpion weed *(Phacelia tanacetifolia)*, sunflower *(Helianthus annuus)* and wheat *(Triticum spec.)* were rotationally grown on C3 plots; lovegrass *(Eragrostis curvula)*, maize *(Zea mays)*, amaranth *(Amaranthus spec.)* and sorghum *(Sorghum spec.)* were rotationally grown on C4 plots. All plots were equally managed, namely annually in autumn the total shoot biomass was harvested and equal amounts were re-distributed on each plot considering the vegetation type. From October to April, i.e. over the non-growing season, plots were covered with a water permeable sheet that allowed plant litter decomposition but prevented weed germination. In the following spring the plots were sown again with their respective vegetation type.

Table 2.1. Mean (n= 3 ± standard deviation) values of soil characteristics and root biomass in sandy and clayey soils from the C3-C4 vegetation change experiment.

Characteristic	**Sandy soil**		**Clayey soil**	
	C3 plots	**C4 plots**	**C3 plots**	**C4 plots**
Sand content [%]	50.4		9.2	
Silt content [%]	43.8		75.1	
Clay content [%]	5.9		15.7	
Soil moisture [%]				
Non-growing season	23.0 ± 1.8	23.5 ± 0.8	19.5 ± 0.9	19.9 ± 0.5
Growing season	16.8 ± 2.3	19.6 ±1.5	21.0 ± 2.1	19.7 ± 0.7
Mean annual	19.9 ± 1.5	21.6 ± 0.9	20.3 ± 0.1	19.8 ± 0.4
DOC [mg L^{-1}]				
Non-growing season	12.4 ± 0.1	25.5 ± 0.2	10.8 ± 0.1	6.6 ± 0.01
Growing season	10.6 ± 0.2	28.1 ± 0.3	21.9 ± 0.01	15.1 ± 0.01
Mean annual	11.5 ± 0.1	26.8 ± 0.2	16.4 ± 0.1	10.9 ± 0.01
SOC [%]	5.0 ± 0.6	4.6 ± 0.3	2.2 ± 0.2	2.0 ± 0.2
Root biomass [g m^{-2}]	53.7 ± 1.0		63.1 ± 2.7	

2.3.2 Soil sampling and preparation

For the present study, soil samples were collected in 2012 in the non-growing season and the growing season. Using a stainless steel core cutter (inner Ø= 4.8 cm, 60 cm long Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) we collected 3 independent samples to a depth of 10 cm in each plot. Soil samples were sieved (<2 mm) and cleaned by

removing remaining plant material and stones, and subsequently stored at -20ºC until PLFA analysis.

2.3.3 PLFA extraction, analysis and compound specific measurement

A mixture of CH_4O, $CHCl_3$ and 0.05 M K_2HPO_4 buffer (2:1:0.8 v/v/v) was used to extract the total lipid fraction from 50 g of wet soil (Bligh & Dyer (1959); modified by Kramer and Gleixner (2006)). PLFAs were purified from the total lipid extraction by eluting a silica-filled solid phase extraction column (Bond Elut ® SPE, Varian US.) with methanol; the elution was hydrolyzed and methylated with a 0.2 M KOH methanolic solution. Subsequently, methylated fatty acids (PLFA-ME) were split up into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in an aminoproply modified SPE column (Bond Elut ® SPE, Düren, Germany) impregnated with $AgNO_3$. After adding the fatty acid 19:0 (as an internal standard) to all fractions, PLFAs were quantified with a GC-FID system (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies, United States) using a HP Ultra column (50 m x 0.32 mm internal diameter, 0.52 mm film thickness) and Helium as a carrier gas. Following the initial conditions, 140ºC held for 1 min, the temperature increased at a rate of 2º min^{-1} until reaching 270ºC (6 min isotherm). Afterwards, the heating rate increased to 30ºC min^{-1} to reach a final temperature of 320ºC that was held during 3 min. PLFA identification was performed by comparison of the retention times with known standards and mass spectral data from an in house database (Thoms et al., 2010).

Total PLFA concentration was calculated from the sum of the all identified PLFA markers. Based on their predominant microbial origin, individual PLFA markers were assigned to different microbial groups: G+ bacteria: 15:0i, 15:0a, 16:0i, 17:0i and 17:0a (Zelles, 1997); actinobacteria: 16:0(10Me), 19:0(10Me) (Kroppenstedt, 1985); G- bacteria: 15:1, 16:1ω7, 16:1ω5, 16:1, 17:0cy, 17:1, 18:1ω9, 18:1ω7 and 19:0cy (Zelles, 1997); and saprotrophic fungi: 18:2ω6,9. Non-specific markers (14:0, 16:0 and 18:0 (Zelles, 1997)) were also accounted for the total PLFA concentration.

Isotope C ratios of individual PLFA markers were measured in a GC-IRMS system (HP5890 GC, Agilent Technologies, Palo Alto USA; IRMS: Deltaplus XL, Finnigan MAT, Bremen, Germany). Isotope ratios are expressed as $\delta^{13}C$ value in per mil [‰] relative to the international reference standard Vienna-PeeDee Belemnite (V-PDB) (Equation 6) using NBS 19 (Werner and Brand, 2001). The software ISODAT NT 2.0 (SP 2.67, Thermo Fisher, USA) was used for data evaluation.

$$\delta^{13}\mathrm{C\ value}[‰]_{\mathrm{V-PDB}} = \frac{[(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sa}} - (^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{std}}]}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{std}}} \times 1000 \qquad \text{(Equation 6)}$$

where $(^{13}C/^{12}C)_{sa}$ is the $^{13}C/^{12}C$ ratio of the sample and $(^{13}C/^{12}C)_{std}$ the $^{13}C/^{12}C$ ratio of the reference standard (0.011202). $\delta^{13}C$ values were also corrected for the methyl carbon added during methylation (Equation 7) (Kramer and Gleixner, 2006).

$$\delta^{13}C_{PLFA} = \frac{[N \times \delta^{13}C_{PLFA-ME} - \delta^{13}C_{MeOH}]}{(N-1)} \qquad \text{(Equation 7)}$$

where $\delta^{13}C_{PLFA}$ is the isotope ratio of the phospholipid fatty acid, $\delta^{13}C_{PLFA\text{-}ME}$ the isotope ratio of the phospholipid fatty acid methyl ester, $\delta^{13}C_{MeOH}$ that of methanol used for derivatization and N is the number of carbon atoms of the PLFA-ME.

2.3.3.1 Assessing the C origin in PLFA

The fraction of plant C derived to individual PLFAs (Fp_{PLFA}; Equation 8) was calculated according to Kramer and Gleixner (2006):

$$Fp_{PLFA} = \frac{[\delta^{13}C_{PLFA-C4} - \delta^{13}C_{PLFA-C3}]}{[\delta^{13}C_{Plant-C4} - \delta^{13}C_{Plant-C3}]} \qquad \text{(Equation 8)}$$

Where $\delta^{13}C_{PLFA\text{-}C4}$ and $\delta^{13}C_{PLFA\text{-}C3}$ represent the isotopic values of individual PLFA measured in soils with C4 and C3 vegetation, respectively, and $\delta^{13}C_{Plant\text{-}C4}$ and $\delta^{13}C_{Plant\text{-}C3}$ indicate the isotopic values of different vegetation types analyzed in this study.

2.3.4 Explanatory variables

In addition to the experimental design variables (vegetation type, soil type and season) ecologically important covariates were assessed. Soil moisture [%] was determined gravimetrically (Black, 1965) from 5 g of soil (wet weight) that were collected as subsamples from the soil cores taken for PLFA analysis. Root biomass [g m^2] was quantified as the average of roots collected from three quadrants (0.5 × 0.5 m) in each plot. For SOC [%] measurements, soils were dried at 40ºC until constant weight and homogenized by grinding in a ball mill. Concentration of SOC was calculated from the difference between total C and inorganic C. Total C and inorganic C were measured by elemental analysis at 1150ºC (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany), inorganic C was obtained after burning organic C for 16 h at 450ºC in a muffle furnace (Steinbeiss et al., 2008b). DOC [mg L^{-1}] was measured from soil water samples that were collected in the non-growing and growing seasons, in one day close to the soil sampling for each vegetation season. Soil water samples were collected at 10 cm depth by extraction with borosilicate glass suction plates (thickness 9 mm, diameter 120 mm, pore size 1 µm; UMS, Germany). For DOC analysis, 1.5 ml of soil water was transferred to brown glass vials (silanized) and dissolved inorganic carbon (DIC) was removed acidifying the water samples with 20 µl of 8.5% phosphoric acid

(Merck, Germany). Samples were then gently purged with nitrogen (N_2) for 10 minutes in a 12-port chamber (VisiprepTM, Visidry™, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) (Malik et al., 2012; Scheibe et al., 2012).

2.3.5 Statistical analyses

Analyses of variance (ANOVA) were performed to test for the effects of experimental factors, i.e. soil type, vegetation type and season on the total microbial biomass and on the δ^{13}C values of individual PLFA markers. ANOVA was also used to test the effect of soil type and season on the uptake of plant-derived C among microbial groups. In this ANOVA individual PLFAs were tested based on the assignment to specific soil microbial groups (see section 2.3.3) followed by Tukey's Honestly Significant Difference (HSD) test.

The effect and the relative importance of the experimental factors on the microbial community composition in terms of PLFA concentrations and δ^{13}C values of PLFAs were tested by Permutational Multivariate Analyses of Variance (PERMANOVAs: 'adonis' function in vegan package in R (Oksanen et al., 2011). For PERMANOVA the "Euclidian" distance measured was applied. Redundancy analyses (RDAs) were carried out to identify the underlying drivers of the experimental factors by assessing the impact of root biomass, SOC, DOC, clay and sand content and soil moisture on the soil microbial community composition (PLFA concentration) and its plant-derived C uptake.

PERMANOVAs, ANOVAs and Tukey's HSD tests were performed with R for windows (R Development Core Team, 2014). RDAs were carried out using CANOCO 5.0 for windows (ter Braak and Šmilauer, 2012).

CHAPTER III

PLANT DIVERSITY GENERATES ENHANCED SOIL MICROBIAL ACCESS TO RECENTLY PHOTOSYNTHESIZED CARBON IN THE RHIZOSPHERE[1]

Abstract

Plant diversity positively impacts ecosystem services such as biomass production and soil organic matter (SOM) storage. Both processes counteract increasing atmospheric CO_2 concentration and global warming and consequently need better understanding. In general it is assumed that complementary resource use is driving the positive biomass effect and that the rhizospheric microbial community provides the necessary nutrients mineralizing SOM. So far however, it remains unclear how this link between the above and the belowground system is functioning; in detail it remains unclear if a more efficient CO_2 uptake at higher diversity levels leads to higher root exudation that stimulate the microbial mineralization. Contrastingly we show here for the first time that more diverse grassland communities provide a better access to root exudates for the rhizospheric community. We applied a continuous $^{13}CO_2$ label in a controlled environment (The Montpellier European Ecotron) to ecosystem monoliths from the long-term The Jena Experiment and showed analyzing the $\delta^{13}C$ content of phospholipid fatty acids and neutral lipid fatty acid that plant diversity increased the plant-derived C uptake of Gram negative bacteria and arbuscular mycorrhizal fungi (AMF). Root biomass but not the amount and $\delta^{13}C$ content of root sugars positively influenced the plant diversity effect observed on Gram negative bacteria whereas the specific interaction between plant and AMF was independent from any plant trait. Our results demonstrate that plant diversity facilitated the accessibility of plant derived C but not the above-belowground transfer rates. This facilitating effect enabled more diverse plant communities to use complementary C and most likely nutrient resources both from soil organic matter mineralization for better growth. We anticipate from our results that plant diversity effects are less driven by the performance of individuals in mixtures (trait plasticity) but by the combination of individuals that interact independently (trait complementarity).

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3.1 Introduction

Biodiversity is an important regulator of ecosystem functioning (Hooper et al., 2005; Cardinale et al., 2012; Bardgett and van der Putten, 2014). During the last two decades numerous studies have demonstrated negative effects of biodiversity loss on key ecosystem processes (reviewed in Hooper et al., 2012) such as primary productivity and soil organic carbon (SOC) storage (Tilman et al., 2001; Fornara and Tilman, 2008; Steinbeiss et al., 2008a; Marquard et al., 2009). Yet, primary productivity and SOC storage are not independent from each other, as enhanced belowground inputs of plant-derived carbon (C) associated with increased biomass production at higher diversity levels might lead to increased SOC accumulation (Steinbeiss et al., 2008a; Lange et al., 2015). This plant-soil relationship is likely to be mediated by soil microorganisms, since they are the main agents transferring C from plants to SOC (Gleixner, 2013). Soil microorganisms can be classified in several ways, for instance the soil microbial community has been divided into ecological categories related to their preferentially decomposed C sources: copiotrophs are able to decompose labile C sources (e.g. root exudates (Wardle, 2002)) and oligotrophs have higher nutrient affinity and are capable of decomposing SOC and plant-derived litter (Fierer et al., 2007). Another commonly way of classifying soil microorganisms is by their abundance in different compartments of the soil, such as rhizosphere and bulk soil (Denef et al., 2007; Elfstrand et al., 2008; Denef et al., 2009; Esperschutz et al., 2009; Bird et al., 2011; De Deyn et al., 2011; Bahn et al., 2013; Fanin et al., 2015). In general, Gram negative (G-) bacteria are recognized as rhizosphere microorganisms (Denef et al., 2009), which preferentially decompose simple substrates; such as recently fixed plant-derived C (i.e. root exudates). Gram positive (G+) bacteria are slow growing and stress tolerant microorganisms (Waldrop et al., 2000) that dominate the bacterial community in the bulk soil. They are known to decompose complex substrates and are able to use older and more stabilized SOC for growth (Kramer and Gleixner, 2008; Bahn et al., 2013). Saprotrophic fungi can actively access their C substrates through hyphal growth (Strickland and Rousk, 2010). They have extensive enzymatic capabilities (Denef et al., 2007) and are able to decompose a wider variety of C substrates like root exudates, plant litter (Treonis et al., 2004) and SOC (Garcia-Pausas and Paterson, 2011). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that actively form associations with most of the known terrestrial plants (Engels et al., 2000). AMF colonize the intracellular space of plant roots and actively trade C for nutrients with the host plant (Bonfante and Genre, 2008; Drigo et al., 2010).

The final mechanisms how plant diversity interacts through the soil microbial community on soil C storage remain unclear so far. Several possible mechanisms have been put forward to explain how more diverse plant communities could increase the C transfer into the soil microbial community. For instance, more diverse plant communities could be more effective in photosynthetic assimilation (Milcu et al., 2014) resulting in higher root exudation rates (Amos and Walters, 2006; Lange et al., 2015; Shahzad et al., 2015), which might promote growth of the soil microbial community (Chung et al., 2009). Alternatively, more diverse plant communities do not only produce higher aboveground biomass, but also higher root biomass (Ravenek et al., 2014). The increased root biomass might, in turn, foster soil microorganisms, in particularly root-associated microbes in several ways, for instance via a higher input of root litter (Phillipson et al., 1975; Latz et al., 2015) which would act on the longer term; through increased rhizodeposition (Van Der Krift et al., 2001) and by facilitating a better access to recently fixed plant derived C (Guo et al., 2005).

Labelling experiments have been extensively used to determine the C transfer from plants to soil microorganisms. Different experimental approaches have been developed, for example, labelling experiments can be based on the application of either enriched ^{13}C substrates that mimic root exudates (Lemanski and Scheu, 2014) or enriched $^{13}CO_2$ that label all photosynthetic products (Denef et al., 2007; Staddon et al., 2014). However, different outcomes might be obtained by the use of distinct labelling techniques, *e.g.* pulse labelling or continuous labelling. Metabolic fluxes that are dependent on turnover of different pools are best investigated using pulse labelling (Wolfe and Chinkes, 2005). However, the results depend on the duration of the labelling as slow pools are not completely labelled and this is known to bias for example the release of plant C to the soil system (Paterson et al., 1997; Malik et al., 2015). In contrast, continuous labelling experiments allow even slow pools to reach steady-state and are therefore more suitable to determine C resource availability and C sources for different compartments of the ecosystem (*e.g.* soil, soil microorganisms, plants) (Schimel, 1993).

In order to identify the influence of plant diversity and the mechanisms behind the C transfer from plants to soil microbial communities, we took advantage of a long-term biodiversity experiment (The Jena Experiment (Roscher et al., 2004)) and an advanced controlled environment facility for ecosystem research (The Montpellier European Ecotron (Milcu et al., 2014)). Atmospheric $^{13}CO_2$ labelling of large monoliths originating from The Jena Experiment with four and 16 species allowed us to investigate how the flow of C between the different ecosystem compartments was affected by plant diversity. We extracted

phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) from all soil samples to account for differences in δ^{13}C values of distinct soil microbial groups and to identify changes in both the microbial biomass and the isotopic ratios of the microbial groups related to increased plant diversity. Following the argumentation by Lange et al. (2015) that emphasized the importance of increased microbial activity in the rhizosphere under high plant diversity for triggering the C cycling and ultimately promoting SOC accumulation, we explored more deeply the mechanisms of C transfer from aboveground to belowground through the soil microbial community and hypothesize that 1) higher photosynthetic activity at higher plant diversity results in increased microbial C uptake, as a result of a better access to recently photosynthesized plant derived C (i.e. root exudates) and 2) this plant diversity effect is more pronounced in root-associated soil microorganisms.

3.2 Results

3.2.1 Total concentration of PLFA and NLFA

The total PLFA concentration was significantly higher (mean = 134.22, SD = 36.93 nmol g^{-1}) in the topsoil (0-5 cm) than in the deeper soil layer (5-10 cm) (73.64 ± 19.27 nmol g^{-1}; $F_{1,\ 10}$= 20.66, P < 0.001; Fig. 3.1, Table 3.1a). The mean PLFA concentration increased by 13% in the topsoil layer and by 3% in the deeper soil layer with increasing plant diversity, however, this increase was not significant ($F_{1,\ 10}$= 0.565, P = 0.470; Fig. 3.1, Table 3.1a). Concentrations of PLFAs grouped into different microbial groups were significantly higher in the topsoil layer (Appendix A3.1: G+ bacteria=26.73 ± 8.46 nmol g^{-1}; Actinobacteria= 8.87 ± 3.35 nmol g^{-1}; G- bacteria (cyclo) = 10.54 ± 3.68 nmol g^{-1}; G- bacteria= 69.49 ± 19.05 nmol g^{-1} and saprotrophic fungi= 3.13 ± 1.18 nmol g^{-1}) than in the deeper soil layer (Appendix A3.1: G+ bacteria=17.09 ± 4.75 nmol g^{-1}; Actinobacteria= 6.57 ± 1.84 nmol g^{-1}; G- bacteria (cyclo) = 2.50 ± 1.52 nmol g^{-1}; G- bacteria= 35.59 ± 12.88 nmol g^{-1} and saprotrophic fungi= 1.56 ± 0.54 nmol g^{-1}). No significant changes between plant diversity levels were found in any of the microbial groups (Appendix A3.1).

The NLFA concentration showed similar patterns to the total PLFA concentration (Fig. 3.1, Table 3.1b), *i.e.* significantly higher in the top soil layer (107.75 ± 34.69 nmol g^{-1}) than in the deeper soil layer (80.95 ± 28.50 nmol g^{-1}; $F_{1,\ 10}$= 10.01, P = 0.010) and increasing concentrations with increasing plant diversity (low plant diversity: topsoil layer = 82.94 ± 30.03; deeper soil layer 61.90 ± 17.34 nmol g^{-1}. High plant diversity: topsoil layer = 132.56 ± 16.39 nmol g^{-1}; deeper soil layer 100.01 ± 24.80 nmol g^{-1} (Fig. 3.1). The difference of NLFA concentrations between plant diversity levels was significant ($F_{1,\ 10}$= 18.84, P < 0.001).

Table 3.1. ANOVA (analyses of variance) results from the differences in a) total PLFA concentration; b) NLFA concentration; c) weighted means (δ^{13}C values weighted by concentration) of total PLFA and d) weighted means of NLFA related to plant diversity. Stars indicate significant differences of δ^{13}C values between plant diversity levels and soil layers (**:p≤ 0.01; *: p≤ 0.05; $^{+}$: p≤ 0.1).

a) Total PLFA concentration					
Error:plot					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plant diversity	1	533.4	533.4	0.565	0.470
Residuals	10	9437	943.7		
Error: Within					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Layer	1	16857	16857	20.66	0.001**
Plant div:Layer	1	399	399	0.49	0.500
Residuals	10	8159	815.9		

b) NLFA concentration					
Error:plot					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plant diversity	1	11543	11543	18.84	0.001**
Residuals	10	6126	612.6		
Error: Within					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Layer	1	4308	4308	10.007	0.010*
Plant div:Layer	1	199	199	0.462	0.512
Residuals	10	4305	430.5		

Table 3.1. (Continuation)

c) δ^{13}C-PLFA					
Error: plot					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plant diversity	1	1.462	1.462	0.52	0.487
Residuals	10	28.105	2.811		
Error: Within					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Layer	1	5.991	5.991	4.109	0.070^{+}
Plant div:Layer	1	0.476	0.476	0.326	0.580
Residuals	10	14.579	1.458		

d) δ^{13}C-NLFA					
Error:plot					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Plant diversity	1	43.88	43.88	3.567	0.088^{+}
Residuals	10	123.01	12.3		
Error: Within					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Layer	1	1.236	1.236	0.752	0.406
Plant div:Layer	1	9.261	9.261	5.637	0.039*
Residuals	10	16.429	1.643		

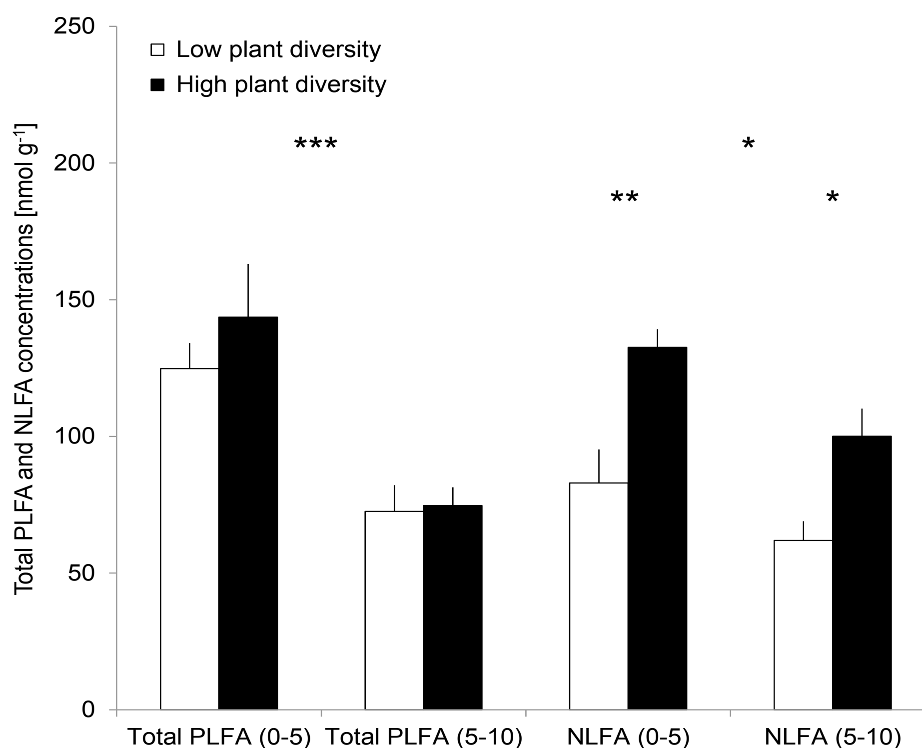

Figure 3.1. Mean (n=6 ± standard error) total phospholipid fatty acids (PLFA) concentration and neutral lipid fatty acid (NLFA) concentration in soils at two different soil depths (0-5 cm and 5-10 cm) as affected by low and high plant diversity. Stars in the first row (closer to bars) indicate significant differences between plant diversity levels; stars in the second row indicate significant differences between soil depths (*: p≤ 0.05; **: p≤ 0.01; ***: p≤ 0.001).

3.2.2 Compound specific δ^{13}C of PLFA and NLFA

In the top soil layer (Fig. 3.2), the most enriched δ^{13}C values were found for the fatty acids 15:0a, 17:0a (G+ bacteria), 16:1ω7, 16:1ω5 (G- bacteria), 18:2ω6,9 (saprotrophic fungi); and 16:1ω5N (AMF). Plant diversity affected significantly only a minority of the compound specific δ^{13}C values. The δ^{13}C values of 16:1ω7 and 18:1ω9 (G- bacteria) and 16:1ω5N (AMF) significantly increased (P < 0.05) with increasing plant diversity (Fig. 3.2; Appendix A2.3a). Moreover, with increasing plant diversity PLFA 17:1 and 16:1ω5 (G- bacteria) increased marginally significantly (P < 0.1. Fig. 3.2, Appendix A2.3a). The PLFA 16:1 and 18:1ω7 (G- bacteria) were higher in plots of high plant diversity, but this difference was not significant (Fig. 3.2; Appendix A2.3a). Contrarily, δ^{13}C values of G+ bacteria (14:0i, 15:0i, 15:0a, 16:0i, 17:0i. 17:0a), actinobacteria (16:0(10Me), 18:0(10Me)) and saprotrophic fungi (18:2ω6,9) were not affected by plant diversity and had often slightly lower values in plots with higher diversity (Fig. 3.2; Appendix A2.3a).

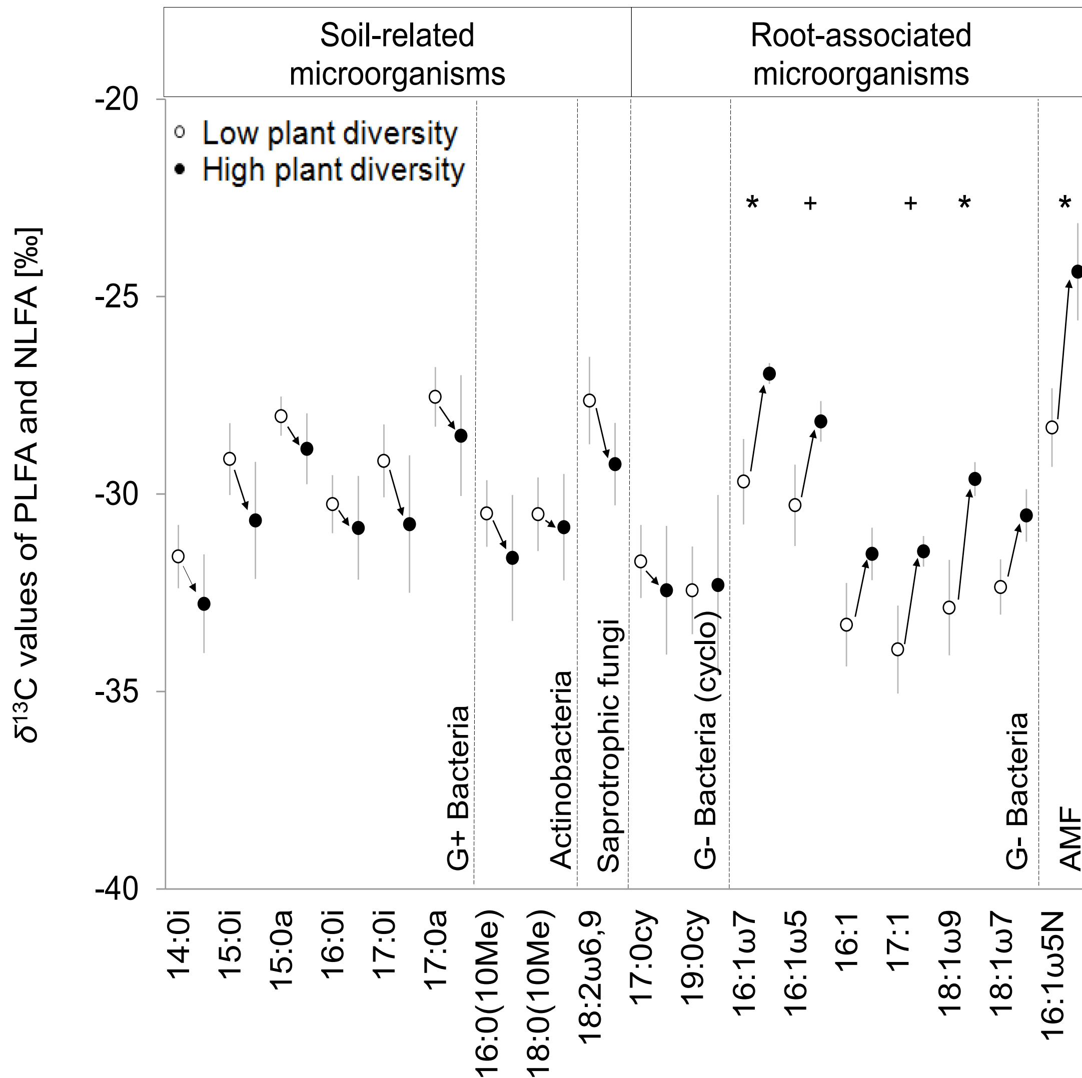

Figure 3.2. Mean (n=6 ± standard error) $\delta^{13}C$ values (‰) of individual phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) in top soils (0-5 cm depth) with low and high plant diversity. Fatty acids are grouped into Gram positive (G+) bacteria, actinobacteria, saprotrophic fungi, Gram negative (G-) bacteria (cyclo), Gram negative (G-) bacteria and arbuscular mycorrhizal fungi (AMF). Arrows pointing up represent an increase of $\delta^{13}C$ values with plant diversity; whereas arrows pointing down indicate an opposite trend. Stars and plus signs indicate significant and marginally significant differences of $\delta^{13}C$ values between plant diversity levels (*: $p \leq 0.05$; +: $p \leq 0.1$), respectively.

Furthermore, the average $\delta^{13}C$ values of total PLFA was not significantly different between plant diversity levels and soil layers (Fig. 3.3; Table 3.1c). The $\delta^{13}C$ values of G+ bacteria, actinobacteria, cyclic G- bacteria and saprophytic fungi (Appendix A2.3b) did not differ between plant diversity levels. In contrast $\delta^{13}C$ values of G- bacteria were significantly higher in plots with high plant diversity (Appendix A2.3b). The $\delta^{13}C$ values of NLFA was significantly higher in high plant diversity (Fig. 3.3; Table 3.1d), but no differences were observed between soil layers (Fig. 3.3; Table 3.1d).

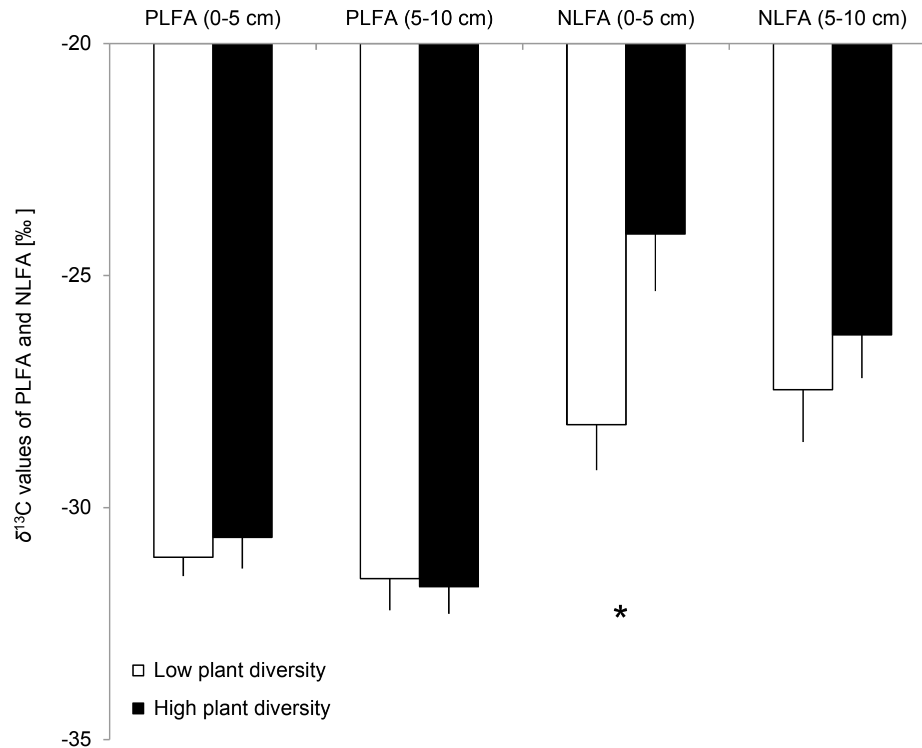

Figure 3.3. Average δ13C values (n=6 ± standard error) of total phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) in top (0-5 cm) and deeper soils (5-10 cm) from communities with low and high plant diversity.

3.2.3 Response of plant and soil related covariates to plant diversity

The majority of the considered covariates were positively correlated with plant diversity. However, only SOC concentration was significantly ($p \leq 0.05$, Appendix A2.4a and b) and root biomass and soil moisture were marginally significant ($p \leq 0.1$, Appendix A2.4a and b). The $\delta^{13}C$ values of roots and mean $\delta^{13}C$ of root sugars (calculated from $\delta^{13}C$ values of sucrose and raffinose-family) were negatively correlated to plant diversity (significantly and marginally significantly, respectively: Appendix A2.4a and b). All other covariates studied, namely canopy leaf nitrogen, shoot biomass, root length, specific root length, root sugar concentration and $\delta^{13}C$-SOC) did not varied significantly between plant diversity levels (Appendix A2.4a and b).

3.2.4 Plant and soil related impacts on microbial group specific $\delta^{13}C$ values

The positive relation of $\delta^{13}C$ values of G- bacteria to plant diversity was mainly mediated by plant related variables. The $\delta^{13}C$ values of G- bacteria were significantly correlated with root biomass (Fig. 3.4a; Tables 3.2 and 3.3 and Appendix A2.5), which was higher in more diverse plant communities. In contrast the $\delta^{13}C$ values of the other bacterial

groups, *i.e.* G+ bacteria, actinobacteria and G- bacteria (cyclo), were not affected by plant diversity. Their $\delta^{13}C$ values were best explained by soil related covariates, such as $\delta^{13}C$ values of SOC (Table 3.3 and Appendix A2.5; G+ bacteria: Fig. 3.4b). The isotopic signature of saprotrophic fungi (Table 3.3 and Appendix A2.5) was significantly explained by $\delta^{13}C$ values of soil and by $\delta^{13}C$ values of root material ($P < 0.1$). The higher $\delta^{13}C$ values of AMF was only positively related to plant diversity itself (Table 3.3 and Appendix A2.5), indicating that plant diversity effect on AMF was not mediated by the studied covariates (Table 3.3 and Appendix A2.5).

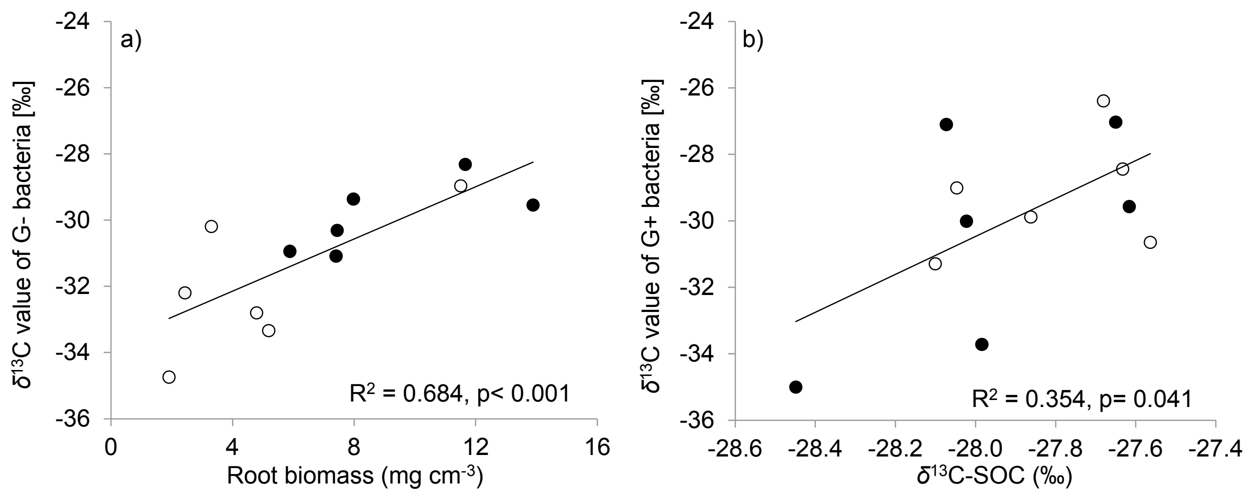

Figure 3.4. Linear correlations between: a) $\delta^{13}C$ values of Gram negative (G-) bacterial markers and root biomass; b) $\delta^{13}C$ values Gram positive (G+) bacterial markers and $\delta^{13}C$ values of soil organic carbon ($\delta^{13}C$ - SOC). Filled and empty circles represent high and low plant diversity, respectively. Significant differences are indicated by p values lower than 0.05.

Table 3.2. Results of automated selection of three best models (‘dredge’ function in ‘MuMIn’ package). Possible explanatory variables included were: soil moisture, soil organic carbon (SOC), canopy leaf nitrogen (N.area), root biomass, root length per volume (root length), specific root length, plant diversity (Div), δ^{13}C values of soil organic carbon (δ^{13}C-SOC), root material (δ^{13}C-Root) and root sugars (δ^{13}C-Sugars).

Microbial group	**Intercept**	**Soil moisture**	**SOC**	**N.area**	**Root biomass**	**Root length**	**Specific root length**	**Div**	**δ^{13}C-SOC**	**δ^{13}C-Root**	**δ^{13}C-Sugars**	**R^2**	**df**	**logLik**	**AICc**	**delta**	**Weight**
a) Gram positive bacteria																	
Best model	122.20								5.45			0.35	3	-24.64	58.3	0	0.17
2nd best model	145.90			-0.85					6.22			0.48	4	-23.35	60.4	2.13	0.06
3rd best model	137.30		3.19		-0.47				6.19			0.67	5	-20.68	61.4	3.07	0.04
b) Actinobacteria																	
Best model	134.30								5.93			0.30	3	-27.07	63.1	0	0.14
2nd best model	-46.88		5.83				0.02	-0.30				0.68	5	-22.40	64.8	1.67	0.06
3rd best model	-43.11		3.45				0.02					0.42	4	-25.97	65.6	2.51	0.04
c) Gram negative bacteria (cyclo)																	
Best model	169.80								7.25			0.31	3	-29.32	67.6	0	0.09
2nd best model	-27.72		5.07				0.03			0.89		0.72	5	-23.99	68.0	0.33	0.08
3rd best model	-43.32	0.51										0.24	3	-29.90	68.8	1.16	0.05
d) Gram negative bacteria																	
Best model	-34.07				0.42							0.68	3	-17.69	44.4	0	0.28
2nd best model	-97.34				0.46				-2.26			0.77	4	-15.70	45.1	0.73	0.19
3rd best model	-36.80				0.42						-0.14	0.71	4	-17.11	47.9	3.57	0.05

Table 3.2. (Continuation)

Microbial group	Intercept	Soil moisture	SOC	N.area	Root biomass	Root length	Specific root length	Div	δ^{13}C-SOC	δ^{13}C-Root	δ^{13}C-Sugars	R^2	df	logLik	AICc	delta	Weight
e) Saprotrophic fungi																	
Best model	164.70								6.93			0.49	3	-24.15	57.3	0	0.29
2nd best model	173.80						<0.01		7.22			0.56	4	-23.26	60.2	2.94	0.07
3rd best model	170.80					<0.01			7.12			0.54	4	-23.48	60.7	3.37	0.05
f) Arbuscular mycorrhizal fungi																	
Best model	-29.63							0.33				0.38	3	-28.04	65.1	0	0.19
2nd best model	-35.65		3.37									0.28	3	-28.99	67.0	1.91	0.07
3rd best model	-29.13				0.4							0.21	3	-29.51	68.0	2.94	0.04
g) Non-specific marker																	
Best model	164.60								6.96			0.41	3	-26.22	61.4	0	0.19
2nd best model	152.80			-0.71			0.01		6.56			0.51	4	-25.13	64.0	2.53	0.05
3rd best model	-45.22		5.84				0.02	-0.32				0.7	5	-22.06	64.1	2.67	0.05

Table 3.3. Results of the best ANOVA model (see Table 3.2) for each microbial group. Stars indicate significant differences of δ^{13}C values between plant diversity levels (***: p≤ 0.001; **: p≤ 0.01; *: p≤ 0.05; $^{+}$: p≤ 0.1)

Microbial group		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Gram positive bacteria						
	δ^{13}C-SOC	1	23.4	23.41	5.482	0.041*
	Residuals	10	42.7	4.27		
Actinobacteria						
	δ^{13}C-SOC	1	27.7	27.702	4.33	0.064^{+}
	Residuals	10	64	6.397		
Gram negative bacteria (cyclo)						
	δ^{13}C-SOC	1	41.4	41.41	4.445	0.061^{+}
	Residuals	10	93.2	9.317		
Gram negative bacteria						
	Root biomass	1	29	28.974	21.6	0.001***
	Residuals	10	13.4	1.339		
Saprotrophic fungi						
	δ^{13}C-SOC	1	37.8	37.83	9.619	0.011*
	Residuals	10	39.3	3.933		
Arbuscular mycorrhizal fungi						
	Plant diversity	1	46.7	46.73	6.218	0.032*
	Residuals	10	75.2	7.515		
Non-specific marker						
	δ^{13}C-SOC	1	38.2	38.17	6.877	0.026*
	Residuals	10	55.5	5.551		

3.3 Discussion

Undoubtedly, soil microorganisms exert an important control on C cycling in soils. It has been hypothesized that increasing C inputs to soils would potentially cause a positive priming of SOC (Fontaine et al., 2007). However, Lange et al. (2015) have shown that although increased C input to soil with high plant diversity does promote the soil microbial activity, the positive impact on SOC priming is neglectable when compared to SOC accumulation. In the study by Lange et al. (2015), increased root exudation was suspected as a possible mechanism of higher SOC accumulation accompanied with higher plant diversity. Here we were able to investigate the underlying mechanism of increased microbial carbon uptake. In contrast to the findings of earlier studies (Chung et al., 2007; Chung et al., 2009; Lange et al., 2015) we did not find evidence that plant diversity increases higher microbial C uptake at higher plant diversity by higher root exudation. Instead, root biomass was the best predictor for the observed patterns. This indicates that higher plant diversity increases the

access to plant-derived C for root-associated microorganisms, which are more capable of utilize easily decomposable C inputs (i.e. root exudates) in the rhizosphere, through more root biomass.

3.3.1 Effects of plant diversity and soil depth on the PLFA and NLFA concentrations

Previous studies from The Jena Experiment have reported a significant plant diversity effect on microbial biomass (Habekost et al., 2008b; Lange et al., 2014). In the present study, however, working with a subset of the plant diversity levels and a smaller number of replicates this positive effect of plant diversity on the total microbial biomass (measured as total PLFA concentration) was present but not significant. Nonetheless, the concentration of 16:1ω5N, which is a biomarker for the presence of AMF, was significantly higher in plots with higher plant diversity. AMF are intrinsically related to plants and trade plant C for nutrients. It has been suggested that in grassland ecosystems more than 10% of the photosynthetically fixed C is allocated to AMF. Therefore, AMF are likely to possess a high sensitivity for changes in photosynthetic assimilation, such as an increased C fixation observed in more diverse plant communities (Milcu et al., 2014). This in turn might result in increased AMF concentrations in ecosystems that support higher plant diversity, as our study shows. We also confirmed that concentrations of both total PLFA and NLFA decreased with increasing soil depth (Fig. 3.1) (Kramer and Gleixner, 2008; Huang et al., 2011).

3.3.2 Response of plant and soil related covariates to plant diversity

Plant diversity positively and significantly (or marginally significantly) influenced SOC concentrations, root biomass and soil moisture in our experiment. Increased belowground inputs of plant-derived C have been shown to enhance the SOC accumulation in soils with high plant diversity (Fornara and Tilman, 2008; Steinbeiss et al., 2008a; Lange et al., 2015). Furthermore, plant diversity increased root standing biomass (Ravenek et al., 2014) through increased primary productivity (Milcu et al., 2014). And finally, higher soil moisture in high plant diversity plots are likely the result of higher leaf area index, which provides shading to the upper layers of the soil, thus preventing evaporation (Lange et al., 2014). Moreover, previous investigations have shown that plant diversity influences positively and significantly other plant and soil related factors (e.g. aboveground biomass (Cardinale et al., 2006; Ravenek et al., 2014)). In our experiment, plant diversity was positively correlated to canopy leaf nitrogen, aboveground biomass and root length. However, the plant diversity

effect on those covariates was small and not significant, which was probably due to the small number of replicated in the Ecotron facility.

3.3.3 Plant and soil related impacts on ^{13}C uptake into soil microbial groups

We observed that the δ^{13}C values of G- bacteria and AMF increased with plant diversity. G- bacteria and AMF are commonly known as root-associated soil microorganisms (Treonis et al., 2004; Olsson and Johnson, 2005; Drigo et al., 2010) that are more abundant in the rhizosphere, especially G- bacteria (Denef et al., 2009). G- bacteria rely more on fresh rhizodeposits (Kramer and Gleixner, 2008; Esperschutz et al., 2009; De Deyn et al., 2011; Balasooriya et al., 2012; Staddon et al., 2014) as energy source and are therefore very likely to monitoring the C transfer from the aboveground to the belowground system. Remarkably, our results provide evidence that only root biomass as a plant related variable is a significant driver for the observed increased δ^{13}C values of G- bacteria with increasing plant diversity (Fig. 3.4a; Appendix A2.5). We suggest that higher root biomass facilitates the access of root-associated microorganisms to recently fixed plant-derived C. Our results are in line with previous investigations (De Graaff et al., 2013; De Graaff et al., 2014) that have emphasized the importance of roots for inputs of plant-derived C into soil. However, in contrast to our results the previous studies identified specific root length as a driver of carbon transfer into soils. These different findings might reflect differences in the study design and study object. While previous studies investigated the impact of root architecture of individual plants on carbon transfer, we worked with plant species mixtures. Furthermore, in our experiment root biomass was the only root trait, which significantly increased with plant diversity (Appendix A2.4b). This might be another reason why in our study root biomass was tested as best predictor underlying the plant diversity effect on soil microbial C uptake. However, irrespective of the specific morphological root covariate, explaining the plant diversity effect, higher exudation rates seemed not to be the mechanism responsible for the G- bacteria enrichment at higher plant diversity. Root exudates comprise several low and high molecular weight compounds (such as organic acids, sugars, amino acids and proteins (Nguyen, 2003)), it has been observed that the chemical composition of the root exudates might change to fulfil specific plants' needs (e.g. under low P availability (Zhang et al., 1997)). However, to our knowledge there are not yet investigations that report changes in the chemistry of root exudates with changing plant diversity. Sugars are among the most abundant (Jones and Darrah, 1996) and easily decomposable C sources exuded by the roots (Paterson et al., 2007), therefore we expected that changes in root exudation would be evidenced by a linkage in the

changes of the C assimilated by root-associated microorganisms (that commonly decompose the most labile C resources) and the changes in the ^{13}C incorporated to root sugars; although our results demonstrated that this process was not occurring in our system. We found neither the total amount of root sugars nor their $\delta^{13}C$ values, which would indicate higher flux rates, affected G- bacteria. This is also in line with our observation that higher $\delta^{13}C$ values of AMF at higher diversity were not related to any of the plant-related covariates (Table 3.3 and Appendix A2.5). AMF actively approach their host plant using chemotactic growth and actively trade C for nutrients (Engels et al., 2000; Drigo et al., 2010). Therefore AMF are less dependent on root development and accessibility than bacteria that are less mobile in soil. We hypothesize that the increased $\delta^{13}C$ content of AMF yields from an increased C-nutrient trading following a higher mineralization rate at higher diversity (see discussion below).

We observed that the $\delta^{13}C$ values of roots and root sugars (raffinose-family) were significantly higher in low plant diversity (Fig. 3.5; Appendix A2.4a and b). Additionally, $\delta^{13}C$ values of CO_2 ecosystem respiration (plant and soil combined respiration) were marginally significantly higher in low plant diversity (Appendix A2.2); therefore we suggest that the lower enrichment of $\delta^{13}C$ in roots and root sugars (raffinose-family) at higher plant diversity is an effect of complementary use of soil respired CO_2. In high plant diversity plots, soil respiration is greater due to a higher microbial biomass (Lee and Jose, 2003; Eisenhauer et al., 2010), therefore the resulting $\delta^{13}C$-depleted CO_2 from SOC mineralization might cause a “dilution effect” of the $\delta^{13}C$ values of CO_2 when re-assimilated by plants (Busch et al., 2013). This suggests that at higher plant diversity additional resources are used for growth.

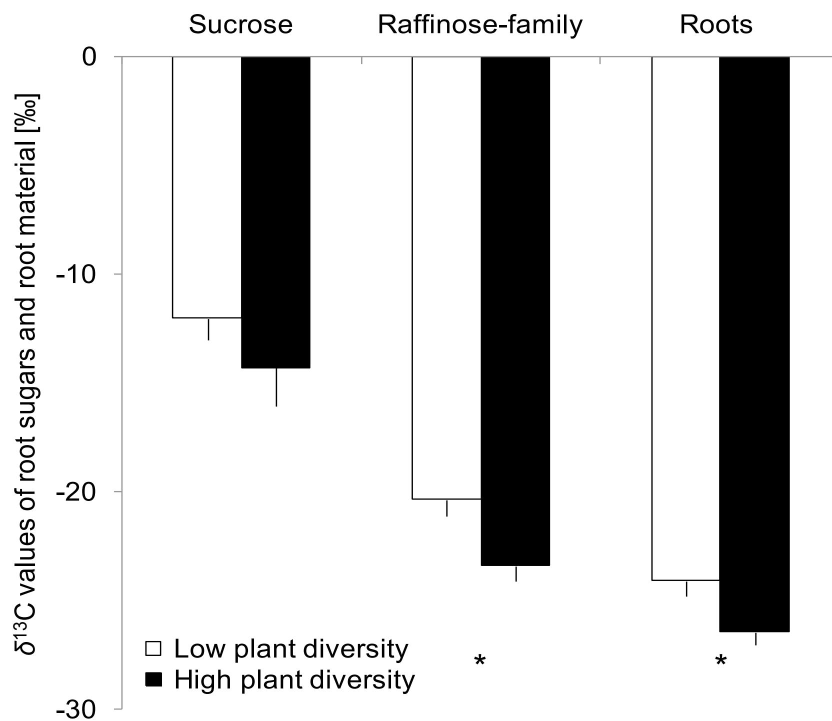

Figure 3.5. Mean (n=6 ± standard error) δ^{13}C values (‰) of root sugars (sucrose and raffinose-family) and root material in top soils (0-5 cm) with low and high plant diversity. Stars indicate significant differences of δ^{13}C values between plant diversity levels (*: $p \leq 0.05$).

The G- bacteria (cyclo) markers did not respond as other G- bacteria PLFA, as their δ^{13}C values and trends were more similar to those of G+ bacteria PLFA (Table 3.3 and Appendix A2.5). Similar results have been previously observed (Bird et al., 2011), and are likely attributed to the dormancy state of G- bacteria (cyclo) (Butler et al., 2003; Elfstrand et al., 2008). The δ^{13}C values of G+ bacteria, actinobacteria and saprotrophic fungi were not affected by plant diversity. Instead their δ^{13}C values were positively correlated to δ^{13}C values of soil (Table 3.3 and Appendix A2.5) indicating that SOC is their major C source (Elfstrand et al., 2008; Kramer and Gleixner, 2008; Bird et al., 2011; Bahn et al., 2013). The interpretation of saprotrophic fungi is more complex, as this microbial group might be related to a number of functions in soil. For instance, saprotrophic fungi could use different C substrates, like dead biomass (Klamer and Hedlund, 2004), SOC (Garcia-Pausas and Paterson, 2011) and root exudates (Lemanski and Scheu, 2014). The positive correlation of δ^{13}C values of saprotrophic fungi and δ^{13}C of soil indicates a preferred consumption of SOC as C source in this experimental set up. Moreover, similarly to G+ bacteria, the high δ^{13}C values observed in saprotrophic fungi points to C recycling through the microbial food web. We suggest that δ^{13}C values of saprotrophic fungi and their lack of response to plant diversity in the system we

investigated are the result of a combined utilization of different sources of C, namely plant-derived C, recycled microbial C and SOC.

3.4 Conclusion

This study presents evidence that high plant diversity promotes an increased C utilization to and cycling in the belowground system. Possible mechanisms are 1) better access to recently photosynthesized plant-derived C resources for root-associated microorganisms but not higher exudation rates and 2) increased C allocation to AMF. Additionally, by increasing the access to plant-derived C deposited in soils, high plant diversity has positive impacts on root-associated microorganisms (G- bacteria and AMF), which are capable of utilizing newly photosynthesized C and are, therefore triggering the cycling of new C entering the soil system.

CHAPTER IV

THE POSITIVE PLANT DIVERSITY EFFECT ON SOIL MICROBIAL COMMUNITY AND SOIL BACTERIAL DIVERSITY IS DRIVEN BY PLANT- AND SOIL-RELATED FACTORS

Abstract

In this study, we investigated the plant diversity effect on the soil microbial biomass, soil microbial community and bacterial diversity. We investigated soils from 82 plots spanning over a wide gradient of plant diversity, from 1 to 60 plant species. Phospholipid fatty acid (PLFA) analyses and terminal-restriction fragment length polymorphism (T-RFLP) measures described how the microbial community and the bacterial diversity, respectively, were influenced by plant diversity. Our results showed that soil microbial biomass, soil microbial community and bacterial diversity were positively and significantly affected by plant diversity, namely plant species richness and abundance of specific plant functional groups. These positive plant diversity effects were attributed mainly to higher availability and diversity of resources provided by improved vegetation conditions, such as higher root biomass, and enhanced soil properties, such as increased soil moisture and soil organic carbon (SOC) concentrations. Moreover, plant diversity influenced similarly the amounts of root-associated and soil-related soil microorganisms thereby triggering the cycling of new and old carbon (C) in the belowground system. However the evenness of the microbial community markers decreased with increasing plant diversity. Soil microbial markers indicative of root-associated microorganisms dominated in high plant diversity which could indicate that plant diversity favors specific functions in the soil system.

4.1 Introduction

Plant diversity, including plant species richness, functional group richness and presence and abundance of specific plant functional groups, play a key role in controlling ecosystem functions relevant for the cycling of C in terrestrial ecosystems (Hooper et al., 2005; Cardinale et al., 2012; Hooper et al., 2012). The effect of plant diversity on the C cycle in soils has been vastly investigated because soil C sequestration might counteract increasing atmospheric CO_2 concentrations that are triggering the global climate change (Batjes, 1998; Schmidt et al., 2011; Batjes, 2014). The transfer of C between plants and soils is mainly mediated by the soil microbial activity. The input of plant-derived C to soils triggers the microbial C cycling firstly by providing fresh substrates for soil microorganisms growth (Gleixner, 2013) and secondly by stimulating the mineralization of already present soil organic matter (SOM) (Fontaine et al., 2003). The latter process releases additional nutrients that are essential for plant growth. Plant diversity influences plant- and soil-related properties which are relevant for the microbial C cycling in the belowground system. For instance, higher root biomass (Ravenek et al., 2014) and greater leaf area index (LAI), as well as greater soil moisture (Lange et al., 2014) and higher soil organic carbon (SOC) (Fornara and Tilman, 2008; Steinbeiss et al., 2008b) have been linked to more abundant soil microbial communities, consequently impacting the processes that are mediated by soil microorganisms, such as microbial C uptake, microbial C cycling and ultimately soil C accumulation (Gleixner, 2013; Lange et al., 2015; Mellado-Vázquez et al., 2016).

It is known that the effect of plant diversity on soil microbial dynamics impacts the cycling of majorly important elements, such as C, however the processes that are controlled by these interactions are still not fully understand. Phospholipid fatty acids (PLFA) analyses are greatly used in soil ecology to quantify the total microbial biomass and to classify the microbial community (Chowdhury and Dick, 2013; Kramer et al., 2013; Wu et al., 2013; Ng et al., 2014; Watzinger, 2015). Most commonly, PLFA markers are classified into different microbial groups based in their chemical structure, in general branched saturated PLFAs represent Gram positive (G+) bacteria (Zelles, 1997), monounsaturated PLFAs are indicative of Gram negative (G-) bacteria (Zelles, 1997) and polyunsaturated PLFAs represent fungi and higher organisms (Zelles, 1997). Following the classification given by PLFA analyses, bacterial and fungal organisms are often designated to different functions related to their preferred C substrates as root-associated and soil-related microorganisms: G- bacteria and AMF are commonly designated as root-associated microorganisms because they are more likely to feed on readily decomposable sources of C, such as rhizodeposits (Denef et al., 2009)

and via direct plant C uptake (Drigo et al., 2010), respectively. G+ bacteria and saprotrophic fungi are usually denominated as soil-related microorganisms, they have a high affinity for complex compounds, such as stabilized soil organic carbon (SOC) and plant litter (Kramer and Gleixner, 2008; Garcia-Pausas and Paterson, 2011; Bahn et al., 2013). Although every part of the soil microbial community is particularly adapted to perform distinct specific functions (related to C cycling), their functions complement each other allowing the most efficient cycling of elements belowground. In that sense, high microbial diversity is crucial for assuring an effective soil functioning, because different microbial species perform slightly distinct functions mainly through complementary use of resources (Bell et al., 2005) belowground. Highly diverse plant communities might impact positively the diversity of microorganisms in soils through increased resource heterogeneity (Loreau and Hector, 2001), thereby imposing an indirect control on soil microbial functions. Phylogenetic techniques, such as terminal-restriction fragment length polymorphism (T-RFLP) analysis are very useful to measure microbial diversity in soils (Schütte et al., 2008). In contrast, analysis that provide less resolved results, such as PLFA analysis are less suited to provide information on the microbial diversity in the environment (Frostegård et al., 2011). However, the evenness of PLFA markers allows identifying the dominant microbial markers in soils, which can be further assign to specific functions in soils.

We hypothesized firstly, that high plant diversity modifies plant- and soil-related conditions that promote increased resource availability that benefits the soil microbial functions by impacting soil microbial biomass, community composition and diversity; and secondly that this greater resource input activates all parts of the soil microbial community (root-associated and soil-related microorganisms). However higher amounts of new C resources favors slightly more the functions performed by root-associated microorganisms. In order to test our hypotheses, we explored how plant diversity shapes the microbial community in soils from The Jena Experiment (Roscher et al., 2004), a biodiversity experiment which comprises a wide gradient of plant diversity levels. We analysed PLFA profiles from all soil samples to identify changes in microbial biomass and microbial community composition related to plant diversity. The impact of plant diversity on bacterial diversity was measured with T-RFLP.

4.2 Results

4.2.1 Influence of plant diversity on plant and soil-related covariates

Plant species richness influenced significantly and positively the plant and soil related covariates (Appendices A2.6 and A4.1). Plant functional group richness and abundance of legumes (Appendix A4.1) had a positive and significant impact on LAI and soil moisture, respectively. With increasing plant diversity, mean values of root biomass increased from 1.86 ± 1.88 to 10.29 ± 4.10 g m^{-2}; soil moisture increased from 12.44 ± 2.61 to 17.80 ±1.08 %; SOC increased from 2.24 ± 0.34 to 2.70 ± 0.13 % and lastly the LAI increased from 0.57 ± 0.52 to 3.50 ± 0.22 (Appendix A2.6).

4.2.2 Soil microbial biomass

Total PLFA concentration (henceforth termed as microbial biomass) was significantly influenced by block and plant species richness (Table 4.1). Microbial biomass increased significantly with increasing plant species richness, (Fig. 4.1a; Table 4.1). Number of functional groups (Fig. 4.1b; Table 4.1) and abundance of grasses (Fig. 4.2a; Table 4.1) small (Fig. 4.2b; Table 4.1) and tall herbs (Fig. 4.2c; Table 4.1) and legumes (Fig. 4.2d; Table 4.1) did not affect significantly the microbial biomass. Root biomass, soil moisture and SOC drove significantly the plant species richness effect on the microbial biomass (Table 4.1).

4.2.3 Plant diversity effect on different soil microbial groups

Plant diversity, namely plant species richness, number functional groups and abundance of specific functional groups affected similarly all parts of the soil microbial community. Root-associated and soil-related microorganisms were positively and significantly affected by plant species richness (Table 4.1) and presence of small herbs (Table 4.1). Number of functional groups and abundance of grasses, tall herbs and legumes were not significantly related to any microbial group (Table 4.1). Interestingly, the positive plant species richness effect was significantly explained by root biomass, soil moisture and SOC (Table 4.1). Moreover, the small herbs effect could be only partially attributed to root biomass, soil moisture and SOC because the mere effect of small herbs continued to be significant after testing the driving force of all covariates.

Table 4.1. ANOVA (Analysis of variance) and ANCOVA (analysis of covariance) results from the changes in microbial biomass, root-associated and soil-related microorganisms, bacterial diversity and PLFA evenness related to plant diversity measures: plant species richness (PSR); functional group (FG); grasses, small and tall herbs and legumes. Root biomass, leaf area index (LAI), soil moisture and soil organic carbon (SOC) were set as covariates to test for their influence on the changes in microbial biomass, root-associated and soil-related microorganisms, bacterial diversity and PLFA evenness. Bold numbers represent significant differences (P values ≤ 0.05).

		ANOVA		**ANCOVA**	
	Df	**F value**	**P**	**F value**	**P**
Microbial biomass					
Block	1	24.96	< **0.001**	29.37	< **0.001**
Root biomass	1			6.74	**0.011**
LAI	1			0.00	0.978
Soil moisture	1			13.22	< **0.001**
SOC	1			5.53	**0.021**
PSR	1	8.14	**0.006**	1.18	0.280
FG	1	0.76	0.386		
Grasses	1	0.22	0.637		
Small herbs	1	3.33	0.072		
Tall herbs	1	0.10	0.759		
Legumes	1	0.17	0.679		
Root-associated					
Block	1	16.52	< **0.001**	19.63	< **0.001**
Root biomass	1			7.23	**0.009**
LAI	1			0.04	0.840
Soil moisture	1			15.60	< **0.001**
SOC	1			5.98	**0.017**
PSR	1	9.57	**0.003**	1.43	0.236
FG	1	0.63	0.428		
Grasses	1	0.10	0.753		
Small herbs	1	6.98	**0.010**	5.19	**0.026**
Tall herbs	1	0.00	0.980		
Legumes	1	0.24	0.623		

Table 4.1. Continuation

		ANOVA		ANCOVA	
	Df	F value	P	F value	P
Soil-related	1				
Block	1	24.48	< **0.001**	29.35	< **0.001**
Root biomass	1			6.27	**0.015**
LAI	1			0.04	0.843
Soil moisture	1			13.07	< **0.001**
SOC	1			6.68	**0.012**
PSR	1	6.96	**0.010**	0.94	0.336
FG	1	1.25	0.268		
Grasses	1	0.06	0.811		
Small herbs	1	7.83	**0.007**	5.97	**0.017**
Tall herbs	1	0.09	0.768		
Legumes	1	0.46	0.501		
Bacterial diversity					
Block	1	1.27	0.264		
Root biomass	1			10.96	**0.001**
LAI	1			12.89	< **0.001**
Soil moisture	1			15.88	< **0.001**
SOC	1			1.13	0.290
PSR	1	21.80	< **0.001**	0.13	0.720
FG	1	4.89	**0.030**	1.56	0.216
Grasses	1	0.35	0.555		
Small herbs	1	1.11	0.295		
Tall herbs	1	2.71	0.104		
Legumes	1	3.30	0.073		
PLFA evenness					
Block	1	19.80	< **0.001**	22.72	< **0.001**
Root biomass	1			10.57	**0.002**
LAI	1			0.27	0.607
Soil moisture	1			10.77	**0.002**
SOC	1			4.74	**0.033**
PSR	1	10.39	**0.002**	0.49	0.485
FG	1	0.06	0.815		
Grasses	1	0.11	0.740		
Small herbs	1	3.53	0.064		
Tall herbs	1	0.03	0.860		
Legumes	1	0.00	0.951		

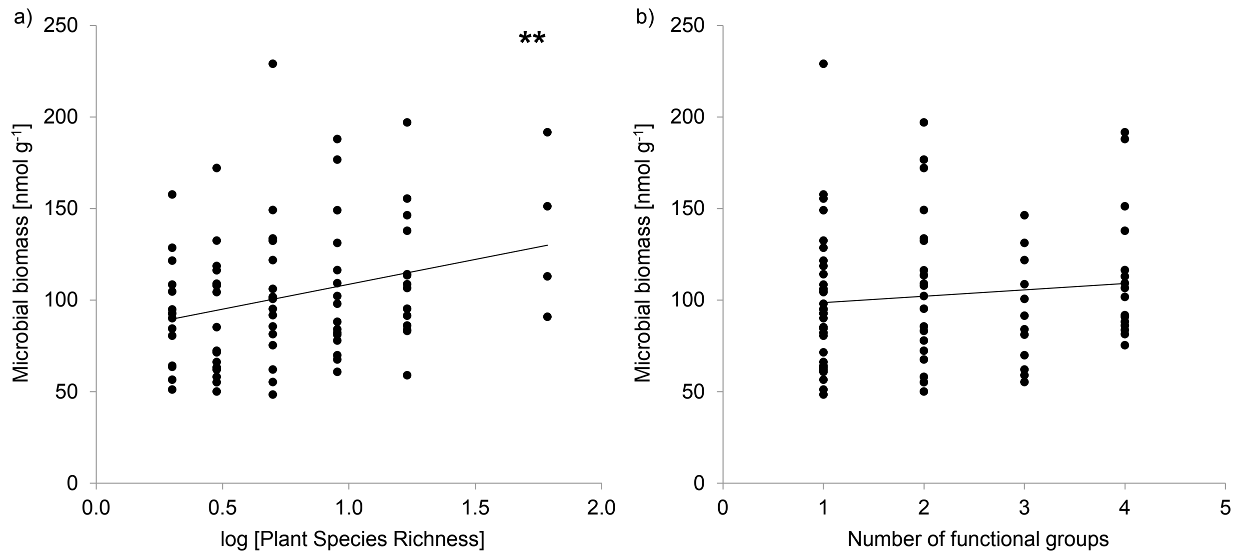

Figure 4.1. Microbial biomass in (a) soils with a gradient in plant diversity (plant species richness) and (b) soils with a gradient of plant functional groups.

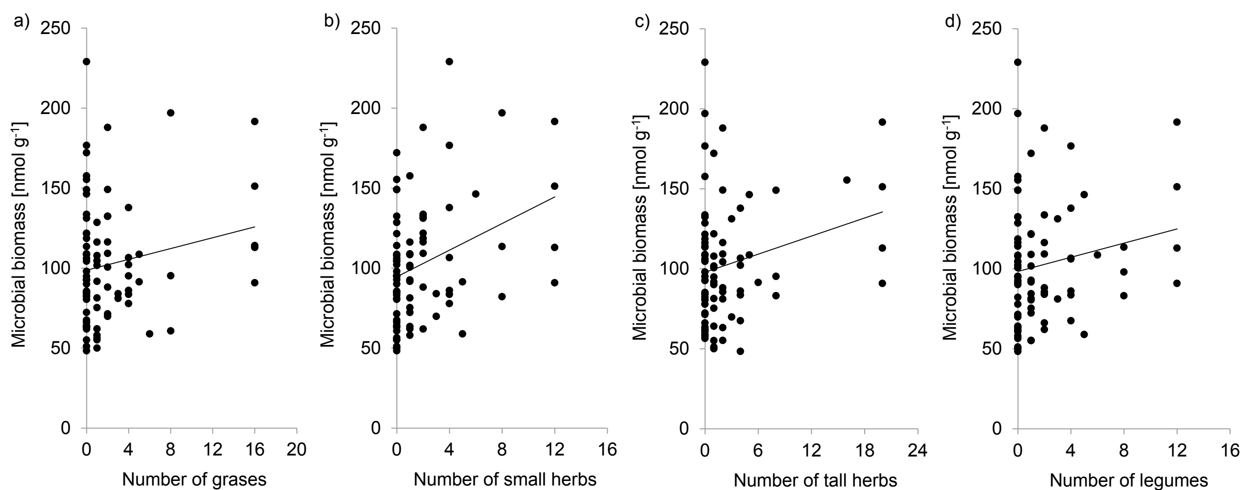

Figure 4.2. Microbial biomass in soils with gradient different plant functional groups: (a) number of grasses; (b) number of small herbs; (c) number of tall herbs and (d) number of legumes.

4.2.4 Bacterial diversity and PLFA evenness

Bacterial diversity increased significantly with increasing plant species richness (Fig.4.3a; Table 4.1) and functional group richness (Table 4.1). These positive plant diversity effects were significantly driven by increasing root biomass, LAI and soil moisture (Table 4.1). Contrastingly, PLFA evenness decreased significantly with increasing plant species richness affected by plant species richness (Fig. 4.3b; Table 4.1), which was attributed to the increased root biomass, soil moisture and SOC (Table 4.1) in higher plant diversity.

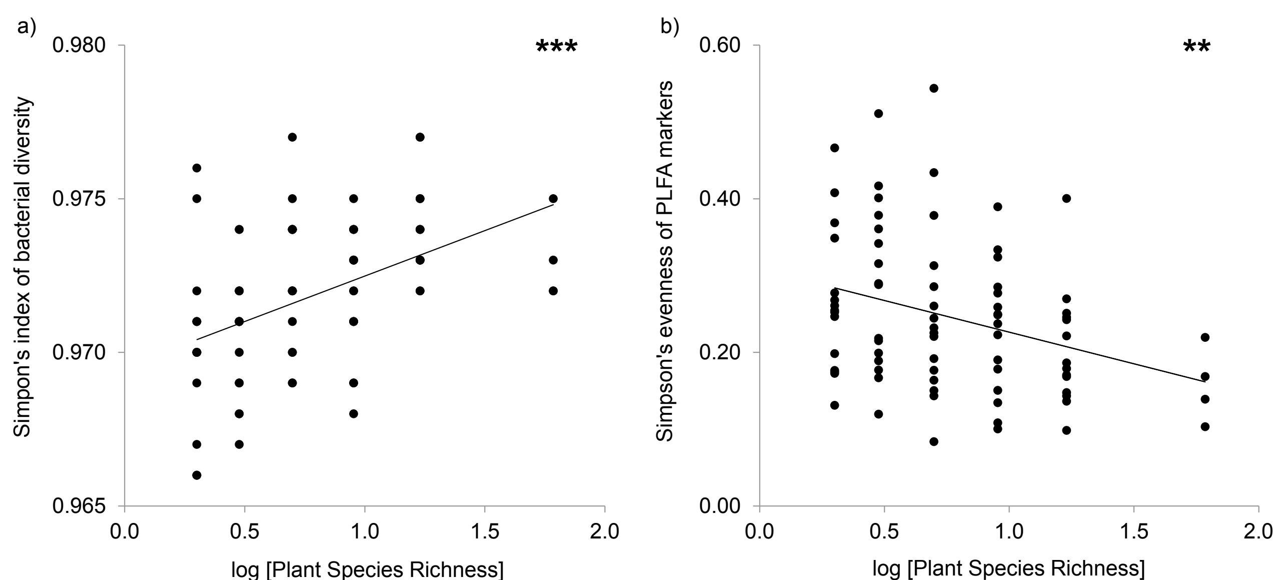

Figure 4.3. a) Bacterial diversity measured from Simpson index of T-RFLP and b) PLFA evenness measured from Simpson's index of diversity. Both a) and b) correspond to soils with a gradient in plant diversity.

4.3 Discussion

4.3.1 Plant diversity effect on plant and soil-related covariates

The plant diversity effect on plant and soil-related factors is inherently correlated. Root biomass, LAI, soil moisture and SOC increased significantly with increasing plant diversity (Appendices A3.1 and A3.2). Plant diversity is known to increase plant biomass above and belowground through the increase of C allocation due to higher photosynthetic assimilation (Milcu et al., 2014). Presumably, LAI is also augmented in higher plant species richness as a result of increasing aboveground biomass. Soil moisture increase in higher plant diversity has been attributed to greater LAI, which increases shading to the upper soil layers avoiding soil evaporation (Rosenkranz et al., 2012; Lange et al., 2014). Lastly, in highly diverse plant communities, there is an increased transfer (due to higher root biomass

(Mellado-Vázquez et al., 2016)) of resources, especially C to the soil system, thereby causing increasing SOC concentrations.

4.3.2 Soil microbial biomass

Microbial biomass was significantly greater in soils with higher plant species richness (Fig. 4.1a; Table 4.1); previous studies of the microbial biomass in soils have demonstrated the relevance of plant diversity for the development of an abundant soil microbial community (e.g.: Zak et al. (2003) and Reich et al. (2012)). Particularly in the Jena Experiment field site, Habekost et al. (2008) were the first to explore this relation and followed by Lange et al. (2014) showed that plant diversity influenced significantly the microbial biomass in these soils, here we additionally observed that the positive impact of plant diversity on the microbial biomass becomes more pronounced over time.

In our study site, greater root biomass and higher SOC concentrations (Table 4.1) drove significantly the plant diversity effect on the microbial biomass; some of the mechanisms behind this positive plant diversity effect are linked to higher availability (SOC (Steinbeiss et al., 2008b)) and accessibility (higher root biomass (Mellado-Vázquez et al., 2016)) of resources belowground, which is consisting with our observations. Additionally, the positive plant diversity effect on the microbial biomass could be the result of improved microhabitat conditions (e.g. increased soil moisture, Lange et al., 2014) in more diverse plant-soil ecosystems. Furthermore, Habekost et al. (2008) reported a significant effect of the abundance of legumes on the microbial biomass, whereas Lange et al. (2014) found no significant effect of any plant functional group. Similarly to Lange et al., (2014), we found that abundance of specific plant functional groups did not influence significantly the microbial biomass in our system.

4.3.3 Plant diversity effect on soil microbial groups

Our results demonstrated that increasing plant diversity promotes the development of different microbial groups in a similar fashion (Tables 4.1). Both, root-associated and soil-related microorganisms were positively and significantly influenced by plant species richness and abundance of small herbs. Root biomass, soil moisture and SOC explained significantly these plant diversity effects. Higher soil moisture facilitates the dissolution and motility of resources belowground (Davidson et al., 2000), thereby enhancing resource availability for microbial C uptake. SOC is comprised by two main fractions: a labile C fraction (e.g. recently photosynthesized and easily decomposable C) and a more stabilized C fraction (Breulmann et

al., 2012). Unfortunately, in the framework of our study, SOC concentrations reflect both fractions together. Thus, possibly the positive driving force of SOC over the plant diversity effects on the two major soil microbial groups (i.e. root-associated and soil-related microorganisms) reflects the influence of recent plant-derived C resources for root-associated microbes (Denef et al., 2009) and older C substrates for soil-related microorganisms (Kramer and Gleixner, 2008; Bahn et al., 2013). Furthermore, since greater root biomass in high plant diversity provides with a more appropriate habitat where root-associated microbes can thrive due to a better access to plant-derived C resources (De Graaff et al., 2013; De Graaff et al., 2014; Mellado-Vázquez et al., 2016) we expected root biomass to explain only the significant effect of plant diversity on root-associated microorganism, however root biomass also explained significantly the plant diversity effect on soil-related microorganisms, we suggest that this might be an indirect response. Apart from feeding microorganisms that are capable to decompose easily decomposable C resources, such as root-associated ones, root-derived C inputs promote the decomposition of SOM (Fontaine et al., 2003), thereby promoting the growth of microorganisms that are better suited to decompose more complex C substrates, such as soil-related microorganisms (Kramer and Gleixner, 2008; Garcia-Pausas and Paterson, 2011; Bahn et al., 2013).

4.3.4 Plant diversity effect on bacterial diversity and PLFA evenness

Plant diversity (plant species and functional group richness) influenced positively and significantly the bacterial diversity in our system (Fig. 4.3a; Table 4.1). In soils with higher plant diversity not only the quantity of resources is higher but more relevant the quality and diversity of those resources is also greater (Loreau and Hector, 2001; Bell et al., 2005), thus complementing the needs of a wider range of microbial species. We further observed that this response was controlled by increased resource availability factors, such as root biomass, LAI and soil moisture (Table 4.1). In contrast, the evenness of PLFA was lower in high plant diversity (Fig. 4.3b; Table 4.1), this indicated that in higher plant diversity levels fewer PLFA markers, mainly root-associated microbial markers, e.g. 16:1ω7, 16:1ω5, 18:1ω7 and 18:1ω9, responded to plant diversity. This suggests that root-associated microorganisms represent a larger part of the bacterial species in our system, and consequently the functions performed by root-associated microorganism are favored in higher plant diversity.

4.4 Conclusion

Plant species richness and the abundance of individual plant functional groups influenced significantly the microbial development in soils by favoring C input related factors, such as root biomass, LAI, soil moisture and SOC. Although the two major groups of soil microorganisms: root-associated and soil-related microbes were similarly affected by the plant and soil related drivers, decreasing evenness of bacterial markers as a response of increasing plant diversity suggested that root-associated microorganisms were more strongly influenced by plant diversity. This could indicate that even though plant diversity plays important role for the cycling of both new and old C resources belowground, the impact of plant diversity on the formation of new C in the soils is stronger.

CHAPTER V

THE SOIL MICROBIAL COMMUNITY AND ITS CARBON UPTAKE ARE MORE AFFECTED BY SOIL TYPE AND SEASON THAN BY VEGETATION TYPE IN A VEGETATION CHANGE EXPERIMENT (C3 AND C4 PLANTS)

Abstract

This study investigates the influence of different vegetation types (C3 and C4 plants), soil type and seasonal changes on the soil microbial biomass, soil microbial community composition and soil microbial carbon (C) uptake. In 2012 we collected soil samples in the growing and non-growing season from an experimental site cropping C3 and C4 plants for 6 years on two different soil types (sandy and clayey). Phospholipid fatty acids (PLFAs) and their compound-specific δ^{13}C values were used to determine microbial biomass and the flow of C from plants to soil microorganisms, respectively. The most important factor controlling the total microbial biomass was season with higher abundances in the growing season. The microbial community composition was mainly explained by soil type. The underlying drivers of soil type were differences in soil organic carbon (SOC) and root biomass. Higher amounts of SOC were driving the predominance of G+ bacteria, actinobacteria and cyclic G- bacteria in sandy soils; whereas root biomass was strongly related to the increased proportions of G- bacterial PLFA markers in clayey soils. The uptake of plant-derived C in G- bacteria increased significantly in clayey soils in the growing season. This increase was positively and significantly driven by root biomass. Moreover, changes in plant-derived C among microbial groups pointed to specific capabilities of different microbial groups to decompose distinct sources of C. We concluded that soil texture and favorable growth conditions driven by rhizosphere interactions are the most important factors controlling the soil microbial community. Our results demonstrated that a change of C3 plants vs. C4 plants has no effect on the soil microbial community and its functioning. Thus, such experiments are well suited to investigate soil organic matter dynamics as they allow tracing the C flow from plants into the soil microbial community without interacting with other experimental factors.

5.1 Introduction

Soils hold a very relevant position in the carbon (C) cycling at a global scale since they store the majority of terrestrial organic carbon (Lal, 2004). Therefore, it is crucial to understand how processes, such as accumulation and decomposition of soil organic carbon (SOC) might be affected by different ecosystem properties and environmental conditions. Soil microorganisms play a key role in controlling formation, decomposition and accumulation of SOC (Gleixner et al., 2002; Balser and Firestone, 2005; Bardgett et al., 2005; Lange et al., 2015). The microbial biomass in soils is primarily controlled by plant-derived C resources (Wardle, 2002). Simultaneously, the microbial community in soils remobilize nutrients that enhance plant growth (Porazinska et al., 2003). Soil microbial communities are highly affected by several abiotic and biotic factors such as soil texture (Merckx et al., 1985; Johnson et al., 2003; de Vries et al., 2012), pH (Thoms et al., 2010), soil moisture (Berg and Steinberger, 2010; Lange et al., 2014), temperature (Medeiros et al., 2006), vegetation type (Grayston et al., 2001; Berg and Steinberger, 2010; Epron et al., 2011), plant diversity (Lange et al., 2014) and seasonality (Habekost et al., 2008; Cao et al., 2011; Thoms and Gleixner, 2013). Though, the impact of these factors on the soil microbial community have been investigated individually; so far little is known about their relative importance for shaping the microbial community composition as well as its functioning. In addition, different soil microbial groups utilize C from different sources, Gram positive (G+) bacteria are most commonly decomposers of SOC (Bahn et al., 2013; Mellado-Vázquez et al., 2016); Gram negative (G-) bacteria have high affinity for plant-derived C, such as root exudates (Denef et al., 2009; Mellado-Vázquez et al., 2016)), while saprotrophic fungi are able to decompose root exudates and plant litter (Treonis et al., 2004) as well as SOC (Garcia-Pausas and Paterson, 2011; Mellado-Vázquez et al., 2016). Analyses of phospholipid fatty acids (PLFAs) are a helpful mean to measure the living soil microbial biomass and to describe the soil microbial community composition through the assignment of individual PLFA markers to different soil microbial groups (e.g. (Frostegard et al., 1991; Bossio and Scow, 1998; Bossio et al., 1998; Zelles, 1999b; Potthoff et al., 2006; Habekost et al., 2008; Thoms et al., 2010)). Generally, distinct PLFA markers are specific for different bacterial and fungal groups, however the microbial origin of some markers might change depending on ecosystem type (soil, sediment, etc.), quality and availability of substrates, therefore the designation of specific microbial origin to PLFA markers must be done with due care. Nonetheless, compound specific isotope ratios of PLFAs, in combination with isotopic labeling, are useful to determine the C dynamics in the soil microbial community (Miltner et al., 2004; Evershed

et al., 2006; Kindler et al., 2009; Garcia-Pausas and Paterson, 2011). Focal advances in the understanding of C flow between plants, microorganisms and soil organic matter derive from vegetation change experiments (Balesdent and Balabane, 1996; Kramer and Gleixner, 2006; Blagodatskaya et al., 2011; Gleixner, 2013). In such experiments, the native plant community of either C3 or C4 plants is exchanged by the respective other plant type. Differences in the photosynthetic pathways of C3 and C4 plants result on naturally distinct δ^{13}C values of both plant types (C3: -25 ‰; C4: -14 ‰ (Degens, 1969; O'Leary, 1981)) that are also reflected in the δ^{13}C of SOC. However, there are uncertainties if such a vegetation change from C3 to C4 plants only changes the isotopic signal of the C source or if it is also changing the soil microbial community composition and their functions in soils.

In this study we investigated the effect and the relative importance of C3-C4 vegetation change, soil types and season on the soil microbial community composition and its functioning in terms of C uptake. We studied total soil microbial biomass, soil microbial community composition as well as their isotopic C composition on a replicated and input controlled C3-C3 experiment, with two distinct soil types (sandy and clayey) in the non-growing and growing seasons. Furthermore, we were able to assess the microbial uptake of plant-derived C for distinct microbial groups depending on plant type and season. Specifically we tested if i) the soil microbial community composition and abundance are affected by vegetation type, soil type and seasonal changes and ii) if the C dynamics of the soil microbial community are affected by these parameters and iii) if microbial uptake of plant-derived C differs among microbial groups.

5.2 Results

5.2.1 Microbial biomass and microbial community composition

Microbial biomass (measured as total PLFA concentration) changed significantly with season and soil type (Table 5.1a). Significantly higher microbial biomass was found in the growing season and in clayey soils (Fig. 5.1). Vegetation type had no significant impact on soil microbial biomass (Table 5.1a).

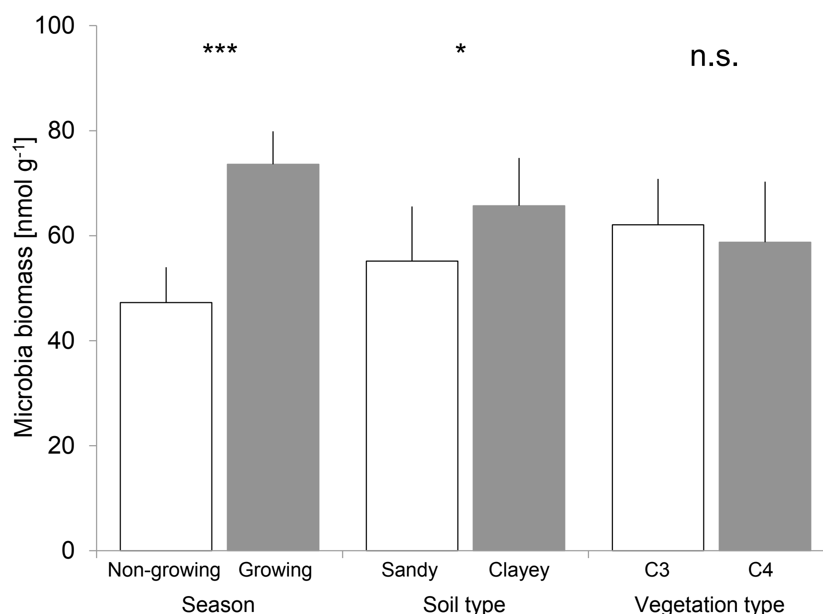

Figure 5.1. Mean (n=3 ± standard error) microbial biomass in sandy and clayey soils sown with C3 and C4 plants in different seasons. Stars indicate significant differences in mean total microbial biomass between seasons and soil type (*: $p \leq 0.05$; ***: $p \leq 0.001$).

Soil type was, with 50% explained variation, the main driver for the microbial community composition of the PLFA concentration. The other experimental factors, vegetation type and season, did not affect significantly the soil microbial community (Table 5.1b). The RDA revealed that the significant effect of soil type was driven by differences in SOC content (explains 8.0 %, Appendix A5.2), while soil moisture, clay content and DOC had no significant impact on the microbial community composition (Appendix A5.2). By far, the most important factor for structuring the soil microbial community was root biomass, explaining 45.2 % of the PLFA composition (Appendix A5.2). Root biomass mainly separates G+ bacteria from G- bacteria (Fig. 5.2).

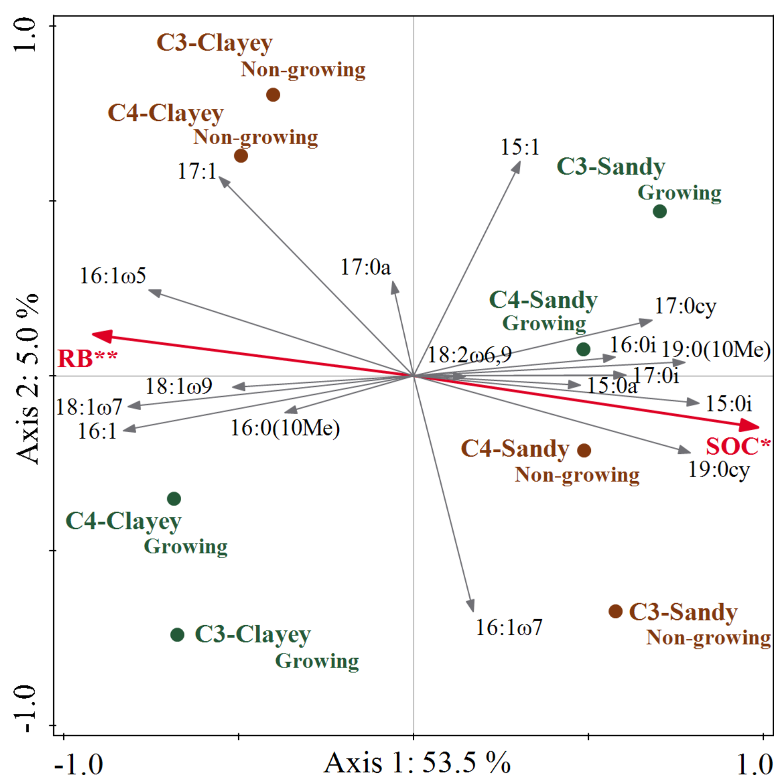

Figure 5.2. Redundancy analysis (RDA) of the mean relative proportions (mol%) of individual phospholipid fatty acids (PLFAs) in sandy and clayey soils sown with C3 and C4 plants. Root biomass (RB) and soil organic carbon (SOC) were set as explanatory variables to explain the variance of individual PLFAs. Stars indicate significant differences between soil types (*: $p \leq 0.05$; **: $p \leq 0.01$).

5.2.2 Isotopic signature of microbial markers and uptake of plant-derived C

All individual PLFAs had significantly higher δ^{13}C values in C4 vegetated plots (Appendices A5.1 and A5.3). Furthermore, the δ^{13}C values of PLFA markers indicative for G+ bacteria, actinobacteria and G- bacteria cyclo (15:0i, 16:0i, 17:0i, 17:0a, 16:0(10Me), 19:0(10Me), 17:0cy and 19:0cy) were higher in the growing season; whereas δ^{13}C values of 15:1, 16:1ω5 and 18:1ω9 (G- bacterial markers) were higher in the non-growing season (Appendices A5.1 and A5.3). These findings are supported by the results of the PERMANOVA – the composition of δ^{13}C values of PLFA markers among plots were significantly influenced by vegetation type and season, while there was no effect of soil type (Tables 5.1c, Appendices A5.1 and A5.3). The prominent role of vegetation type for the microbial isotopic composition was demonstrated as 75 % of the variance in δ^{13}C values of PLFA composition was explained by this factor (Table 5.1c).

Table 5.1. Results of the ANOVA (analysis of variance) from the (a) total microbial biomass and PERMANOVA (Permutational Multivariate Analysis of Variance Using Distance Matrices) from (b) microbial community composition and (c) the δ^{13}C values of individual PLFA obtained from both the sandy and clayey soils of the C3-C4 vegetation change experiment. Stars indicate significant differences between season, vegetation type or soil type (*: p≤ 0.05; **: p≤ 0.01; ***: p≤ 0.001).

(a) Total microbial biomass [nmol g^{-1}]				
	Df	F value	R^2	Pr(>F)
Season	1	40.59	0.59	<0.001 ***
Vegetation type	1	0.64	0.01	0.434
Soil type	1	6.49	0.10	0.021 *
Season: Vegetation type	1	0.05	0.60	0.834
Season: Soil type	1	1.18	0.71	0.294
Vegetation type: Soil type	1	2.49	0.14	0.134
Season: Vegetation type: Soil type	1	0.57	0.76	0.461
Residuals	16			
Total	23			
(b) Microbial community composition (mol%)				
	Df	F.model	R^2	Pr(>F)
Season	1	0.64	0.01	0.492
Vegetation type	1	0.57	0.01	0.546
Soil type	1	22.26	0.50	0.001 **
Season: Vegetation type	1	0.62	0.01	0.516
Season: Soil type	1	3.38	0.08	0.076
Vegetation type: Soil type	1	0.32	0.01	0.767
Season: Vegetation type: Soil type	1	0.92	0.02	0.359
Residuals	16	0.36		
Total	23			
(c) δ^{13}C-PLFA (‰)				
	Df	F.model	R^2	Pr(>F)
Season	1	6.63	0.06	0.014 *
Vegetation type	1	87.71	0.75	0.001 **
Soil type	1	2.45	0.02	0.106
Season: Vegetation type	1	0.95	0.01	0.311
Season: Soil type	1	1.46	0.01	0.213
Vegetation type: Soil type	1	1.03	0.01	0.319
Season: Vegetation type: Soil type	1	0.46	0	0.587
Residuals	16	0.14		
Total	23			

Similarly to the microbial community composition, the composition of plant-derived C in individual PLFA markers was affected by soil type and season (Fig. 5.3). However, plant-derived C in most microbial groups was not significantly different between seasons and soil type, except for plant-derived C in G- bacteria, which significantly increased in clayey soils in

the growing season (Fig. 5.4b; Appendix A5.5). The increased plant-derived C in G- bacterial markers in clayey soils was positively and significantly related to root biomass (Fig. 5.3; Appendix A5.4). Moreover, plant-derived C changed significantly among microbial groups. In the non-growing season, the amount of plant-derived C in G+ bacteria, actinobacteria, cyclic G- bacteria and G-bacteria were not different among them but were significantly lower than plant-derived C in saprotrophic fungi (Fig. 5.4a; Appendices A5.6 and A5.7). In the growing season, only plant-derived C in actinobacteria was significantly lower from plant-derived C in saprotrophic fungi and G- bacteria. No significant differences were observed among plant-derived C in all other microbial groups (Fig. 5.4b; Appendices A5.6 and A5.7).

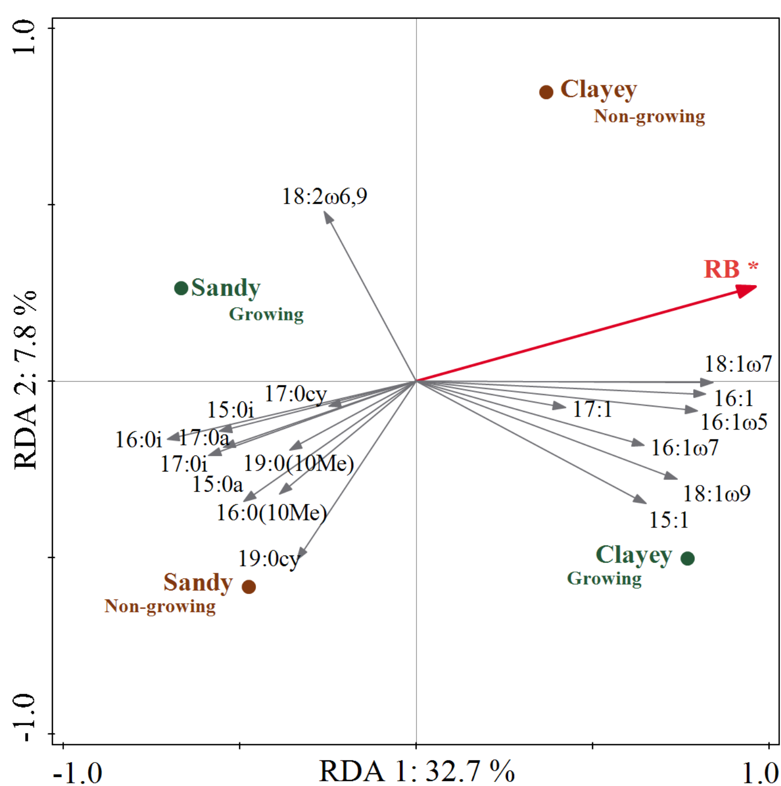

Figure 5.3. Redundancy analysis (RDA) of mean (n= 3 ± standard error) plant-derived C in individual phospholipid fatty acids (PLFAs) in sandy and clayey soils. Root biomass (RB) was set as explanatory variables to explain the variance of plant-derived C in individual PLFAs. Stars indicate significant differences between soil types (*: p≤ 0.05).

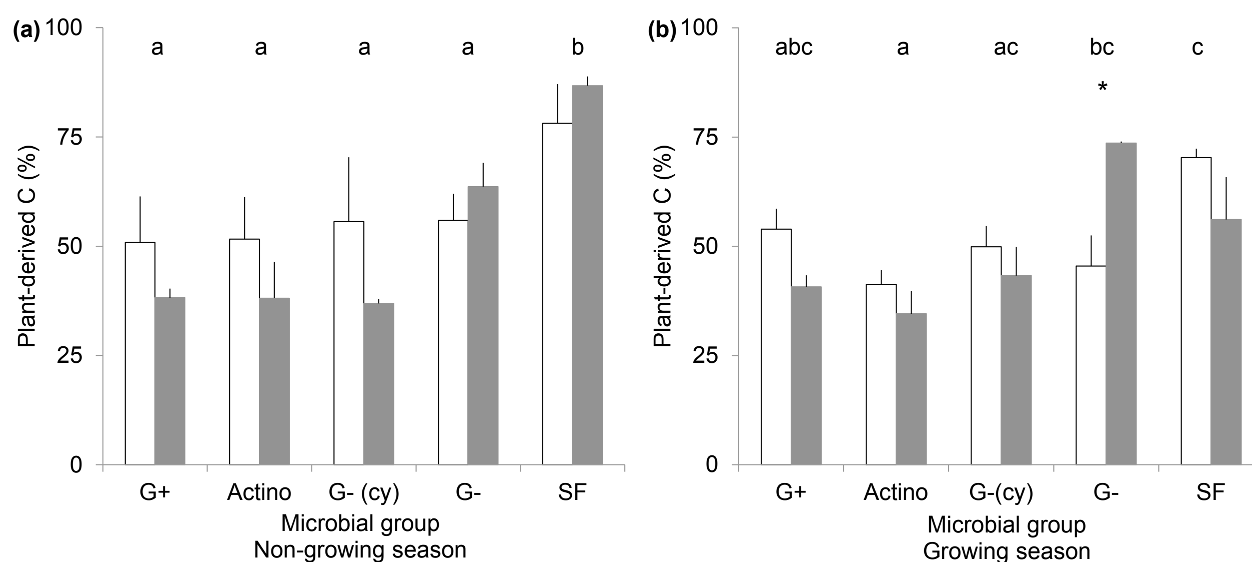

Figure 5.4. Mean (n= 3 ± standard error) plant-derived C in different microbial groups (Gram positive bacteria (G+), actinobacteria (Actino), cyclic Gram negative bacteria (G- (cy)), Gram negative bacteria (G-) and saprotrophic fungi (SF)) in a) non-growing and b) growing season, in sandy (white bars) and clayey (gray bars) soils. Stars indicate significant differences between soil types (*: $p \leq 0.05$). Letters indicate significant differences ($p \leq 0.05$) between plant-derived C in different microbial groups.

5.3 Discussion

In this study we investigated the impact of an experimental vegetation change – from C3 to C4 plants – on the soil microbial community and its functioning. Furthermore, in our input controlled experiment we were able to quantify the relative influence of this vegetation change compared to differences in soil and season.

5.3.1 The effect of the vegetation change

We found no differences in the microbial biomass and in the microbial community composition between plots of different vegetation types. This indicates no or at most a minor impact of C3 and C4 vegetation on soil microbial communities and their functioning. In contrast, vegetation type was the most important factor for variation in the δ^{13}C values of the PLFAs. The significantly higher δ^{13}C values of all individual PLFAs in C4 growing plots reflected the naturally higher δ^{13}C values of C4 plants (Degens, 1969) and evidenced that all soil microbial groups depend directly or indirectly on plant-derived C resources (Wardle, 2002). The C uptake and thus the microbial functioning was independent of the vegetation type itself; indicating that the effects of soil type and season are not affected by the vegetation change and are independent from different plant types.

5.3.2 Effects of soil type on the soil microbial community

The total soil microbial biomass depended on soil type and was significantly higher in clayey soils. The impact of soil type on the microbial community composition was even more pronounced as in our study the PLFA markers of G+ bacteria, actinobacteria, cyclic G- bacteria and saprotrophic fungi were more abundant in sandy soils. However, we do not suspect the coarser texture accounting for this finding but the higher SOC concentrations in the sandy soil of our experiment. The RDA revealed that G+ bacteria, actinobacteria, cyclic G- bacteria and saprotrophic fungi are mainly fostered by the higher SOC content in the sandy soil of our study. These microbial groups mainly depend on SOC as they are better adapted than other microbial groups to degrade high molecular organic molecules present in SOC (Fierer et al., 2003; Kramer and Gleixner, 2008). In contrast, PLFA markers assigned to G- bacteria are more abundant in clayey soils. The RDA showed that G- bacteria are mainly driven by the root biomass. In our experiment more root biomass was found in clayey soils, which form a finer mineralogy and might therefore sustain a better root net resulting in more root biomass than sandy soils (Merckx et al., 1985). G- bacteria depend strongly on plant-derived C resources, e.g. root exudates (Dequiedt et al., 2011). Higher amounts root biomass has been shown to facilitate the uptake of exudates by G- bacteria due to an increase microbial access to the plant derived C (Mellado-Vázquez et al., 2016). However, there was no influence on soil type on the δ^{13}C values of PLFA markers and their composition.

5.3.3 Seasonal differences of the soil microbial community

Differences in δ^{13}C values between growing seasons in some bacterial markers are not in line with the current theory. The enrichment in the δ^{13}C values of G+ bacterial (15:0i, 16:0i, 17:0i, 17:0a), actinobacterial (16:0(10Me), 19:0(10Me)) and cyclic G- bacterial markers (17:0cy and 19:0cy, which are produced under environmental stress conditions by G- bacteria (Kaur et al., 2005) and behave more similarly to G+ bacteria (Treonis et al., 2004; Mellado-Vázquez et al., 2016) might reflect an increased microbial respiration and higher C turnover with increasing soil temperature in the growing season (Creamer et al., 2015). Contrarily, for G- bacterial markers (16:1ω5 and 18:1ω9), the enrichment in their δ^{13}C values in the non-growing season might have resulted from an increased saprotrophic uptake of plant-derived C. Although we assigned these PLFA markers to their most common origin in soils (i.e. G- bacteria (Zelles, 1997)), the fungal origin of both markers is often discussed in the literature (Olsson et al., 1995; Madan et al., 2002; Bååth and Anderson, 2003; Sakamoto et al., 2004; Kaiser et al., 2010) and fungal uptake of plant C is higher in the non-growing

season (Fig. 5.4a). This might suggest, as previously observed (Borga et al., 1994; White et al., 1996; Madan et al., 2002; Wilkinson et al., 2002; Bååth and Anderson, 2003; Ruess et al., 2007), that the microbial origin of some PLFA markers depends strongly on substrate quality and availability, presumably in the non-growing season when there was a limited input of newly photosynthesized plant-derived C (i.e. root exudates), the signal of PLFAs 16:1ω5 and 18:1ω9 represented saprotrophic fungi better than G-bacteria.

5.3.4 Plant-derived C in different soil microbial groups

The incorporation of plant-derived C into most of the microbial groups we studied (G+ bacteria, actinobacteria, cyclic G- bacteria and saprotrophic fungi) was not significantly affected by different soil types and seasons (Fig. 5.4a and b; Appendix A5.5). G- bacteria are more sensitive to differences in soil type in the growing season, higher percentages of plant-derived C in G- bacteria were found in clayey soils (Fig. 5.4a and b). G- bacteria depend stronger, than the rest of the microbial groups, on the plant C substrate that recently incorporates into the soil (Treonis et al., 2004; Mellado-Vázquez et al., 2016). Therefore, G-bacteria were able to thrive in clayey soils under growing conditions firstly because of a more efficient delivery of plant C resources to clayey soils due to a more favorable root environment provided by the mineralogy of this soil type (Merckx et al., 1985); and secondly because of an increased plant C resource availability found in growing seasons (Habekost et al., 2008). We additionally observed that the plant-derived C differed among microbial groups in both seasons (Fig. 5.4a and b; Appendices A5.6 and A5.7). In the non-growing season, when the most important plant C resource was derived from decaying root biomass, the amount of plant C incorporated into saprotrophic fungi was significantly higher than that incorporated to all other microbial groups analyzed. This is likely the result of a better capability of fungi to decompose litter-plant derived C (Klamer and Hedlund, 2004; Singh et al., 2006). In the growing season, when there is a constant supply of newly photosynthesized C and the conditions might be favorable for all microbial groups, the differences were not only driven by their specific ability to decompose distinct C substrates but also by other factors such as plant-derived C availability and soil type, as was previously discussed for G-bacteria.

5.4 Conclusions

Our results demonstrate that the soil microbial community composition and function is independent from C3 and C4 vegetation type. Presence and absence of vegetation in different

seasons controlled the microbial biomass, the microbial community composition and the microbial C uptake. However, soil type was the most important factor influencing the soil microbial communities. In conclusion, contrary to the common idea that vegetation change experiments are biased by the preference of soil microorganisms to specific vegetation types, we show here that vegetation change experiments are useful to understand microbial C dynamics in soil. We also highlighted the importance of soil texture in the functioning of soil microbial communities, however future experiments should include a wider range of soil types and the interactions between different soil types and various abiotic and biotic factors in order to identify and better understand the drivers of the soil microbial C dynamics.

CHAPTER VI

SYNTHESIS

This thesis provides new insights of the influence of several plant- and soil-related factors on soil microbial communities and on soil microbial C dynamics. We identified 1) the mechanisms behind the plant diversity effect on the transfer of C between above and belowground (Chapter 3); 2) the influence of plant diversity on different soil microbial groups, soil bacterial diversity and PLFA evenness (Chapter 4); and 3) the relative importance of the combined effects of soil type, vegetation type and seasonal changes on the soil microbial community composition and soil microbial C uptake (Chapter 5). These new findings provide further knowledge of the effects of changes in ecosystem properties on soil microbial C dynamics. Consequently, our results contribute to the understanding of factors that control the ability of soils to act as C sinks, thereby mitigating increasing atmospheric CO_2 and global climate change.

6.1 Plant diversity effects on soil microorganisms

High plant diversity induces an increased C transfer from above to belowground (Lange et al., 2015). This increased C flow fosters soil microbial communities and the functions they perform in soils. We identified and described possible mechanisms behind the plant diversity effect on the increased C transport from plants to soils (Chapter 3). Our results contrasted with previous investigations that proposed increased root exudation associated to increased photosynthetic assimilation, as the main mechanism explaining the increased C flow in high plant diversity (Chung et al., 2007; Chung et al., 2009). We traced the flow of labelled $^{13}CO_2$ from plants to soil microbial communities. Surprisingly, we observed that the increased CO_2 assimilation does not translate to higher root exudation. Instead, increasing root biomass in higher plant diversity facilitated the access of recently photosynthesized C for root-associated microorganisms favoring soil microbial C uptake and fueling the cycling of new C belowground. These results provide a new understanding of the mechanisms responsible for plant diversity effect on the increased C accumulation in SOC. In addition, our results indicated an indirect plant diversity effect on the ^{13}C enrichment of some PLFA markers indicative of G+ bacteria (i.e. soil-related microorganisms) which pointed to C recycling within the microbial food web. Although the plant diversity effect was stronger on

root-associated microorganisms, this finding suggested that plant diversity impacted indirectly the C assimilation of soil-related microorganisms.

The influence of plant diversity on resource quality and availability (Habekost et al., 2008) and on microclimatic conditions has been related to the structure of the soil microbial community (Lange et al., 2014). Therefore, we expected to find a differentiated plant diversity effect on different soil microbial groups (root-associated and soil-related microorganisms). Unexpectedly, our results (Chapter 4) demonstrated that plant diversity promoted similarly root-associated and soil-related microorganisms. Unexpectedly, we showed that root biomass explained the plant diversity effect on both microbial groups. Different mechanisms were attributed to this effect. We suggested that root biomass drove the plant diversity effect on root-associated and soil-related microorganisms via two different mechanisms. Firstly, root biomass impacted root-associated microorganisms by increasing the access of recently photosynthesized C (as demonstrated in Chapter 3); and secondly, these new plant-derived C inputs triggered SOC mineralization (Fontaine et al., 2003), thereby favoring soil-related microorganisms.

Increased resource heterogeneity in high plant diversity has been linked to more diverse bacterial communities in soils (Loreau and Hector, 2001; Bell et al., 2005). Consistently, our results (Chapter 4) showed that plant diversity impacted positively bacterial diversity in soils. Unexpectedly, we found that PLFA evenness decreased with increasing plant diversity. PLFA bacterial markers indicative of root-associated microorganisms dominated over soil-related PLFA bacterial markers. This might suggest that the plant diversity effect on the processing of recently photosynthesized C dominates over the processes mediated by soil-related microorganisms, such as mineralization of SOC.

6.2 The effects of soil characteristics and vegetation properties on soil microbial communities

Soil microbial communities and the function they perform in soils are influenced by several ecosystem properties. The individual effects of soil type, vegetation type and seasonal changes in vegetation abundance have been widely investigated (e.g. (Merckx et al., 1985; Grayston et al., 2001; Johnson et al., 2003; Berg and Steinberger, 2010; Thoms and Gleixner, 2013)). However, the relative impact of the combined effects of various factors is scarcely investigated. Our results provided new understanding of how the interaction of several factors affects soil microorganisms (Chapter 5). We showed for the first time that the effect of soil type dominated over the effects of vegetation type and seasonal on the soil microbial

community. Higher SOC concentrations in sandy soils and greater root biomass in clayey soils induced shifts in the microbial community composition. G+ bacteria dominated in sandy soils; G- bacteria predominated in clayey soils. Moreover, we demonstrated that higher root biomass in clayey soils favored the rhizospheric C transfer, thereby promoting G- bacteria.

Differences in plant-derived C inputs from different vegetation types can be determine through isotopic analysis of source (plants) and sink (soil microbes) because the C isotope fingerprint of different vegetation types (C3 and/or C4) is recorded in the microbial biomass (Gleixner et al., 1999; Steinbeiss et al., 2008b). Such changes are commonly tracked in vegetation change experiments, where C3 vegetation is replaced by C4 vegetation (or vice versa). However, until now it remained unclear if such a vegetation change impacts the microbial community and the functions soil microorganisms perform in soils. Our results demonstrated for the first time that the microbial C incorporation is not influenced by vegetation type (C3 and C4 plants) and that soil microbial community composition is not subject to changes related to isotopic differences in plant-derived C.

Seasonal changes in vegetation abundance have been linked to changes in the quantity and quality of plant-derived C inputs to soils (Habekost et al., 2008; Thoms and Gleixner, 2013). We demonstrated that seasonal changes were overall not significant for the microbial community composition. However, a shift in the type of C substrate (from plant litter-derived C to recently photosynthesized plant-derived C) between the non-growing and the growing seasons, favored distinctively the C uptake of different microbial groups. In the non-growing season, plant litter-derived C exceeded the amount of recently photosynthesized rhizodeposits, thereby favoring saprotrophic fungi. In contrast, in the growing season the increased root biomass and increased input of root exudates promoted the microbial C uptake of G- bacteria in our soils.

6.3 General conclusions

Overall, the results outlined in this work demonstrated that the effects of plant diversity, soil type, vegetation type and seasonal changes in vegetation abundance influenced differently soil microbial communities. Increased resource availability in higher plant diversity favored soil microbial biomass, but was less relevant for the structure of soil microbial communities. In contrast, soil type and changes in the type (not the amount) of C substrate were more important for shaping soil microbial community composition. However, irrespective of the effect of the different factors studied, we demonstrated that the impact of plant diversity, soil type and vegetation characteristics influences the transfer of C from

aboveground to belowground and the formation and accumulation of new soil SOC, consequently impacting the ability of soils to mitigate increasing atmospheric CO_2 concentrations and global climate change.

6.4 Perspectives for future research

We offered new valuable insights to the mechanisms responsible for the transfer of C between plants and soil microorganisms (Chapter 3), however new investigations are still needed in order to further understand the soil microbial C cycling. We identified root biomass, as a significant driver of the positive plant diversity effect on the incorporation of newly photosynthesized C into the soil microorganisms; additionally higher root exudation in higher plant diversity was excluded as a mechanism explaining this interaction. Undoubtedly, however, root exudation is a very relevant mean for C transfer from plants to soil microorganisms, around 17 % of the C photosynthetically assimilated is actively released by living roots to soils (Nguyen, 2003), in the form of a wide variety of chemical compounds, including sugars, amino acids, organic acids, proteins and enzymes (Nguyen, 2003; Badri et al., 2009). It is known that the chemical composition of root exudates changes in response to changes in environmental conditions, such as nutrient stress (Jones, 1998; Neumann and Martinoia, 2002), increased pathogenic attack (Morrissey and Osbourn, 1999) and based on specific plant species (Grayston et al., 1997; Fan et al., 2001). However to date it is not known if plant species richness influences the nature of root exudation and the effect that this would have on microbial-mediated processes, such as C incorporation and cycling belowground. Therefore in order to provide further mechanistic understanding on the increased soil microbial C uptake and soil microbial C cycling in high plant diversity, future research should be directed to investigate 1) potential changes in the chemical composition of root exudates in response to changes in plant diversity and 2) how/if the chemical composition of root exudates impacts microbial community composition and thereby microbial functioning in soils with increasing plant diversity.

In Chapter 4 we described the positive influence of plant diversity on bacterial diversity and the predominance of microbial markers indicative of root-associated microorganisms; we argued that this effect presumably favors the cycling of freshly photosynthesized C in soils. However, in order to strengthen these conclusions, it is necessary to perform a complete phylogenetic analysis of the bacterial community in soils to assign specific soil functions to specific bacterial species. Commonly, PLFA markers can be related to more than one microbial group (see Appendix A1.1 and references therein). This low

microbial specificity of PLFA markers makes PLFA analysis less suited to differentiate soil microbial functions. Therefore, in order to identify specific functions that are potentially favored by high plant diversity, a comparative analysis of the dominant (i.e. most abundant) PLFA bacterial markers and the bacterial species, and their functions, would allow drawing conclusions on the processes that are being favored by high plant diversity.

Lastly, we showed that soil type (i.e. soil texture) exerted an important control on soil microbial dynamics, and compared to seasonal changes and vegetation types, was the most important mediator of soil microbial communities and soil microbial functions (Chapter 5). Therefore, new investigations should be directed to study a wider range of soil types and to identify possible meaningful interactions between soil types and additional factors that might result in positive or negative effects on the functions that microbial communities perform in soils. For instance, pH has been recognized to have a strong effect on the shaping on the microbial community in soils (Bååth and Anderson, 2003; Thoms et al., 2010; Scheibe et al., 2015), therefore it would be interesting to study how the interaction of soil type and pH would influence the microbial community composition and their functions in soils.

These new considerations would provide further understanding on how changes in ecosystem properties might alter soil microbial C dynamics. Additionally, they would further strengthen our knowledge on the factors that control the ability of soils to act as C sinks, thereby mitigating increasing atmospheric CO_2 and global climate change.

SUMMARY

Soil microbial communities are crucial for the cycling of C in the belowground system. Most of the C that enters the soil system through plant deposition (e.g. plant litter and/or root exudation) is processed by soil microorganisms and contributes to the formation and accumulation of SOC (Gleixner et al., 2002; Balser and Firestone, 2005; Bardgett et al., 2005; Lange et al., 2015). These soil microbial-mediated processes are very sensitive to changes in ecosystem properties, such as plant diversity, soil type and seasonal changes in vegetation abundance; however the drivers of these plant- and soil-related factors on the soil microbial community and soil microbial functions are not fully understood so far. In that context, the main aim of this work was to deepen our knowledge on the influence of plant- and soil-related factors on soil microbial communities and soil microbial C dynamics. We specifically focused in understanding 1) the mechanisms responsible for the influence of plant diversity on the transfer of C from above to belowground (Chapter 3); 2) the influence of plant diversity effect on different soil microbial groups, bacterial diversity and bacterial evenness (i.e. PLFA evenness) (Chapter 4); and 3) the influence of the combined effects of soil type, vegetation type and seasonal changes in vegetation abundance on the microbial community composition and microbial C uptake in soils (Chapter 5).

In Chapter 3, we identified the mechanisms behind the increased microbial C uptake in high plant diversity. A continuous $^{13}CO_2$ labeling was applied in a controlled environment (The Montpellier European Ecotron) to 12 ecosystem samples from the Jena Experiment. Six ecosystem samples held a mixture of 4 plant species, and the other six ecosystem samples had 16 plant species. The transfer of C from plants to soil microorganisms was determined with compound-specific $\delta^{13}C$ from phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analyses. Our results showed that high plant diversity increased the C uptake of root-associated microorganisms, i.e. Gram negative (G-) bacteria and arbuscular mycorrhizal fungi (AMF). In contrast to previous investigations, our results demonstrated that this effect was not driven by increased root exudation rates; instead greater root biomass explained the increased G- bacterial C uptake in high plant diversity. This suggested that greater root biomass in high plant diversity facilitated the access of recently photosynthesized plant-derived C for G- bacteria. Moreover, our results highlighted the ability of AMF to uptake C resources directly from their host plant. In conclusion, high plant diversity promotes an increased soil microbial

assimilation of newly photosynthesized plant-derived C, consequently promoting C cycling and accumulation in soils.

In Chapter 4**,** we analyzed the influence of plant diversity on different soil microbial groups, soil bacterial diversity and soil PLFA evenness. We collected 82 soil samples from a long-term plant diversity experiment (The Jena experiment). We measured total microbial biomass, root-associated microbial biomass and soil-related microbial biomass using phospholipid fatty acid (PLFA) analysis. Bacterial diversity was determined with terminal-restriction fragment length polymorphism (T-RFLP) analysis. Plant diversity impacted similarly root-associated and soil-related microorganisms. Unexpectedly, increased root biomass, higher SOC and greater soil moisture drove the plant diversity effects on both microbial groups. We suggest that the similar response of different microbial groups to plant diversity displays a "chain response" to the studied plant- and soil-related factors. For instance, increased root biomass in high plant diversity influenced first root-associated microorganisms by increasing the access of recently photosynthesized C that is rapidly uptake in the rhizosphere. As a result, these new plant-derived C inputs possibly fueled soil-related microorganisms via provision of microbial remains and by triggering SOC mineralization. Moreover, plant diversity impacted positively the bacterial diversity in our soils, possibly through the input of more diverse resources. In contrast, PLFA evenness decreased with increasing plant diversity. Bacterial PLFA markers indicative of root-associated microorganisms dominated over soil-related bacterial PLFA markers. This might suggest that plant diversity promotes root-associated microbial-mediated processes, however further analysis of specific bacterial species and their functions are still needed in order to generalize these conclusions.

Lastly, in Chapter 5 we described the analysis of the combined effects of soil type, seasonal changes in vegetation abundance and vegetation type (C3 and C4 plants) on soil microbial biomass, soil microbial community composition and soil microbial C turnover. We collected soil samples, in the non-growing season and the growing season in 2012, from a long-term vegetation change experiment which was replicated in clayey and sandy soils. We studied soil microbial community composition and the C flow from above to belowground using phospholipid fatty acids (PLFA) and compound-specific δ^{13}C-PLFA analyses, respectively. We further analyzed the effects of the factors of interest on the microbial dynamics in soils. Higher microbial biomass was found in the growing season. The microbial community composition was mainly explained by soil type. Higher amounts of SOC drove the predominance of PLFA markers indicative of Gram positive bacteria, actinobacteria and

cyclic Gram negative (G-) bacteria in sandy soils; whereas root biomass was strongly related to the increased proportions of G- bacterial PLFA markers in clayey soils. Plant-derived C in G- bacteria increased significantly in clayey soils in the growing season. This increase was driven by root biomass. Moreover, changes in plant-derived C between microbial groups pointed to specific microbial functions in soils. Soil texture and seasonal changes in vegetation abundance driven by rhizospheric interactions were the most important factors controlling soil microbial dynamics in our study site. In addition, our results demonstrated that a change of C3 plants vs. C4 plants has only a minor effect on soil microbial dynamics.

Overall, the effect of plant-related factors, i.e. plant diversity and vegetation abundance, was mostly mediated by increased resource availability that fostered all parts of the soil microbial communities; although high plant diversity favored preferentially the cycling of new C in soils. Furthermore, soil-related characteristics, such as soil type had a stronger influence in shaping of the soil microbial community composition and soil microbial functioning. In conclusion, plant- and soil-related characteristics influenced soil microbial communities and soil microbial C uptake, indirectly controlling soil C cycling and SOC accumulation.

This thesis provides with new understanding of factors that control soil microbial communities and soil microbial C dynamics. However, future research should include more in depth analysis of the identified mechanisms presented in this work. For instance, new research should investigate potential changes in the chemical composition of root exudates with changing plant diversity; and the combined effect of different soil types and additional edaphic factors (such as pH) on soil microorganisms and soil microbial-mediated processes. These new investigations have great potential to strengthen our knowledge on the factors controlling soil microbial communities, soil microbial C dynamics and ultimately C cycling in soils and globally.

ZUSAMMENFASSUNG

Mikrobielle Gemeinschaften sind ein wesentlicher Bestandteil des Kohlenstoff(C)-Kreislaufs im Boden. Der überwiegende Teil an C, welcher in das Bodensystem durch pflanzliche Ablagerungen (z.B. Bodenstreu und/oder Wurzelexsudate) gelangt, wird durch Bodenmikroorganismen verarbeitet und trägt zur Bildung und Akkumulation von organischem C im Boden (engl.: Soil Organic Carbon, kurz: SOC) bei (Gleixner et al., 2002; Balser and Firestone, 2005; Bardgett et al., 2005; Lange et al., 2015). Diese Bodenmikroben-vermittelten Prozesse reagieren sehr empfindlich auf Veränderungen der Ökosystem-Eigenschaften, wie z.B. pflanzliche Diversität, Bodentyp und saisonale Änderungen der Vegetationsfülle. Jedoch sind die zugrundeliegenden Mechanismen dieser Pflanzen- und Boden-bedingten Faktoren und ihrer Wirkung auf mikrobielle Bodengemeinschaften sowie deren Funktionen noch nicht vollständig verstanden. In diesem Zusammenhang war das Hauptziel dieser Arbeit das Wissen über den Einfluss Pflanzen- und Boden-bedingter Faktoren auf mikrobielle Bodengemeinschaften und deren C-Dynamik zu vertiefen. Insbesondere lag unsere Aufmerksamkeit im Verständnis 1) der Mechanismen die für den Einfluss der Pflanzendiversität auf den Transfer von C vom Oberirdischen ins Unterirdische verantwortlich sind (3. Kapitel); 2) des Einflusses von Pflanzendiversitäts-Effekten auf verschiedene Gruppen von Bodenmikroben, die bakterielle Diversität und bakterielle Gleichmäßigkeit (d.h. Gleichmäßigkeit von Phospholipid-Fettsäuren) (4. Kapitel); und 3) des Einflusses der kombinierten Effekte aus Bodentyp, Vegetationstyp und saisonalen Änderungen der Vegetationsfülle auf die Zusammensetzung der mikrobiellen Gemeinschaft sowie auf die mikrobielle C-Aufnahme im Boden (5. Kapitel).

Im 3. Kapitel haben wir die Mechanismen identifiziert die der erhöhten mikrobiellen C-Aufnahme bei hoher pflanzlicher Diversität zugrunde liegen. Eine fortlaufende Markierung mit $^{13}CO_2$ wurde in einer kontrollierten Umgebung (The Montpellier European Ecotron) an 12 Ökosystem-Proben aus dem Jena-Experiment durchgeführt. Sechs dieser Ökosystem-Proben enthielten eine Mischung aus 4 Pflanzenarten und die anderen sechs Ökosystem-Proben hatten 16 Pflanzenarten. Der C-Transfer von Pflanzen zu Bodenmikroorganismen wurde durch die Verbindungs-spezifische δ^{13}C-Analyse von Phospholipid-Fettsäuren (PLFA) und Neutrallipid-Fettsäuren (NLFA) bestimmt. Unsere Ergebnisse haben gezeigt dass hohe pflanzliche Diversität die C-Aufnahme von Wurzel-assoziierten Mikroorganismen, d.h. Gram-negativen (G-) Bakterien und arbuskulären Mykorrhiza-Pilzen (AMF), erhöht. Im

Gegensatz zu vorausgegangenen Untersuchungen haben unsere Ergebnisse gezeigt dass dieser Effekt nicht durch gesteigerte Wurzel-Exsudationsraten angetrieben wurde, sondern dass eine größere Wurzelbiomasse die erhöhte C-Aufnahme durch G- Bakterien bei hoher pflanzlicher Diversität erklärt. Dies weist darauf hin dass die größere Wurzelbiomasse bei hoher pflanzlicher Diversität den Zugriff von G- Bakterien auf kürzlich photosynthetisierten, Pflanzen-basiertem C begünstigt hat. Des Weiteren unterlegen unsere Ergebnisse die Fähigkeit von AMF C-Ressourcen direkt von ihren Wirtspflanzen aufzunehmen. Schlussfolgernd fördert eine hohe pflanzliche Diversität die gesteigerte Assimilation von neu photosynthetisierten Pflanzen-basiertem C durch Bodenmikroben und folglich auch den C-Kreislauf sowie die C-Akkumulation im Boden.

Im 4. Kapitel haben wir den Einfluss der pflanzlichen Diversität auf verschiedene mikrobielle Gruppen, Bodenbakterien-Diversität und Gleichmäßigkeit der Phospholipid-Fettsäuren (PLFAs) im Boden analysiert. Dafür haben wir 82 Bodenproben aus einem Langzeit-Pflanzendiversitäts-Experiment (Jena-Experiment) gesammelt. Die absolute mikrobielle Biomasse, Wurzel-assoziierte mikrobielle Biomasse und Boden-bezogene mikrobielle Biomasse wurde unter Verwendung der PLFA-Analyse gemessen. Die bakterielle Diversität haben wir durch die Analyse des terminalen Restriktionsfragment-Längenpolymorphismus (T-RFLP) bestimmt. Als Ergebnis haben wir gefunden dass sich die pflanzliche Diversität ähnlich auf Wurzel-assoziierte und Boden-bezogene Mikroorganismen ausgewirkt. Unerwarteterweise trugen die erhöhte Wurzelbiomasse, der größere SOC-Gehalt und die höhere Bodenfeuchte zu Pflanzendiversitäts-Effekten auf beide mikrobiellen Gruppen bei. Wir vermuten dass die ähnliche Reaktion verschiedener mikrobieller Gruppen auf die pflanzliche Diversität eine Art „Kettenreaktion“ auf die untersuchten Pflanzen- und Boden-bedingten Faktoren wiederspiegelt. Beispielsweise könnte die größere Wurzelbiomasse bei hoher pflanzlicher Diversität zuerst die Wurzel-assoziierten Mikroorganismen durch einen gesteigerten Zugang zu kürzlich photosynthetisiertem C, welcher schnell in der Rhizosphäre aufgenommen wird, beeinflussen. Daraus resultierend würden die frischen Pflanzen-basierten C-Einträge die Boden-bezogenen Mikroorganismen durch die Bereitstellung von mikrobiellen Überresten und durch das Anstoßen der SOC-Mineralisierung nähren. Außerdem hat sich die pflanzliche Diversität generell positiv auf die bakterielle Diversität in den untersuchten Böden ausgewirkt, möglicherweise durch den Eintrag einer größeren Vielfalt an Ressourcen. Dahingegen hat sich die PLFA-Gleichmäßigkeit bei höherer pflanzlicher Diversität vermindert. Bakterielle PLFA-Marker die bezeichnend für Wurzel-assoziierte Mikroorganismen sind haben über die PLFA-Marker für Boden-bezogene Bakterien

dominiert. Dies deutet darauf hin dass eine hohe pflanzliche Diversität Wurzel-assoziierte mikrobiell-vermittelte Prozesse fördert, jedoch werden noch weitere Analysen von bestimmten Bakterienarten und ihren Funktionen benötigt um diese Schlussfolgerung zu verallgemeinern.

Zuletzt beschreiben wir im 5. Kapitel die Effekte von Bodentyp, saisonalen Änderungen der Vegetationsfülle und des Vegetationstyps (C3- und C4-Pflanzen) auf die mikrobielle Biomasse, die Zusammensetzung der mikrobiellen Bodengemeinschaft und den Umsatz von mikrobiellem C im Boden. Dafür haben wir Bodenproben außerhalb und während der Vegetationsperiode in 2012 aus einem Langzeit-Vegetationswechsel-Experiment, das in tonigen und sandigen Böden repliziert wurde, gesammelt. Daran haben wir die Zusammensetzung der mikrobiellen Bodengemeinschaft und den C-Fluss vom Oberirdischen ins Unterirdische unter Nutzung der quantitativen Analyse sowie der Verbindungs-spezifischen δ^{13}C-Analyse von Phospholipid-Fettsäuren (PLFAs) untersucht. Darüber hinaus haben wir die Effekte der o.g. Faktoren auf die mikrobielle Dynamik im Boden weiter analysiert. In unseren Ergebnissen haben wir, eine höhere mikrobielle Biomasse während der Vegetationsperiode gefunden. Die Zusammensetzung der mikrobiellen Gemeinschaft war hauptsächlich vom Bodentyp bestimmt. Größere Mengen an SOC im sandigen Boden führten zur Prädominanz von PLFAs die für Gram-positive Bakterien, Actinobakterien und Gram-negative (G-) Bakterien mit zyklischen PLFAs bezeichnend sind; wohingegen die höhere Wurzelbiomasse im tonigen Boden stark mit gesteigerten Anteilen von PLFA-Markern für G- Bakterien verbunden war. In den G- Bakterien aus tonigem Boden war der Pflanzen-basierte Kohlenstoff während der Vegetationsperiode signifikant erhöht, wobei dieser Anstieg durch die Wurzelbiomasse angetrieben wurde. Zudem wiesen Unterschiede im Pflanzen-basierten C zwischen verschiedenen mikrobiellen Gruppen auf spezifische mikrobielle Funktionen im Boden hin. Die Bodentextur sowie die saisonale Änderung der Vegetationsfülle, welche die Interaktionen in der Rhizosphäre bestimmen, waren die wichtigsten Einflussfaktoren auf die Dynamik der Bodenmikroben in unserer Testfläche. Zusätzlich demonstrieren unsere Ergebnisse dass der Wechsel zwischen C3- und C4-Pflanzen nur geringfügige Effekte auf die mikrobielle Dynamik im Boden hat.

Insgesamt wurde der Effekt Pflanzen-bedingter Faktoren, d.h. pflanzliche Diversität und Vegetationsfülle, hauptsächlich durch die erhöhte Verfügbarkeit von Ressourcen vermittelt, die alle Teile der mikrobiellen Gemeinschaften gefördert hat; obwohl eine hohe pflanzliche Diversität vorzugsweise den Kreislauf von neuem C im Boden begünstigt hat. Hinzu kommt, dass Boden-zugehörige Charakteristika, wie der Bodentyp, einen stärkeren

Einfluss auf die Ausprägung der mikrobiellen Bodengemeinschaft und der Funktionsweise von Bodenmikroben hatten. Abschließend lässt sich sagen, dass Pflanzen- und Boden-bedingte Charakteristika mikrobielle Bodengemeinschaften und deren C-Aufnahme beeinflusst haben, wodurch indirekt der C-Kreislauf im Boden und die SOC-Akkumulation gesteuert wurde.

Diese Dissertation liefert ein neues Verständnis von Faktoren welche die mikrobielle Bodengemeinschaften und die C-Dynamik von Bodenmikroben kontrollieren. Jedoch sollte die zukünftige Forschung noch eingehendere Analysen der hier identifizierten Mechanismen enthalten. Beispielsweise wird weitere Forschung benötigt, um potenzielle Veränderungen in der chemischen Zusammensetzung von Wurzelexsudaten bei verschiedener pflanzlicher Diversität; und den gemeinsamen Effekt von verschiedenen Bodentypen und zusätzlichen edaphischen Faktoren (wie pH) auf Bodenmikroorganismen und Bodenmikroben-vermittelte Prozesse zu untersuchen. Solche neuen Untersuchungen besitzen ein großes Potenzial unser Wissen über die Faktoren, welche mikrobielle Bodengemeinschaften, die C-Dynamik von Bodenmikroben und letztendlich den C-Kreislauf in Böden und auf globaler Ebene kontrollieren, zu stärken.

REFERENCES

Amos, B., Walters, D.T., 2006. Maize root biomass and net rhizodeposited carbon: An analysis of the literature. Soil Science Society of America Journal 70, 1489-1503.

Angers, D.A., Caron, J., 1998. Plant-induced changes in soil structure: Processes and feedbacks. Biogeochemistry 42, 55-72.

Bååth, E., 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microbial Ecology 45, 373-383.

Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35, 955-963.

Badri, D.V., Weir, T.L., van der Lelie, D., Vivanco, J.M., 2009. Rhizosphere chemical dialogues: plant-microbe interactions. Current Opinion in Biotechnology 20, 642-650.

Bahn, M., Lattanzi, F.A., Hasibeder, R., Wild, B., Koranda, M., Danese, V., Bruggemann, N., Schmitt, M., Siegwolf, R., Richter, A., 2013. Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. New Phytologist 198, 116-126.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms, Annual Review of Plant Biology, pp. 233-266.

Balasooriya, W.K., Denef, K., Huygens, D., Boeckx, P., 2012. Translocation and turnover of rhizodeposit carbon within soil microbial communities of an extensive grassland ecosystem. Plant and Soil, 1-13.

Balesdent, J., Balabane, M., 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biology and Biochemistry 28, 1261-1263.

Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395-415.

Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology and Evolution 20, 634-641.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505-511.

Barton K., 2014. MuMln: Multi-Model Inference. R Package Version 1.10.5.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 47, 151-163.

Batjes, N.H., 1998. Mitigation of atmospheric CO_2 concentrations by increased carbon sequestration in the soil. Biology and Fertility of Soils 27, 230-235.

Batjes, N.H., 2014. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science 65, 10-21.

Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., Lilley, A.K., 2005. The contribution of species richness and composition to bacterial services. Nature 436, 1157-1160.

Berg, N., Steinberger, Y., 2010. Are biological effects of desert shrubs more important than physical effects on soil microorganisms? Microbial Ecology 59, 121-129.

Bezemer, T.M., Lawson, C.S., Hedlund, K., Edwards, A.R., Brook, A.J., Igual, J.M., Mortimer, S.R., Van Der Putten, W.H., 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. Journal of Ecology 94, 893-904.

Bird, J.A., Herman, D.J., Firestone, M.K., 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biology and Biochemistry 43, 718-725.

Black, C.A., 1965. Methods of Soil Analysis: Part I Physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin, USA.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Turnover of soil organic matter and of microbial biomass under C3-C4 vegetation change: Consideration of ^{13}C fractionation and preferential substrate utilization. Soil Biology and Biochemistry 43, 159-166.

Bligh, E.G., Dyer, W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Bonfante, P., Anca, I.A., 2009. Plants, mycorrhizal fungi, and bacteria: A network of interactions, Annual Review of Microbiology, pp. 363-383.

Bonfante, P., Genre, A., 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends in Plant Science 13, 492-498.

Borga, P., Nilsson, M., Tunlid, A., 1994. Bacterial communities in peat in relation to botanical composition as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 26, 841-848.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265-278.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1-12.

Bowman, J.P., Sly, L.I., Nichols, P.D., Hayward, A.C., 1993. Revised taxonomy of the methanotrophs: Description of Methylobacter gen. nov., emendation of Methylococcus, validation of Methylosinus and Methylocystis species, and a proposal that the family Methylococcaceae includes only the group I methanotrophs. International Journal of Systematic Bacteriology 43, 735-753.

Breulmann, M., Schulz, E., Weißhuhn, K., Buscot, F., 2012. Impact of the plant community composition on labile soil organic carbon, soil microbial activity and community structure in semi-natural grassland ecosystems of different productivity. Plant and Soil 352, 253-265.

Busch, F.A., Sage, T.L., Cousins, A.B., Sage, R.F., 2013. C3 plants enhance rates of photosynthesis by reassimilating photorespired and respired CO_2. Plant, Cell and Environment 36, 200-212.

Butler, J.L., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2003. Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793-6800.

Cao, D., Shi, F., Ruan, W., Lu, Z., Chai, M., 2011. Seasonal changes in and relationship between soil microbial and microfaunal communities in a Tamarix chinensis community in the Yellow River Delta. African Journal of Biotechnology 10, 18425-18432.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59-67.

Cardinale, B.J., Srivastava, D.S., Emmett Duffy, J., Wright, J.P., Downing, A.L., Sankaran, M., Jouseau, C., 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443, 989-992.

Carney, K.M., Matson, P.A., 2005. Plant communities, soil microorganisms, and soil carbon cycling: Does altering the world belowground matter to ecosystem functioning? Ecosystems 8, 928-940.

Chamberlain, P.M., Black, H.I.J., 2005. Fatty acid compositions of Collembola: Unusually high proportions of C 20 polyunsaturated fatty acids in a terrestrial invertebrate. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 140, 299-307.

Chapin Iii, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., Diaz, S., 2000. Consequences of changing biodiversity. Nature 405, 234-242.

Chowdhury, T.R., Dick, R.P., 2013. Ecology of aerobic methanotrophs in controlling methane fluxes from wetlands. Applied Soil Ecology 65, 8-22.

Chung, H., Zak, D.R., Reich, P.B., Ellsworth, D.S., 2007. Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Change Biology 13, 980-989.

Chung, H.G., Zak, D.R., Reich, P.B., 2009. Microbial assimilation of new photosynthate is altered by plant species richness and nitrogen deposition. Biogeochemistry 94, 233-242.

Creamer, C.A., de Menezes, A.B., Krull, E.S., Sanderman, J., Newton-Walters, R., Farrell, M., 2015. Microbial community structure mediates response of soil C decomposition to litter addition and warming. Soil Biology and Biochemistry 80, 175-188.

Crowther, T.W., Boddy, L., Hefin Jones, T., 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. The ISME Journal 6, 1992-2001.

Davidson, E.A., Verchot, L.V., Henrique Cattânio, J., Ackerman, I.L., Carvalho, J.E.M., 2000. Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. Biogeochemistry 48, 53-69.

De Deyn, G.B., Quirk, H., Oakley, S., Ostle, N., Bardgett, R.D., 2011. Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8, 1131-1139.

De Graaff, M.A., Jastrow, J.D., Gillette, S., Johns, A., Wullschleger, S.D., 2014. Differential priming of soil carbon driven by soil depth and root impacts on carbon availability. Soil Biology and Biochemistry 69, 147-156.

De Graaff, M.A., Six, J., Jastrow, J.D., Schadt, C.W., Wullschleger, S.D., 2013. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biology and Biochemistry 58, 198-206.

de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15, 1230-1239.

Degens, E.T., 1969. Biogeochemistry of stable carbon isotopes, In: Eglington G., M.M.T.J. (Ed.), Organic Geochemistry. Elsevier, New York, pp. 304-329.

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., Muller, C., 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4, 769-779.

Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry 41, 144-153.

Dequiedt, S., Saby, N.P.A., Lelievre, M., Jolivet, C., Thioulouse, J., Toutain, B., Arrouays, D., Bispo, A., Lemanceau, P., Ranjard, L., 2011. Biogeographical patterns of soil molecular microbial biomass as influenced by soil characteristics and management. Global Ecology and Biogeography 20, 641-652.

Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. Journal of General Microbiology 132, 1815-1825.

Drigo, B., Pijl, A.S., Duyts, H., Kielak, A., Gamper, H.A., Houtekamer, M.J., Boschker, H.T.S., Bodelier, P.L.E., Whiteley, A.S., van Veen, J.A., Kowalchuk, G.A., 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. Proceedings of the National Academy of Sciences of the United States of America 107, 10938-10942.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.M., Jeffrey, S.W., 1993. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35, 155-161.

Edlund, A., Nichols, P.D., Roffey, R., White, D.C., 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from Desulfovibrio species. Journal of Lipid Research 26, 982-988.

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W.W., Scheu, S., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91, 485-496.

Elfstrand, S., Lagerlöf, J., Hedlund, K., Mårtensson, A., 2008. Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling. Soil Biology and Biochemistry 40, 2530-2539.

Engels, C., Neumann, G., Gahoonia, T.S., George, E.a., Schenk, M., 2000. Assessing the ability of roots for nutrient adquisition. In: Root Methods: A handbook. 405-457 pp. Springer-Verlag Berlin Heidelberg, Germany.

Epron, D., Ngao, J., Dannoura, M., Bakker, M.R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J.C., Priault, P., Barthes, L., Loustau, D., 2011. Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labelling of trees. Biogeosciences 8, 1153-1168.

Esperschutz, J., Buegger, F., Winkler, J.B., Munch, J.C., Schloter, M., Gattinger, A., 2009. Microbial response to exudates in the rhizosphere of young beech trees (Fagus sylvatica L.) after dormancy. Soil Biology & Biochemistry 41, 1976-1985.

Evershed, R.P., Crossman, Z.M., Bull, I.D., Mottram, H., Dungait, J.A.J., Maxfield, P.J., Brennand, E.L., 2006. ^{13}C-Labelling of lipids to investigate microbial communities in the environment. Current Opinion in Biotechnology 17, 72-82.

Falkowski, P., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Hogberg, P., Linder, S., Mackenzie, F.T., Moore Iii, B., Pedersen, T., Rosental, Y., Seitzinger, S., Smetacek, V., Steffen, W., 2000. The global carbon cycle: A test of our knowledge of earth as a system. Science 290, 291-296.

Fan, T.W.M., Lane, A.N., Shenker, M., Bartley, J.P., Crowley, D., Higashi, R.M., 2001. Comprehensive chemical profiling of gramineous plant root exudates using high-resolution NMR and MS. Phytochemistry 57, 209-221.

Fanin, N., Hättenschwiler, S., Schimann, H., Fromin, N., 2015. Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest. Functional Ecology 29, 140-150.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354-1364.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167-176.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277-280.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35, 837-843.

Fornara, D.A., Tilman, D., 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96, 314-322.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Frostegard, A., Tunlid, A., Baath, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151-163.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621-1625.

Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology and Biochemistry 43, 1705-1713.

Gleixner, G., 2013. Soil organic matter dynamics: A biological perspective derived from the use of compound-specific isotopes studies. Ecological Research 28, 683-695.

Gleixner, G., Bol, R., Balesdent, J., 1999. Molecular insight into soil carbon turnover. Rapid Communications in Mass Spectrometry 13, 1278-1283.

Gleixner, G., Poirier, N., Bol, R., Balesdent, J., 2002. Molecular dynamics of organic matter in a cultivated soil. Organic Geochemistry 33, 357-366.

Gram, H.C., 1884. Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten. Fortschritte der Medizin 2, 185–189.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33, 533-551.

Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29-56.

Guckert, J.B., Antworth, C.P., Nichols, P.D., White, D.C., 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Letters 31, 147-158.

Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 794-801.

Guckert, J.B., Ringelberg, D.B., White, D.C., Hanson, R.S., Bratina, B.J., 1991. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the Proteobacteria. Journal of General Microbiology 137, 2631-2641.

Guo, L.B., Halliday, M.J., Siakimotu, S.J.M., Gifford, R.M., 2005. Fine root production and litter input: Its effects on soil carbon. Plant and Soil 272, 1-10.

Habekost, M., Eisenhauer, N., Scheu, S., Steinbeiss, S., Weigelt, A., Gleixner, G., 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biology and Biochemistry 40, 2588-2595.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., Van Ruijven, J., Vos, V.C.A., Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218-221.

Hill, M.O., 1973. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 54, 427-432.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Lippincott Williams & Wilkins, Maryland, USA.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105-U129.

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

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75, 3-35.

Huang, Y.M., Michel, K., An, S.S., Zechmeister-Boltenstern, S., 2011. Changes in microbial-community structure with depth and time in a chronosequence of restored grassland soils on the Loess Plateau in northwest China. Journal of Plant Nutrition and Soil Science 174, 765-774.

IPCC, 2007. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. In: Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (Ed.), Cambridge University Press, UK, USA.

Johnson, M.J., Lee, K.Y., Scow, K.M., 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. Geoderma 114, 279-303.

Jones, D.L., 1998. Organic acids in the rhizosphere - A critical review. Plant and Soil 205, 25-44.
Jones, D.L., Darrah, P.R., 1996. Re-sorption of organic compounds by roots of Zea mays L. and its consequences in the rhizosphere. III.Characteristics of sugar influx and efflux. Plant and Soil 178, 153-160.
Kaiser, C., Frank, A., Wild, B., Koranda, M., Richter, A., 2010. Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2ω6,9 and 18:1ω9. Soil Biology and Biochemistry 42, 1650-1652.
Kaur, A., Chaudhary, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89, 1103-1112.
Kerger, B.D., Nichols, P.D., Antworth, C.P., Sand, W., Bock, E., Cox, J.C., Langworthy, T.A., White, D.C., 1986. Signature fatty acids in the polar lipids of acid-producing Thiobacillus spp.: Methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl and branched and normal monoenoic fatty acids. FEMS Microbiology Letters 38, 67-77.
Kindler, R., Miltner, A., Thullner, M., Richnow, H.H., Kästner, M., 2009. Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. Organic Geochemistry 40, 29-37.
Klamer, M., Hedlund, K., 2004. Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism. Soil Biology and Biochemistry 36, 983-988.
Kramer, C., Gleixner, G., 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology & Biochemistry 38, 3267-3278.
Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. Soil Biology & Biochemistry 40, 425-433.
Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L., Kandeler, E., 2013. Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Biology and Biochemistry 61, 76-85.
Kroppenstedt, R.M., 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms., In: Goodfellow, M., Minnikin, D.E. (Ed.), Chemical Methods in Bacterial Systematics. Academic Press, London, pp. 173-199.
Lal, R., 2003. Global potential of soil carbon sequestration to mitigate the greenhouse effect. Critical Reviews in Plant Sciences 22, 151-184.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623-1627.

Lane, D.J., 1991. In Nucleic Acid Techniques in Bacterial Systematics. (eds Stackebrandt, E. & Goodfellow, M.) 115-175 Wiley.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6, 6707.

Lange, M., Habekost, M., Eisenhauer, N., Roscher, C., Bessler, H., Engels, C., Oelmann, Y., Scheu, S., Wilcke, W., Schulze, E.D., Gleixner, G., 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. PLoS ONE 9, e96182.

Latz, E., Eisenhauer, N., Scheu, S., Jousset, A., 2015. Plant identity drives the expression of biocontrol factors in a rhizosphere bacterium across a plant diversity gradient. Functional Ecology. Article in Press.

Leckie, S.E., 2005. Methods of microbial community profiling and their application to forest soils. Forest Ecology and Management 220, 88-106.

Lee, K.H., Jose, S., 2003. Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management 185, 263-273.

Lemanski, K., Scheu, S., 2014. Incorporation of ^{13}C labelled glucose into soil microorganisms of grassland: Effects of fertilizer addition and plant functional group composition. Soil Biology and Biochemistry 69, 38-45.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72-76.

Lu, Y.H., Abraham, W.R., Conrad, R., 2007. Spatial variation of active microbiota in the rice rhizosphere revealed by in situ stable isotope probing of phospholipid fatty acids. Environmental Microbiology 9, 474-481.

Madan, R., Pankhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biology and Biochemistry 34, 125-128.

Madigan, M.T., Martinko, J., Parker, J., 2004. Brock Biology of Microorganisms 10 ed. Lippincott Williams & Wilkins, Baltimore, USA.

Makula, R.A., 1978. Phospholipid composition of methane-utilizing bacteria. Journal of Bacteriology 134, 771-777.

Malik, A., Scheibe, A., Lokabharathi, P.A., Gleixner, G., 2012. Online stable isotope analysis of dissolved organic carbon size classes using size exclusion chromatography coupled to an isotope ratio mass spectrometer. Environmental Science and Technology 46, 10123-10129.

Malik, A.A., Dannert, H., Griffiths, R.I., Thomson, B.C., Gleixner, G., 2015. Rhizosphere bacterial carbon turnover is higher in nucleic acids than membrane lipids: implications for understanding soil carbon cycling. Frontiers in Microbiology 6.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J., Wade, W.G., 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Applied and Environmental Microbiology 64, 795-799.

Marquard, E., Weigelt, A., Roscher, C., Gubsch, M., Lipowsky, A., Schmid, B., 2009. Positive biodiversity-productivity relationship due to increased plant density. Journal of Ecology 97, 696-704.

Medeiros, P.M., Fernandes, M.F., Dick, R.P., Simoneit, B.R.T., 2006. Seasonal variations in sugar contents and microbial community in a ryegrass soil. Chemosphere 65, 832-839.

Mellado-Vázquez, P.G., Lange, M., Bachmann, D., Gockele, A., Karlowsky, S., Milcu, A., Piel, C., Roscher, C., Roy, J., Gleixner, G., 2016. Plant diversity generates enhanced soil microbial access to recently photosynthesized carbon in the rhizosphere. Soil Biology and Biochemistry 94, 122-132.

Merckx, R., den Hartog, A., van Veen, J.A., 1985. Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biology and Biochemistry 17, 565-569.

Milcu, A., Roscher, C., Gessler, A., Bachmann, D., Gockele, A., Guderle, M., Landais, D., Piel, C., Escape, C., Devidal, S., Ravel, O., Buchmann, N., Gleixner, G., Hildebrandt, A., Roy, J., 2014. Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes. Ecology Letters 17, 435-444.

Miltner, A., Richnow, H.H., Kopinke, F.D., Kästner, M., 2004. Assimilation of CO_2 by soil microorganisms and transformation into soil organic matter. Organic Geochemistry 35, 1015-1024.

Mitchell, J.F.B., Johns, T.C., Gregory, J.M., Tett, S.F.B., 1995. Climate response to increasing levels of greenhouse gases and sulphate aerosols. Nature 376, 501-504.

Morrissey, J.P., Osbourn, A.E., 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiology and Molecular Biology Reviews 63, 708-724.

Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2010. Harvest impacts on soil carbon storage in temperate forests. Forest Ecology and Management 259, 857-866.

Neumann, G., Martinoia, E., 2002. Cluster roots - An underground adaptation for survival in extreme environments. Trends in Plant Science 7, 162-167.

Ng, E.L., Patti, A.F., Rose, M.T., Schefe, C.R., Wilkinson, K., Smernik, R.J., Cavagnaro, T.R., 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biology and Biochemistry 70, 54-61.

Nguyen, C., 2003. Rhizodeposition of organic C by plants: Mechanisms and controls. Agronomie 23, 375-396.

Nichols, P., Stulp, B.K., Jones, J.G., White, D.C., 1986. Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria Vitreoscilla, Flexibacter, Filibacter. Archives of Microbiology 146, 1-6.

Nordby, H.E., Nemec, S., Nagy, S., 1981. Fatty acids and sterols associated with citrus root mycorrhizae. Journal of Agricultural and Food Chemistry 29, 396-401.

O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553-567.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H., 2011. vegan: Community Ecology Package. R package version 1.17-9. .

Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. Fems Microbiology Ecology 29, 303-310.

Olsson, P.A., Baath, E., Jakobsen, I., Soderstrom, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623-629.

Olsson, P.A., Johnson, N.C., 2005. Tracking carbon from the atmosphere to the rhizosphere. Ecology Letters 8, 1264-1270.

Olsson, P.A., Larsson, L., Bago, B., Wallander, H., Van Aarle, I.M., 2003. Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytologist 159, 7-10.

Pacala, S., Socolow, R., 2004. Stabilization wedges: Solving the climate problem for the next 50 years with current technologies. Science 305, 968-972.

Parkes, R.J., Taylor, J., 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuarine, Coastal and Shelf Science 16, 173-174,IN175,175-189.

Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytologist 173, 600-610.

Paterson, E., Hall, J.M., Rattray, E.A.S., Griffiths, B.S., Ritz, K., Killham, K., 1997. Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. Global Change Biology 3, 363-377.

Phillipson, J., Putman, R.J., Steel, J., Woodell, S.R.J., 1975. Litter input, litter decomposition and the evolution of carbon dioxide in a beech woodland-Wytham woods, Oxford. Oecologia 20, 203-217.

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

Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M., Joergensen, R.G., 2006. Soil microbial community composition as affected by restoration practices in California grassland. Soil Biology and Biochemistry 38, 1851-1860.

R Development Core Team, 2014. A language and environment for statistical computing, 3.0.3 ed. R Foundation for Statistical Computing, Vienna, Austria, 2014.

Ravenek, J.M., Bessler, H., Engels, C., Scherer-Lorenzen, M., Gessler, A., Gockele, A., De Luca, E., Temperton, V.M., Ebeling, A., Roscher, C., Schmid, B., Weisser, W.W., Wirth, C., de Kroon, H., Weigelt, A., Mommer, L., 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos.

Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B., Eisenhauer, N., 2012. Impacts of biodiversity loss escalate through time as redundancy fades. Science 336, 589-592.

Ringelberg, D.B., Davis, J.D., Smith, G.A., Pfiffner, S.M., Nichols, P.D., Nickels, J.S., Henson, J.M., Wilson, J.T., Yates, M., Kampbell, D.H., Read, H.W., Stocksdale, T.T., White, D.C., 1989. Validation of signature polarlipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. FEMS Microbiology Letters 62, 39-50.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., Schmid, B., Schulze, E.D., 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5, 107-121.

Rosenkranz, S., Wilcke, W., Eisenhauer, N., Oelmann, Y., 2012. Net ammonification as influenced by plant diversity in experimental grasslands. Soil Biology and Biochemistry 48, 78-87.

Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E., Scheu, S., 2007. Lipid composition of Collembola and their food resources in deciduous forest stands-Implications for feeding strategies. Soil Biology and Biochemistry 39, 1990-2000.

Sakamoto, K., Iijima, T., Higuchi, R., 2004. Use of specific phospholipid fatty acids for identifying and quantifying the external hyphae of the arbuscular mycorrhizal fungus Gigaspora rosea. Soil Biology and Biochemistry 36, 1827-1834.

Saranpää, P., Nyberg, H., 1987. Lipids and sterols of Pinus sylvestris L. sapwood and heartwood. Trees 1, 82-87.

Scheibe, A., Krantz, L., Gleixner, G., 2012. Simultaneous determination of the quantity and isotopic signature of dissolved organic matter from soil water using high-performance liquid chromatography/isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 26, 173-180.

Scheibe, A., Steffens, C., Seven, J., Jacob, A., Hertel, D., Leuschner, C., Gleixner, G., 2015. Effects of tree identity dominate over tree diversity on the soil microbial community structure. Soil Biology and Biochemistry 81, 219-227.

Scheurbrandt, G., Bloch, K., 1962. Unsaturated fatty acids in microorganisms. The Journal of biological chemistry 237, 2064-2068.

Schimel, D.S., 1993. Theory and application of tracers. Academic Press, Inc., San Diego, CA.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schnitzer, M., 1991. Soil organic matter—the next 75 years. Soil Science 151, 41-58.

Schoug, Å., Fischer, J., Heipieper, H.J., Schnürer, J., Håkansson, S., 2008. Impact of fermentation pH and temperature on freeze-drying survival and membrane lipid composition of Lactobacillus coryniformis Si3. Journal of Industrial Microbiology and Biotechnology 35, 175-181.

Schütte, U.M.E., Abdo, Z., Bent, S.J., Shyu, C., Williams, C.J., Pierson, J.D., Forney, L.J., 2008. Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. Applied Microbiology and Biotechnology 80, 365-380.

Schutter, M., Dick, R., 2001. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biology and Biochemistry 33, 1481-1491.

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., Fontaine, S., 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the

rhizosphere priming effect induced by grassland species. Soil Biology and Biochemistry 80, 146-155.

Simpson, E.H., 1949. Measurement of diversity [16]. Nature 163, 688.

Singh, B.K., Munro, S., Reid, E., Ord, B., Potts, J.M., Paterson, E., Millard, P., 2006. Investigating microbial community structure in soils by physiological, biochemical and molecular fingerprinting methods. European Journal of Soil Science 57, 72-82.

Staddon, P.L., Reinsch, S., Olsson, P.A., Ambus, P., Lüscher, A., Jakobsen, I., 2014. A decade of free-air CO_2 enrichment increased the carbon throughput in a grass-clover ecosystem but did not drastically change carbon allocation patterns. Functional Ecology 28, 538-545.

Steenwerth, K.L., Jackson, L.E., Calderón, F.J., Stromberg, M.R., Scow, K.M., 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology and Biochemistry 34, 1599-1611.

Steer, J., Harris, J.A., 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of Agrostis stolonifera. Soil Biology and Biochemistry 32, 869-878.

Steinbeiss, S., Bessler, H., Engels, C., Temperton, V.M., Buchmann, N., Roscher, C., Kreutziger, Y., Baade, J., Habekost, M., Gleixner, G., 2008a. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Global Change Biology 14, 2937-2949.

Steinbeiss, S., Temperton, V.M., Gleixner, G., 2008b. Mechanisms of short-term soil carbon storage in experimental grasslands. Soil Biology and Biochemistry 40, 2634-2642.

Strickland, M.S., Rousk, J., 2010. Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385-1395.

Tefs, C., Gleixner, G., 2012. Importance of root derived carbon for soil organic matter storage in a temperate old-growth beech forest - Evidence from C, N and ^{14}C content. Forest Ecology and Management 263, 131-137.

ter Braak, C.J.F., Šmilauer, P., 2012. Canoco reference manual and user's guide: software for ordination (version 5.0). Ithaca, New York, USA. Microcomputer Power.

Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G., 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology & Biochemistry 42, 1558-1565.

Thoms, C., Gleixner, G., 2013. Seasonal differences in tree species' influence on soil microbial communities. Soil Biology and Biochemistry 66, 239-248.

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

Treonis, A.M., Ostle, N.J., Stott, A.W., Primrose, R., Grayston, S.J., Ineson, P., 2004. Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biology and Biochemistry 36, 533-537.

Trumbore, S.E., 1997. Potential responses of soil organic carbon to global environmental change. Proceedings of the National Academy of Sciences of the United States of America 94, 8284-8291.

Trumbore, S.E., Davidson, E.A., Barbosa De Camargo, P., Nepstad, D.C., Martinelli, L.A., 1995. Belowground cycling of carbon in forests and pastures of eastern Amazonia. Global Biogeochemical Cycles 9, 515-528.

Turgeon, R., Medville, R., 2004. Phloem loading. A reevaluation of the relationship between plasmodesmatal frequencies and loading strategies. Plant physiology 136, 3795-3803.

Van Der Krift, T.A.J., Kuikman, P.J., Möller, F., Berendse, F., 2001. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. Plant and Soil 228, 191-200.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535-541.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry 32, 1837-1846.

Wardle, D.A., 2002. Linking the aboveground and belowground components, In: Levin, S.A., Horn, H.S. (Ed.), Monographs in Population Biology. Princeton University Press, New Jersey, p. 392 pp.

Watzinger, A., 2015. Microbial phospholipid biomarkers and stable isotope methods help reveal soil functions. Soil Biology and Biochemistry 86, 98-107.

Werner, R.A., Brand, W.A., 2001. Referencing strategies and techniques in stable isotope ratio analysis. Rapid Communications in Mass Spectrometry 15, 501-519.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the Sedimentary Microbial Biomass by Extractable Lipid Phosphate. Oecologia 40, 51-62.

White, D.C., Stair, J.O., Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17, 185-196.

Wild, B., Wanek, W., Postl, W., Richter, A., 2010. Contribution of carbon fixed by Rubisco and PEPC to phloem export in the Crassulacean acid metabolism plant Kalanchoë daigremontiana. Journal of Experimental Botany 61, 1375-1383.

Wilkinson, S.C., Anderson, J.M., Scardelis, S.P., Tisiafouli, M., Taylor, A., Wolters, V., 2002. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biology and Biochemistry 34, 189-200.

Wolfe, R., Chinkes, D.L., 2005. Isotope Tracers in Metabolic Research. Principles and Practice of Kinetic Analysis, 2nd Edition Wiley-Liss, New York, NY.

Wu, L., Li, Z., Li, J., Khan, M.A., Huang, W., Zhang, Z., Lin, W., 2013. Assessment of shifts in microbial community structure and catabolic diversity in response to Rehmannia glutinosa monoculture. Applied Soil Ecology 67, 1-9.

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

Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275-294.

Zelles, L., 1999a. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. Biology and Fertility of Soils 29, 111-129.

Zelles, L., 1999b. Identification of single cultured micro-orgnisms based on their whole-community fatty acid profiles, using an extended extraction procedure. Chemosphere 39, 665-682.

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

Zhang, F.S., Ma, J., Cao, Y.P., 1997. Phosphorus deficiency enhances root exudation of low-molecular weight organic acids and utilization of sparingly soluble inorganic phosphates by radish (Raghanus satiuvs L.) and rape (Brassica napus L.) plants. Plant and Soil 196, 261-264.

SELBSTÄNDIGKEITSERKLÄRUNG

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

Jena, Juli 2016

Perla G. Mellado Vázquez

CURRICULUM VITAE

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International Max Planck Research School for Global Biogeochemical Cycles, Jena, Germany (IMPRS-gBGC)

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Experimental Interaction Ecology Group

German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

01. 2009 – 03. 2011 **Master in Science (Aquatic Chemistry). Awarded with Honors**

Instituto de Ciencias del Mar y Limnología (ICML) Universidad Nacional Autónoma de México (UNAM), Mazatlán, México

Thesis title: Recent geochronology with ^{210}Pb of the organic carbon and persistent organic pollutant (PAH's and PCB's) accumulation in two sites of the Mexican Pacific Continental Shelf

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SCIENTIFIC PUBLICATIONS

1- Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., **Mellado-Vázquez, P.G.**, Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications 6,6707*

2- **Mellado-Vázquez P.G.,** Lange M., Bachmann D., Gockele A., Karlowsky S., Milcu A., Piel C., Roscher C., Roy J. and Gleixner G., 2016. Plant diversity generates enhanced soil microbial access to recently photosynthesized carbon in the rhizosphere. *Soil Biology & Biochemistry 94, 122-132*.

3- Malik A.A., Chowdhury S., Schlager V., Oliver A., **Mellado-Vázquez P.G.**, Jehmlich N., von Bergen M., Griffiths R. I., Gleixner G. Soil fungal:bacterial ratios are linked to carbon storage potential. *Under review.*

4- **Mellado-Vázquez P.G.**, Lange Markus and Gleixner Gerd. The soil microbial community composition and soil microbial carbon uptake are more affected by soil type than by different vegetation types (C3 and C4 plants) and seasonal changes. *Ready for submission to Soil Biology and Biochemistry*

SCIENTIFIC PRESENTATIONS

April 17-22, 2016 **Mellado-Vázquez P.G.**, Lange M. and Gleixner G. The soil microbial community composition and soil microbial carbon uptake are more affected by soil type than by different vegetation types (C3 and C4 plants) and seasonal changes. European Geosciences Union General Assembly 2016. April 17-22, 2016, Vienna, Austria. PICO presentation

Dec. 14-18, 2015 **Mellado-Vázquez P.G.**, Lange M., Griffiths R., Malik A., Ravenek A., Strecker T., Eisenhauer N. and Gleixner G. Mechanisms controlling the plant diversity effect on soil microbial community composition and soil microbial diversity. 2015 American Geophysical Union Fall Meeting. San Francisco, California, USA. Poster presentation

March 12-14, 2014 **Mellado-Vázquez P.G.**, Lange M., Gleixner G. Tracing the recently photosythesized carbon into soil microorganisms: A plant diversity experiment. PhD-EES Conferece. Jena, Germany. Poster presentation

Sept. 09-13, 2013 **Mellado-Vázquez P.G.**, Lange M., Landais D., Piel C., Escape C., Devidal S., Ravel O., Milcu A., Roy J., Gleixner G. Different microbial groups contribute differentially to carbon cycling in soils. GfOe. Potsdam, Germany. Oral presentation

Aug. 18-23, 2013 **Mellado-Vázquez P.G.**, Lange M., Landais D., Piel C., Escape C., Devidal S., Ravel O., Milcu A., Roy J., Gleixner G. Effects of biodiversity on the flow of carbon from aboveground to belowground systems. INTECOL. Into the next 100 years. London, UK. Poster presentation

Nov. 5 - 8, 2012 **Mellado-Vázquez P.G.**, Lange M. and Gleixner G. Seasonal variations in the microbial community structure in soil in a vegetation change experiment. 3rd International Student Conference on Microbial Communication. Jena, Germany. Poster presentation

APPENDIX

A1.1. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) assigned to specific microbial groups

Marker	Origin	Marker	Origin
12:0	Bacteria[1]	**17:0i**	Gram + bacteria[2,3,4]
13:0	Bacteria[1]		Gram – bacteria and anaerobic bacteria[3]
14:0i	Gram + bacteria[1,2,3,4,5]	**17:0a**	Gram + bacteria[2,3,4]
	Gram – bacteria and anaerobic bacteria[3]		Gram – bacteria and anaerobic bacteria[3]
14:0	Bacteria[1]	**17:0cy**	Gram – bacteria made in stationary phase[2,4]
15:1	Gram – bacteria[5]		Aerobic bacteria in sediments[21]
15:0i	Gram + bacteria[1,2,3,4,5]		Anaerobic bacteria in soils[22]
	Gram – bacteria and anaerobic bacteria[3]		Starvation[23]
15:0a	Gram + bacteria[2,4,7]		Gram + bacteria[24]
15:0	Bacteria[1]	**17:0**	Bacteria[1]
16:0br	Gram + bacteria[2]	**18:0(10Me)**	Actinobacteria and sulfate- reducing bacteria[3]
16:0i	Gram + bacteria[1,2,3,4]		Actinobacteria[25,6]
	Gram – bacteria and anaerobic bacteria[3]		Sulfate-reducing bacteria[17,18]
16:2	Plants[2]	**18:0br**	Mostly Gram + bacteria[2]
16:1ω11	Gram – bacteria[2]	**18:3**	AMF[14]
	Type I Methanotrophs[8,9]	**18:2**	Plants and fungi[2]
16:1ω7	Bacteria[10,2]	**18:2ω6,9**	Saprotrophic fungi[26,1,2,3,27]
	Gram – bacteria[11,2,6]		Saprotrophic and EMF[28,4]
	Unknown[7]		Plant residues (Pinus sylvestris)[29]
	Type I Methanotrophs[8,9]	**18:1ω9**	Gram – bacteria in grassland/agricultural soil[11,2,27]
16:1ω5 (PLFA)	Gram – bacteria[12,11,13,2]		Fungi (forest soil)[26,30,31,6,27]
16:1ω5 (PLFA and NLFA)	AMF[14,13,15,16,36]		AMF when combined with 16:1ω5 and 20:1ω9[32]
16:1	Type I Methanotrophs[8,9]		Gram + bacteria[14]
16:0(10Me)	Sulfate-reducing bacteria[17,18]		Plants[33]
16:0	Bacteria[1]	**18:1ω8**	Type II Methanotrophs[34,35]
	Saprotrophic fungi[1]	**18:1ω7**	Gram – bacteria[11,2,6,7]
	Roots[1]		AMF[36]
17:1	Gram – bacteria and anaerobic bacteria[3]	**18:1ω5**	Unspecific
17:1ω7i	Desulfovibrio[19]	**18:0**	Bacteria[1]
	Desulfovibrio desulfuricans[20]	**18:1**	Unspecific
17:1ω6	Desulfobulbus[21]	**19:0(10Me)**	Actinobacteria and sulfate- reducing bacteria[2,6]
17:0(10Me)	Actinobacteria and sulfate- reducing bacteria[2,6]		Actinobacteria[6]
	Actinobacteria[6]	**19:0br**	Mostly Gram + bacteria[2]
17:0br	Mostly Gram + bacteria[2]		

A1.1. Continuation

Marker	Origin	Marker	Origin
19:0cy	Gram – bacteria made in stationary phase[2,4]	**20:3**	AMF[14]
	Aerobic bacteria in sediments[21]		Soil protozoa[1]
	Anaerobic bacteria in soils[22]	**20:1ω9**	AMF when combined with 16:1ω5 and 18:1ω9[32]
	Starvation[23]		AMF (Gigaspora)[16]
	Gram + bacteria[24]	**21:0-24:0**	Microeukaryotes[1]
20:5ω3,6,9,12,15	Algae[37]		Plants[4,33]
	Collembola[38]	**22:0br**	Microeukaryotes[1]
20:4	AMF[14]		

1 (White et al., 1996); 2(Zelles, 1997); 3 (Wilkinson et al., 2002); 4(Zelles, 1999a); 5 (Steenwerth et al., 2002); 6 (Bååth and Anderson, 2003); 7 (Carney and Matson, 2005); 8 (Makula, 1978); 9 (Bowman et al., 1993); 10 (Guckert et al., 1991); 11 (Borga et al., 1994); 12 (Nichols et al., 1986); 13 (Olsson et al., 1995), 14 (Nordby et al., 1981); 15 (Olsson et al., 2003); 16 (Sakamoto et al., 2004); 17 (Dowling et al., 1986); 18 (Kerger et al., 1986); 19 (Edlund et al., 1985); 20 (Scheurbrandt and Bloch, 1962); 21 (Parkes and Taylor, 1983); 22 (Guckert et al., 1985); 23 (Guckert et al., 1986); 24 (Schoug et al., 2008); 25 (Kroppenstedt, 1985); 26 (Vestal and White, 1989); 27 (Kaiser et al., 2010); 28 (Frostegård and Bååth, 1996); 29 (Saranpää and Nyberg, 1987); 30 (Schutter and Dick, 2001); 31 (Bååth, 2003); 32 (Madan et al., 2002); 33 (Ruess et al., 2007); 34 (Bossio and Scow, 1998); 35 (Ringelberg et al., 1989); 36 (Olsson, 1999); 37 (Dunstan et al., 1993); 38 (Chamberlain and Black, 2005)

A2.1. Plant diversity (Div) and functional group (G = grasses, H = herbs and L = legumes) composition of the twelve selected plots from the Jena Experiment. The species present in the lysimeters at the final harvest are marked in bold in the table.

Plot ID	Div	G	H	L	Species composition	Dome
B2A22	16	5	5	6	***Am,*** *Cc*, ***Fp***, ***Tf***, ***Pp***, ***Pt***, ***Cj***, ***Ra***, *So*, *CAp* , *Th*, ***Lc***, ***VIc***, ***Tr***, ***Lp***, ***Ov***	1
B4A04	4	1	2	1	***Ae, Pl, As,*** *Tc*	2
B1A01	16	4	8	4	***AVp***, ***Pp***, ***Ao***, ***Bh***, ***Pl***, ***To***, ***Ar***, ***Rr***, *As*, ***Gp***, *TRp*, ***CAc***, *Tc*, ***VIc***, ***Lp***, ***Lc***	3
B1A04	4	1	2	1	***Fp***, ***Pl***, *CAp*, *Ov*	4
B3A23	4	1	2	1	*Bh*, ***Rr***, ***Lv***, ***TRf***	5
B2A18	16	4	8	4	***Ap***, *Bh*, ***Pp***, ***Cc***, ***Rr***, ***Pm***, ***Ar***, *Pv*, ***CAp***, ***Gp***, *As*, *Cp*, *Ml*, ***Tr***, ***Td***, *Tc*	6
B4A18	16	4	8	4	*Cc*, *LUc*, ***Ap***, *Bh*, *La*, ***Pm***, ***Vc***, ***To***, ***Cb***, *CAc*, ***PIm***, ***Hs***, *Th*, *Tc*, ***Lp***, ***Ov***	7
B2A01	4	1	2	1	***Ao***, ***Pv***, ***Ka***, ***Tp***	8
B3A22	16	4	8	4	***PHp***, ***Fr***, *Ao*, ***Be***, ***Rr***, ***Ar***, ***Bp***, ***Vc***, ***Gp***, ***Cb***, ***Ra***, ***Gm***, ***VIc***, ***Ov***, *TRf*, *Td*	9
B2A16	4	0	3	1	***Pm***, *La*, ***Ka***, ***VIc***	10
B3A24	16	6	5	5	***Fp***, *Bh*, ***Ap***, *Ao*, ***Pt***, ***Ae***, ***To***, ***Rr***, ***Ar***, *Pv*, ***Gh***, ***Lc***, ***Tp***, *Tr*, ***VIc***, ***Ms***	11
B4A11	4	1	2	1	***Tf***, ***TRp***, ***Hs***, ***Ms***	12

Grasses (G): Ae = Arrhenatherum elatius L. (J. et C. PRESL), Ao= Anthoxantum odoratum L., Ap= Alopecurus pratensis L., AVp = Avenula pubescens HUDS. (DUM.), Be= Bromus erectus HUDS., Bh= Bromus hordeaceus L., Cc= Cynosurus cristatus L., Dg= Dactylis glomerata L., Fp= Festuca pratensis HUDS., Fr= Festuca rubra L., Hl= Holcus lanatus L., LUc = Luzula campestris L (DC.), PHp= Phleum pratense L., Pp= Poa pratensis L., Pt= Poa trivialis L., Tf= Trisetum flavescens L. (P. BEAUV.);

Herbs (H): Am= Achillea millefolium L., Bp= Bellis perennis L., Cb= Crepis benis L., CAc= Carum carvi L., CAp = Campanula patula L., Cj= Centaurea jacea L., Co= Cirsium oleraceum L., Cp= Cardamine pratensis L., Dc= Daucus carota L., Gm= Galium mollugo L., Gh= Glechoma hederacea L., Hs = Heracleum sphondylium L., Ka= Knautia arvensis L., La= Leontodon autumnalis L., Lh= Leontodon hispidus L., Lv= Leucanthemum vulgare Lam., PIm = Pimpinella major L. (HUDS.), Pl= Plantago lancelolata L., Pm= Plantago media L., PRv = Primula veris L., Pv= Prunella vulgaris L., Ra= Rumex acetosa L., To= Taraxacum officinale WEBER, TRp= Tragopogon pratensis L., Vc= Veronica chamaedrys L;

Legumes (L): Lp= Lathyrus pratensis L., Lc= Lotus corniculatus L., Ml= Medicago lupulina L., Ms=Medicago x varia MARTYN, Ov= Onobrychis viciifolia SCOP., Td= Trifolium dubium SIBTH., TRf = Trifolium fragiferum L., Th= Trifolium hybridum L., Tr= Trifolium repens L., Tp= Trifolium pratense L., VIc= Vicia cracca L.

A2.2. a) $\delta^{13}CO_2$ values ($\delta^{13}CO_2$-respiration, [‰] in night-time ecosystem respiration (plant plus soil respiration) and b) ANOVA (analysis of variance) from the differences of $\delta^{13}CO_2$-respiration between plant diversity levels. Plus symbol indicates marginally significant differences ($^+$: $p \leq 0.1$).

a)

Dome	**Plant diversity**	**$\delta^{13}CO_2$-respiration**			
		T0	**T1**	**T2**	**T3**
D1	16	-13.6	-11.7	-12.4	-12.6
	16	-12.3	-11.6	-12.5	-12.7
D2	4	-12.4	-12.9	-14.5	-15.3
	4	-11.5	-13.0	-14.6	-14.9
D3	16	-11.9	-14.0	-15.4	-15.7
	16	-11.2	-14.2	-15.3	-15.7
D4	4	-10.1	-9.7	-10.8	-11.8
	4	-10.2	-9.5	-12.1	-11.6
D5	4	-10.6	-11.1	-12.3	-12.4
	4	-11.8	-11.9	-12.3	-12.3
D6	16	-10.2	-10.3	-11.3	-14.0
	16	-9.9	-10.3	-12.2	-12.6
D7	16	-11.1	-9.7	-11.0	-11.5
	16	-11.1	-9.6	-11.1	-12.2
D8	4	-10.3	-13.0	-13.9	-14.5
	4	-10.4	-13.0	-14.0	-15.0
D9	16	-10.2	-12.8	-14.8	-14.2
	16	-9.7	-12.9	-14.6	-14.4
D10	4	-10.0	-8.2	-9.9	-11.1
	4	-9.8	-8.2	-9.9	-10.2
D11	16	-9.8	-13.0	-13.4	-13.7
	16	-10.0	-12.2	-13.0	-13.0
D12	4	-10.5	-10.5	-11.3	-12.1
	4	-10.6	-10.6	-12.0	-12.9

b)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	10.02	10.02	3.27	0.074 $^+$
Residuals	94	287.60	3.06		

A2.3. Results of linear models to test the effect of plant diversity on a) the $\delta^{13}C$ values of each individual compound and b) weighted means (average $\delta^{13}C$ value multiplied by the average concentration of each microbial group) in both soil depths (0-5 cm and 5-10 cm depth). Stars indicate significant differences of $\delta^{13}C$ values between plant diversity levels (*: p≤ 0.05; $^{+}$: p≤ 0.1).

a) $\delta^{13}C$ values of individual compounds					
Marker	**Microbial group**	**LM (0-5 cm)**		**LM (5-10 cm)**	
		t-value	**p-value**	**t-value**	**p-value**
14:0i	Gram + bacteria	-0.804	0.440	-0.287	0.780
15:0i	Gram + bacteria	-0.831	0.425	-0.411	0.690
15:0a	Gram + bacteria	-0.809	0.438	0.529	0.608
16:0i	Gram + bacteria	-0.397	0.700	-0.210	0.838
17:0i	Gram + bacteria	-0.813	0.435	-0.220	0.830
17:0a	Gram + bacteria	-0.575	0.578	-0.385	0.708
16:0(10Me)	Actinobacteria	-0.622	0.548	-0.016	0.987
18:0(10Me)	Actinobacteria	-0.201	0.845	0.177	0.863
17:0cy	Gram - bacteria cyclo	-0.390	0.705	0.204	0.843
19:0cy	Gram - bacteria cyclo	0.053	0.959	0.157	0.878
16:1ω7	Gram - bacteria	2.458	0.034*	0.330	0.748
16:1ω5	Gram - bacteria	1.844	0.095^{+}	-0.708	0.495
16:1	Gram - bacteria	-0.397	0.700	-0.210	0.838
17:1	Gram - bacteria	2.109	0.061^{+}	1.589	0.143
18:1ω9	Gram - bacteria	2.467	0.033*	2.174	0.055^{+}
18:1ω7	Gram - bacteria	1.777	0.106	0.813	0.435
16:0	Non-specific marker	-0.862	0.409	0.045	0.965
18:0	Non-specific marker	-0.181	0.860	0.240	0.815
18:2ω6,9	Saprotrophic fungi	-1.058	0.315	-1.196	0.259
16:1ω5N	AM fungi-NLFA	2.494	0.032*	0.999	0.341
b) Weighted means					
	Microbial group	**LM (0-5 cm)**		**LM (5-10 cm)**	
		t-value	**p-value**	**t-value**	**p-value**
	Gram + bacteria	-0.760	0.465	-0.153	0.882
	Actinobacteria	-0.540	0.601	-0.669	0.519
	Gram - bacteria cyclo	-0.106	0.918	0.132	0.897
	Gram - bacteria	2.254	0.048*	1.056	0.316
	Non-specific marker	-0.718	0.489	0.109	0.915

A2.4. a) Mean values (n=6, standard error is given in parenthesis) of all possible explanatory variables measured in soils from 0-5 cm depth: soil moisture, soil organic carbon (SOC), canopy leaf nitrogen (N.area), root biomass, root length per volume of soil (root length), specific root length (specific RL), δ^{13}C values of soil organic carbon (δ^{13}C-SOC), root material (δ^{13}C-Root) and root sugars (δ^{13}C-Sugars) as well as concentration of sugars in root tissues and aboveground (AG) biomass. b) Pearson's correlation coefficients of all possible explanatory variables, concentration of sugars in root tissues measured in soils from 0-5 cm depth and aboveground (AG) biomass. Stars and plus symbols indicate significant (and marginally significant) correlation between a given covariate and plant diversity (*: p≤ 0.05; $^{+}$: p≤ 0.1).

a)

Plant diversity	Soil moisture [%]	SOC [%]	N.area [g m^{-2}]	Root biomass [mg cm^{-3}]	Root length [cm cm^{-3}]	Specific RL [m g^{-1}]	δ^{13}C-SOC [‰]	δ^{13}C-Sugars [‰]	δ^{13}C-Root [‰]	Root sugars [µg mg^{-1}]	AG biomass [g m^{-2}]
Low	20.03	2.45	2.19	4.28	57.36	163.59	-27.81	-16.20	-24.07	141.17	148.72
	(1.08)	(0.15)	(0.45)	(1.43)	(16.89)	(47.63)	(0.09)	(0.48)	(0.73)	(16.93)	(24.93)
High	23.38	3.07	2.88	9.04	110.81	118.59	-27.97	-18.84	-26.43	139.93	193.93
	(1.35)	(0.20)	(0.39)	(1.25)	(30.14)	(27.71)	(0.13)	(1.20)	(0.59)	(17.50)	(17.68)

b)

	Plant diversity	Soil moisture	SOC	N.area	Root biomass	Root length	Specific RL	δ^{13}C-SOC	δ^{13}C-Root	δ^{13}C-Sugars	Root sugars	AG biomass
Plant diversity	1.000											
Soil moisture	0.523^{+}	1.000										
SOC	0.618*	0.681	1.000									
N.area	0.343	0.505	0.190	1.000								
Root biomass	0.572^{+}	0.768	0.680	0.541	1.000							
Root length	0.439	0.520	0.068	0.513	0.421	1.000						
Specific RL	-0.234	-0.090	-0.481	-0.046	-0.397	0.524	1.000					
δ^{13}C-SOC	-0.295	0.221	0.144	0.234	0.246	0.082	0.110	1.000				
δ^{13}C-Root	-0.621*	0.163	-0.042	0.267	0.197	-0.198	-0.166	0.645	1.000			
δ^{13}C-Sugars	-0.543^{+}	0.181	-0.064	0.023	0.079	0.077	0.254	0.821	0.704	1.000		
Root sugars	-0.016	-0.036	0.255	-0.536	-0.078	-0.253	0.181	-0.138	-0.222	-0.044	1.000	
AG biomass	0.417	0.614	0.541	0.759	0.490	0.105	-0.413	0.141	0.211	-0.038	-0.358	1.000

A2.5. Results of second and third best ANOVA models (see Table 2 in main text) for each microbial group. Stars indicate significant differences of δ^{13}C values between plant diversity levels (***: p≤ 0.001; **: p≤ 0.01; *: p≤ 0.05; $^{+}$: p≤ 0.1).

Microbial group		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Gram negative bacteria						
2nd best model	**Root biomass**	1	28.97	28.974	27.126	0.001***
	δ^{13}C-SOC	1	3.78	3.78	3.539	0.093^{+}
	Residuals	9	9.613	1.068		
3rd best model	**δ^{13}C-Sugars**	1	1.589	1.589	1.175	0.307
	Root biomass	1	28.608	28.608	21.155	0.001**
	Residuals	9	12.171	1.352		
Gram negative bacteria (cyclo)						
2nd best model	**Soil moisture**	1	31.95	31.95	3.113	0.108
	Residuals	10	102.63	10.263		
3rd best model	**Soil moisture**	1	31.95	31.95	3.864	0.081^{+}
	δ^{13}C-SOC	1	28.22	28.22	3.413	0.098^{+}
	Residuals	9	74.41	8.268		
Gram positive bacteria						
2nd best model	**N.area**	1	2.802	2.802	0.732	0.414
	δ^{13}C-SOC	1	28.87	28.87	7.546	0.023*
	Residuals	9	34.44	3.827		
3rd best model	**SOC**	1	5.108	5.108	1.853	0.211
	Root biomass	1	10.639	10.639	3.861	0.085^{+}
	δ^{13}C-SOC	1	28.32	28.32	10.276	0.013*
	Residuals	8	22.047	2.756		
Actinobacteria						
2nd best model	**SOC**	1	8.689	8.689	1.047	0.330
	Residuals	10	82.99	8.299		
3rd best model	**δ^{13}C-Sugars**	1	8.021	8.021	0.959	0.351
	Residuals	10	83.65	8.365		
Saprotrophic fungi						
2nd best model	**Root Length**	1	2.33	2.33	0.597	0.459
	δ^{13}C-SOC	1	39.66	39.66	10.148	0.011*
	Residuals	9	35.17	3.908		
3rd best model	**δ^{13}C-Root**	1	23.97	23.97	4.505	0.059^{+}
	Residuals	10	53.2	5.32		

A2.5 (continuation)

Arbuscular mycorrhizal fungi						
2nd best model	**SOC**	1	33.75	33.75	3.829	0.079^{+}
	Residuals	10	88.14	8.814		
3rd best model	**Root biomass**	1	25.86	25.86	2.693	0.132
	Residuals	10	96.02	9.602		
Non-specific marker						
2nd best model	**N.area**	1	0.78	0.78	0.141	0.716
	δ^{13}C-SOC	1	43.07	43.07	7.78	0.021*
	Residuals	9	49.83	5.537		
3rd best model	**Root biomass**	1	0.47	0.47	0.085	0.778
	δ^{13}C-SOC	1	42.91	42.91	7.676	0.022*
	Residuals	9	50.31	5.59		

A2.6. Mean values (± standard deviation) plant and soil related covariates (root biomass, leaf area index (LAI), soil moisture and soil organic carbon (SOC)) in each plant species richness (PSR) level

PSR	Root biomass	LAI	Soil moisture	SOC
	[g m^{-2}]		[%]	[%]
1	1.86 ± 1.88	0.57 ± 0.52	12.44 ± 2.61	2.24 ± 0.34
2	2.96 ± 2.46	0.88 ± 0.65	13.25 ± 2.40	2.25 ± 0.31
4	3.55 ± 2.30	1.87 ± 1.25	15.66 ± 2.78	2.48 ± 0.38
8	5.06 ± 3.26	1.64 ± 0.81	15.14 ± 1.79	2.44 ± 0.22
16	6.72 ± 4.17	2.37 ± 0.71	16.59 ± 2.13	2.64 ± 0.33
60	10.29 ± 4.10	3.50 ± 0.22	17.80 ± 1.08	2.70 ± 0.13

A2.7. Pearson's correlations coefficients of the relations between plant and soil related covariates: root biomass, leaf area index (LAI), soil moisture and soil organic carbon (SOC). Stars represent significant correlations between variables (**: p≤ 0.01; *** p≤ 0.001)

	Root biomass	LAI	Soil moisture	SOC
Root biomass	1.000			
LAI	0.370 ***	1.000		
Soil moisture	0.288 **	0.401 ***	1.000	
SOC	0.298 **	0.316 **	0.601 ***	1.000

A3.1. ANOVA (analyses of variance) results from the differences in concentration of different microbial groups related to plant diversity. Stars indicate significant differences between plant diversity levels (***:p≤ 0.001; **:p≤ 0.01; *: p≤ 0.05).

Gram positive bacteria					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	24.00	24.00	0.51	0.483
Layer	1	556.90	556.90	11.89	0.002 **
Plant div:Layer	1	74.10	74.10	1.58	0.222
Residuals	20	936.70	46.84		
Actinobacteria					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	2.18	2.18	0.31	0.584
Layer	1	31.79	31.79	4.52	0.046 *
Plant div:Layer	1	17.72	17.72	2.52	0.128
Residuals	20	140.68	7.03		
Gram negative bacteria (cyclo)					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	14.05	14.05	1.02	0.325
Layer	1	176.26	176.26	12.79	0.002 **
Plant div:Layer	1	0.01	0.01	0.01	0.944
Residuals	20	275.62	13.78		
Gram negative bacteria					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	28.00	28.00	0.13	0.722
Layer	1	10192.00	10192.00	46.89	<0.001 ***
Plant div:Layer	1	713.00	713.00	3.28	0.085
Residuals	20	4347.00	217.35		
Saprotrophic fungi					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
Plant diversity	1	0.27	0.27	0.30	0.593
Layer	1	14.81	14.81	16.14	<0.001 ***
Plant div:Layer	1	0.01	0.01	0.01	0.941
Residuals	20	18.35	0.92		

A4.1. ANOVA (analysis of variance) results from changes in the plant and soil-related covariates (root biomass, soil moisture, soil organic carbon (SOC) and leaf area index (LAI)) and plant diversity measures: plant species richness (PSR); functional group (FG); grasses, small and tall herbs and legumes. Significant differences between variables are represented by P values ≤ 0.05.

		Root biomass		**LAI**		**Soil moisture**		**SOC**	
	Df	F value	P	F value	P	F value	P	F value	P
Block	1	4.16	0.045	3.29	0.073	0.00	0.981	0.01	0.928
PSR	1	45.42	<0.001	71.78	<0.001	32.91	<0.001	18.07	<0.001
FG	1	0.38	0.540	8.74	0.004	2.12	0.150	1.81	0.183
Grasses	1	3.12	0.082	0.02	0.882	0.08	0.774	0.32	0.572
Small herbs	1	0.16	0.693	2.23	0.139	0.10	0.757	1.35	0.248
Tall herbs	1	0.06	0.815	0.39	0.535	0.53	0.470	2.00	0.162
Legumes	1	0.123	0.727	1.40	0.240	4.76	0.032	3.65	0.060

A5.1. Mean relative proportions (mol%, n= 3 ± standard deviation) and mean isotope C values (δ^{13}C [‰], n= 3 ± standard deviation) of individual phospholipid fatty acids (PLFAs) in sandy and clayey soils sown with C3 and C4 plants.

Marker	Microbial group	Season	Sandy				Clayey			
			C3		C4		C3		C4	
			mol%	δ^{13}C [‰]	mol%	δ^{13}C [‰]	mol%	δ^{13}C [‰]	mol%	δ^{13}C [‰]
15:0i	Gram + bacteria									
		Non-growing season	8.5 ± 0.7	-29.1 ± 1.2	7.3 ± 0.9	-21.6 ± 0.8	6.1 ± 0.4	-28.6 ± 0.6	6.5 ± 0.7	-22.5 ± 1.1
		Growing season	8.3 ± 0.4	-28.5 ± 1.4	8.4 ± 0.1	-20.3 ± 2.4	6.2 ± 1.5	-26.3 ± 1.4	5.8 ± 1.3	-19.8 ± 0.9
15:0a	Gram + bacteria									
		Non-growing season	7.0 ± 0.6	-28.3 ± 1.6	5.9 ± 0.6	-20.4 ± 1.6	6.0 ± 0.3	-27.0 ± 0.2	6.0 ± 0.8	-21.1 ± 0.7
		Growing season	6.6 ± 0.2	-27.7 ± 2.4	6.3 ± 0.2	-19.2 ± 1.9	5.9 ± 1.5	-26.2± 0.5	5.4 ± 1.2	-19.3 ± 0.9
16:0i	Gram + bacteria									
		Non-growing season	3.0 ± 0.4	-29.4 ± 1.1	2.7 ± 0.5	-22.4 ± 0.9	2.7 ± 0.2	-28.5 ± 0.3	2.5 ± 0.3	-23.2 ± 1.3
		Growing season	2.7 ± 0.1	-28.2 ± 1.4	2.9 ± 0.1	-20.9 ± 1.7	2.4 ± 0.5	-26.7 ± 1.7	2.2 ± 0.5	-21.6 ± 1.2
17:0i	Gram + bacteria									
		Non-growing season	2.2 ± 0.3	-31.8 ± 1.9	2.2 ± 0.5	-24.1 ± 1.3	2.0 ± 0.1	-29.3 ± 0.5	1.9 ± 0.3	-23.9 ± 0.4
		Growing season	2.4 ± 0.2	-29.5 ± 2.1	2.5 ± 0.2	-21.7 ± 2.1	2.0 ± 0.4	-25.9 ± 0.5	1.9 ± 0.3	-20.3 ± 1.3
17:0a	Gram + bacteria									
		Non-growing season	2.2 ± 0.3	-29.4 ± 1.2	2.1 ± 0.5	-21.7 ± 2.5	2.4 ± 0.1	-27.8 ± 0.6	2.3 ± 0.3	-22.0 ± 1.0
		Growing season	2.1 ± 0.1	-27.9 ± 3.5	2.2 ± 0.1	-19.4 ± 3.2	2.1 ± 0.5	-25.0 ± 0.6	2.0 ± 0.4	-18.9 ± 1.4
16:0(10Me)	Actinobacteria									
		Non-growing season	5.0 ± 0.4	-31.7 ± 1.3	4.6 ± 0.5	-25.5 ± 1.7	5.3 ± 0.3	-30.3 ± 1.3	5.2 ± 0.6	-25.9 ± 1.8
		Growing season	4.7 ± 0.9	-30.1 ± 1.6	4.9 ± 0.5	-24.4 ± 1.7	5.6 ± 1.1	-28.7 ± 0.9	4.8 ± 0.7	-23.9 ± 0.8
19:0(10Me)	Actinobacteria									
		Non-growing season	2.2 ± 0.4	-30.3 ± 1.3	1.9 ± 0.5	-21.1 ± 1.1	1.7 ± 0.2	-34.0 ± 1.2	1.5 ± 0.2	-25.8 ± 2.1
		Growing season	1.9 ± 0.1	-29.2 ± 0.8	1.9 ± 0.1	-22.6 ± 1.4	1.3 ± 0.2	-28.4 ± 0.2	1.3 ± 0.1	-22.9 ± 1.5

A5.1. Continuation

Marker	Microbial group	Season	Sandy				Clayey			
			C3		C4		C3		C4	
			mol%	δ13C [‰]	mol%	δ13C [‰]	mol%	δ13C [‰]	mol%	δ13C [‰]
17:0cy	G- bacteria (cyclo)									
		Non-growing season	3.0 ± 0.2	-32.7 ± 1.7	2.8 ± 0.6	-24.4 ± 2.6	2.6 ± 0.1	-32.2 ± 1.0	2.7 ± 0.4	-24.4 ± 0.7
		Growing season	3.6 ± 0.1	-30.7 ± 3.8	3.5 ± 0.1	-22.1 ± 2.1	2.5 ± 0.7	-27.7 ± 0.1	2.4 ± 0.5	-21.6 ± 0.2
19:0cy	G- bacteria (cyclo)									
		Non-growing season	7.1 ± 0.8	-37.9 ± 1.0	5.9 ± 0.8	-27.4 ± 1.7	4.8 ± 0.4	-33.1 ± 0.8	4.8 ± 0.6	-29.8 ± 0.9
		Growing season	6.2 ± 0.9	-32.1 ± 2.4	6.2 ± 0.5	-25.8 ± 1.8	4.8 ± 1.2	-30.2 ± 0.1	4.3 ± 0.7	-25.4 ± 0.2
15:1	G- bacteria									
		Non-growing season	2.5± 0.9	-27.6 ± 1.5	2.0 ± 0.4	-17.4 ± 1.9	3.4 ± 0.8	-29.1 ± 0.5	3.2 ± 0.8	-19.1 ± 2.1
		Growing season	3.8 ± 0.2	-29.2 ± 1.4	4.0 ± 0.2	-22.8± 1.5	1.4 ± 0.1	-30.4 ± 0.4	2.8 ± 0.6	-18.9 ± 2.6
16:1ω7	G- bacteria									
		Non-growing season	2.1 ± 0.2	-27.3 ± 1.3	2.5 ± 0.2	-19.9 ± 2.1	1.5 ± 0.4	-25.0 ± 0.9	1.5 ± 0.2	-16.7 ± 2.6
		Growing season	1.6 ± 0.5	-26.4 ± 1.7	1.7 ± 0.5	-20.7 ± 2.2	2.0 ± 0.3	-27.8 ± 0.2	2.0 ± 0.3	-17.4 ± 1.1
16:1ω5	G- bacteria									
		Non-growing season	8.6 ± 1.8	-28.5 ± 1.6	9.3 ± 1.9	-20.6 ± 1.1	11.7 ± 0.3	-30.6 ± 1.2	11.7 ± 0.9	-21.0 ± 1.5
		Growing season	8.9 ± 0.5	-30.5 ± 1.5	8.6 ± 0.1	-23.6 ± 1.0	11.1 ± 2.2	-32.4 ± 0.3	10.9 ± 1.4	-21.4 ± 0.2
16:1	G- bacteria									
		Non-growing season	4.6 ± 0.3	-26.9 ± 1.1	5.2 ± 0.4	-18.8 ± 1.4	5.9 ± 0.8	-27.4 ± 1.2	6.2 ± 0.6	-16.8 ± 2.0
		Growing season	4.6 ± 0.9	-28.1 ± 1.6	3.9 ± 0.3	-20.3 ± 1.0	7.1 ± 1.7	-29.1 ± 0.2	7.7 ± 0.5	-16.6 ± 0.5
17:1	G- bacteria									
		Non-growing season	5.4 ± 1.4	-27.4 ± 0.8	5.5 ± 0.5	-18.5 ± 4.0	7.7 ± 0.2	-30.0 ± 2.5	7.5 ± 0.4	-21.0 ± 1.8
		Growing season	6.1 ± 0.5	-29.4 ± 1.1	6.3 ± 0.5	-22.8 ± 2.8	6.3 ± 0.8	-30.4 ± 0.1	6.4 ± 0.7	-20.7 ± 0.1
18:1ω9	G- bacteria									
		Non-growing season	8.1 ± 1.1	-26.5 ± 1.3	9.5 ± 1.2	-18.3 ± 0.6	9.3 ± 0.4	-27.8 ± 1.0	9.5 ± 1.3	-18.8 ± 1.0
		Growing season	8.4 ± 1.0	-28.3 ± 1.7	8.2 ± 0.4	-21.4 ± 1.1	10.3 ± 1.7	-30.4 ± 0.8	9.3 ± 1.4	-19.5 ± 0.3

A5.1. Continuation

Marker	Microbial group	Season	Sandy				Clayey			
			C3		C4		C3		C4	
			mol%	$\delta^{13}C$ [‰]	mol%	$\delta^{13}C$ [‰]	mol%	$\delta^{13}C$ [‰]	mol%	$\delta^{13}C$ [‰]
18:1ω7	G- bacteria									
		Non-growing season	12.0 ± 1.5	-29.7 ± 1.2	13.6 ± 2.2	-22.0 ± 1.1	15.6 ± 1.0	-30.2 ± 1.0	15.9 ± 2.5	-20.3 ± 1.2
		Growing season	12.2 ± 1.1	-30.5 ± 1.4	11.5 ± 0.6	-23.4 ± 0.6	17.7 ± 3.5	-31.0 ± 0.3	18.1 ± 2.7	-20.2 ± 0.0
18:2ω6,9	Saprotrophic fungi									
		Non-growing season	2.7 ± 0.3	-28.1 ± 1.9	4.1 ± 1.5	-16.4 ± 0.4	2.7 ± 0.4	-28.1 ± 0.3	2.4 ± 1.0	-15.1 ± 0.4
		Growing season	2.6 ± 0.8	-28.5 ± 0.9	2.6 ± 1.2	-18.1 ± 0.4	2.0 ± 0.5	-26.7 ± 1.5	3.5 ± 0.9	-18.3 ± 1.3
14:0	Non-specific									
		Non-growing season	2.2 ± 0.4	-30.8 ± 0.9	2.2 ± 0.3	-24.4 ± 1.4	1.2 ± 0.2	-29.5 ± 1.1	1.0 ± 0.1	-23.4 ± 2.1
		Growing season	2.1 ± 0.2	-32.2 ± 2.2	2.2 ± 0.1	-28.1 ± 2.1	1.1 ± 0.2	-28.0 ± 1.3	1.1 ± 0.1	-21.2 ± 0.8
16:0	Non-specific									
		Non-growing season	8.6 ± 0.9	-29.8 ± 1.2	7.7 ± 0.5	-24.9 ± 1.2	5.8 ± 0.4	-30.0 ± 1.0	5.9 ± 0.6	-24.9 ± 2.0
		Growing season	8.5 ± 0.2	-28.9 ± 1.9	8.9 ± 0.2	-22.3 ± 2.0	6.4 ± 1.4	-27.1 ± 0.8	6.3 ± 1.4	-20.8 ± 1.0
18:0	Non-specific									
		Non-growing season	3.1 ± 0.4	-22.5 ± 0.6	3.1 ± 0.5	-20.4 ± 1.4	1.9 ± 0.2	-24.1 ± 2.0	1.8 ± 0.3	-13.9 ± 1.2
		Growing season	2.9 ± 0.2	-28.4 ± 1.8	3.2 ± 0.1	-22.8 ± 2.1	1.9 ± 0.4	-27.2 ± 1.6	1.8 ± 0.3	-21.5 ± 0.9

A5.2. Results of forward selection of the redundancy analysis (RDA) to calculate the effect of root biomass, SOC, DOC, clay and sand content and soil moisture on the drivers of the soil microbial community composition (The order of the variables in the first column represents the sequence of fitting in the analysis). P values $p \leq 0.05$ indicate significant influence of an explanatory variable on the microbial community composition.

Variable	**Explains [%]**	**Contribution [%]**	**pseudo-F**	**P**
Root biomass	45.2	73.4	24.7	0.001
SOC	8.0	12.9	4.9	0.021
DOC	2.1	3.5	1.3	0.377
Soil moisture	2.3	3.8	1.5	0.251
Clay content	3.9	6.4	2.7	0.101

A5.3. Analyses of variance (ANOVA) of the changes of $\delta^{13}C$ values of individual phospholipid fatty acids (PLFA) related to different seasons (non-growing and growing) and vegetation types (C3 and C4 plants). Stars indicate significant differences between season and vegetation type (*: p≤ 0.05; **: p≤ 0.01; ***: p≤ 0.001).

$\delta^{13}C$ values of individual compounds					
Marker	**Microbial group**		**Df**	**F value**	**Pr(>F)**
15:0i	Gram + bacteria	Vegetation type	1	160.99	<0.001 ***
		Season	1	9.25	0.006 **
		Vegetation type: Season	1	0.27	0.607
		Residuals	20		
15:0a	Gram + bacteria	Vegetation type	1	163.87	<0.001 ***
		Season	1	3.72	0.067 .
		Vegetation type: Season	1	0.51	0.482
		Residuals	20		
16:0i	Gram + bacteria	Vegetation type	1	148.44	<0.001 ***
		Season	1	8.97	0.007 **
		Vegetation type: Season	1	0.00	0.995
		Residuals	20		
17:0i	Gram + bacteria	Vegetation type	1	81.35	<0.001 ***
		Season	1	15.98	<0.001 ***
		Vegetation type: Season	1	0.0	0.974
		Residuals	20		
17:0a	Gram + bacteria	Vegetation type	1	70.75	<0.001 ***
		Season	1	8.39	0.009 **
		Vegetation type: Season	1	0.14	0.713
		Residuals	20		
16:0(10Me)	Actinobacteria	Vegetation type	1	84.39	<0.001 ***
		Season	1	7.53	0.013 *
		Vegetation type: Season	1	0.0	0.960
		Residuals	20		
19:0(10Me)	Actinobacteria	Vegetation type	1	70.25	<0.001 ***
		Season	1	4.62	0.045 *
		Vegetation type: Season	1	1.81	0.195
		Residuals	19		

A5.3. Continuation

Marker	Microbial group		Df	F value	Pr(>F)
17:0cy	Gram - bacteria cyclo	Vegetation type	1	103.41	<0.001 ***
		Season	1	12.96	0.002 **
		Vegetation type: Season	1	0.00	0.979
		Residuals	20		
19:0cy	Gram - bacteria cyclo	Vegetation type	1	55.18	<0.001 ***
		Season	1	17.03	<0.001 ***
		Vegetation type: Season	1	0.02	0.887
		Residuals	18		
15:1	Gram - bacteria	Vegetation type	1	159.89	<0.001 ***
		Season	1	7.23	0.0141 *
		Vegetation type: Season	1	0.68	0.420
		Residuals	20		
16:1ω7	Gram - bacteria	Vegetation type	1	88.21	<0.001 ***
		Season	1	1.11	0.305
		Vegetation type: Season	1	0.01	0.920
		Residuals	20		
16:1ω5	Gram - bacteria	Vegetation type	1	230.68	<0.001 ***
		Season	1	9.54	0.006 **
		Vegetation type: Season	1	0.05	0.832
		Residuals	20		
16:1	Gram - bacteria	Vegetation type	1	215.15	<0.001 ***
		Season	1	2.24	0.150
		Vegetation type: Season	1	0.36	0.553
		Residuals	20		
17:1	Gram - bacteria	Vegetation type	1	88.93	<0.001 ***
		Season	1	3.00	0.099 .
		Vegetation type: Season	1	0.17	0.683
		Residuals	20		
18:1ω9	Gram - bacteria	Vegetation type	1	281.66	<0.001 ***
		Season	1	15.77	<0.001 ***
		Vegetation type: Season	1	0.10	0.751
		Residuals	20		

A5.3. Continuation

Marker	Microbial group		Df	F value	Pr(>F)
18:1ω7	Gram - bacteria	Vegetation type	1	267.49	<0.001 ***
		Season	1	1.78	0.198
		Vegetation type: Season	1	0.03	0.859
		Residuals	20		
18:2ω6,9	Saprotrophic fungi	Vegetation type	1	546.41	<0.001 ***
		Season	1	4.31	0.051 .
		Vegetation type: Season	1	9.54	0.006 **
		Residuals	20		

A5.4. Results of forward selection of the redundancy analysis (RDA) analyzing how root biomass, DOC and soil moisture influenced the distribution of plant-derived C in different PLFA markers (The order of the variables in the first column represents the sequence of fitting in the analysis). P values $p \leq 0.05$ indicate significant influence of an explanatory variable on the plant-derived C.

Variable	Explains [%]	pseudo-F	P
Root biomass	30.9	6.2	0.006
DOC	8.2	1.8	0.399
Soil moisture	6.5	1.4	0.458

A5.5. Results of ANOVA (analysis of variance) of the changes in plant-derived C in distinct microbial groups related to differences between soil types (sandy and clayey) and seasons (non-growing and growing season). Stars indicate significant differences between soil types or seasons (*: p≤ 0.05).

Plant-derived C in Gram positive bacteria			
	Df	F value	Pr (>F)
Season	1	0.22	0.656
Soil type	1	4.61	0.064
Season:Soil type	1	0.00	0.963
Residuals	8		
Plant-derived C in Actinobacteria			
	Df	F value	Pr (>F)
Season	1	0.97	0.355
Soil type	1	2.07	0.189
Season:Soil type	1	0.23	0.644
Residuals	8		
Plant-derived C in Cyclic Gram negative bacteria			
	Df	F value	Pr (>F)
Season	1	0.00	0.972
Soil type	1	2.26	0.171
Season:Soil type	1	0.52	0.491
Residuals	8		
Plant-derived C in Gram negative bacteria			
	Df	F value	Pr (>F)
Season	1	0.00	0.969
Soil type	1	11.10	0.010 *
Season:Soil type	1	3.58	0.095
Residuals	8		
Plant-derived C in Saprotrophic fungi			
	Df	F value	Pr (>F)
Season	1	8.10	0.022 *
Soil type	1	0.172	0.689
Season:Soil type	1	2.85	0.130
Residuals	8		

A5.6. Mean (%, n= 3 ± standard deviation) plant-derived C to different microbial groups in sandy and clayey soils.

Microbial group	Season	Sandy	Clayey
		[%]	[%]
Gram + bacteria	Non-growing	50.9 ± 18.3	38.2 ± 3.6
	Growing	53.9 ± 8.1	40.7 ± 4.6
Actinobacteria	Non-growing	51.6 ± 16.6	38.1 ± 14.4
	Growing	41.3 ± 5.6	34.5 ± 9.1
G- bacteria (cyclo)	Non-growing	55.6 ± 25.5	36.9 ± 1.8
	Growing	49.9 ± 8.3	43.3 ± 11.4
G- bacteria	Non-growing	55.9 ± 10.6	63.7 ± 9.4
	Growing	45.5 ± 12.2	73.6 ± 0.6
Saprotrophic fungi	Non-growing	78.1 ± 15.5	86.7 ± 3.7
	Growing	70.3 ± 3.5	56.1 ± 16.8

A5.7. Results of ANOVA (analysis of variance) and Tukey's HSD (Honestly Significant Difference) test of the changes in plant-derived C between different microbial groups in the non-growing and in the growing season. Stars indicate significant differences between microbial groups (*: p≤ 0.05; **: p≤ 0.01; ***: p≤ 0.001).

ANOVA

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Season	1	325	325	1.82	0.183
Microbial group	4	7984	1996.1	11.20	<0.001 ***
Residuals	54	9634	178.4		

Tukey HSD: Non-growing season

Microbial groups		diff	lwr	upr	p adj
Gram negative bacteria	Actinobacteria	14.93	-13.02	42.88	0.445
Gram neg. bacteria (cyclo)	Actinobacteria	1.43	-26.52	29.38	0.999
Gram positive bacteria	Actinobacteria	-0.33	-28.28	27.62	0.999
Saprotrophic fungi	Actinobacteria	37.57	9.62	65.52	0.009 **
Gram neg. bacteria (cyclo)	Gram negative bacteria	-13.50	-41.45	14.45	0.535
Gram positive bacteria	Gram negative bacteria	-15.27	-43.22	12.68	0.425
Saprotrophic fungi	Gram negative bacteria	22.63	-5.32	50.58	0.130
Gram positive bacteria	Gram neg. bacteria (cyclo)	-1.77	-29.72	26.18	0.999
Saprotrophic fungi	Gram neg. bacteria (cyclo)	36.13	8.18	61.08	0.011 *
Saprotrophic fungi	Gram positive bacteria	37.90	9.95	65.85	0.008 **

Tukey HSD: Growing season

Microbial groups		diff	lwr	upr	p adj
Gram negative bacteria	Actinobacteria	21.63	4.28	38.99	0.014 **
Gram neg. bacteria (cyclo)	Actinobacteria	8.67	-8.69	26.02	0.505
Gram positive bacteria	Actinobacteria	9.43	-7.92	26.79	0.429
Saprotrophic fungi	Actinobacteria	25.33	7.98	42.69	0.005 **
Gram neg. bacteria (cyclo)	Gram negative bacteria	-12.97	-30.32	4.39	0.177
Gram positive bacteria	Gram negative bacteria	-12.20	-29.56	5.16	0.217
Saprotrophic fungi	Gram negative bacteria	3.70	-13.66	21.06	0.951
Gram positive bacteria	Gram neg. bacteria (cyclo)	0.77	-16.59	18.12	0.999
Saprotrophic fungi	Gram neg. bacteria (cyclo)	16.67	-0.69	34.02	0.061
Saprotrophic fungi	Gram positive bacteria	15.90	-1.46	33.26	0.077