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Seasonal Variations in the Microbial Community Structure and Rate of Carbon Flow in a Vegetation Change Experiment

Master's Thesis

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1 Introduction

1.1 The interaction between soil-plant-microbes

The soil domain is among the most populated ecosystem on Earth hosting bacteria, Fungi, nematodes, soil animals and plants to name a few (Bonkowski *et al.* 2009). Soil microbes play relevant role in soil ecosystem processes, such as, nutrient cycling, organic matter decomposition, and bioremediation. Indeed, the main bridge between soil and plant is made by microbes via assimilation of photosynthetic plant-derived carbon and transferring that to the soil organic carbon (SOC) pools (Gleixner 2013). Most soil microorganisms are heterotrophs and dependent on the labile carbon supply by plants through root exudation or root turnover (Dijkstra *et al.* 2013). But from an evolutionary perspective there should be a strong reason for plants to provide microbes with the expensive energy-rich carbon compounds. The soil microbes play a pivotal role in supplying the requisite nutrient for plants through decomposition of stable component and mobilising them in the soil profile (Dijkstra *et al.* 2013).

Many biotic and abiotic factors can influence the microbial community composition either solely or simultaneously such as plant species (Berg and Smalla 2009), plant biodiversity (Lange *et al.* 2014), soil texture (de Vries *et al.* 2012, Mellado-Vazquez *et al.* in press) soil pH (Thoms *et al.* 2010), soil moisture (Williams and Rice 2007, Lange *et al.* 2014), soil temperature (Medeiros *et al.* 2006) and seasonality (Thoms and Gleixner 2013, Mellado-Vazquez *et al.* in press). In addition to them, it has been observed that rhizosphere soil is much richer than the bulk soil in terms of soil microbial community composition. Microorganism that are more abundant in the rhizosphere soil can have a profound effect on the growth rate of plants by improving nutrient availability and contamination control (Marschner 2012). The first observation that microbes are more abundant in the rhizosphere than in distant soil was made by Hiltner (1904). Hiltner observed that the number and activity of microorganisms increased in the vicinity of plant roots. Root exudation is part of the rhizo-deposition process, which is a major source of soil organic carbon released by plant roots (Hutsch *et al.* 2002). The quantity and quality of root exudates are determined by plant species, the age of an individual plant and external factors like biotic and abiotic stresses. Exudation clearly represents a significant carbon cost to the plant (Marschner 2012). For example, young seedlings typically exuding

about 30 – 40 % of their fixed carbon as root exudates (Whipps 2001). Exudation rates also vary with plant developmental stages. Plants during their seedlings produce the lowest amounts of root exudates; this gradually increases until flowering and decreases again at maturity (Aulakh *et al.* 2001). Although the relationship between microbial community structure or species richness and ecosystem function has attracted considerable research interest (Jumpponen *et al.* 2002), so far there is not enough studies determine whether soil microbial activity and abundance also change during the plant developmental stages in line with root exudation variation or not.

1.2 The role of soil microbial communities in the carbon cycle in the soil

Carbon is the main chemical building block of the all living forms on the Earth. The global carbon reservoir is ca. 2,500 Gt comprise of 1,550 Gt soil organic carbon (SOC) and 950 Gt of soil inorganic carbon (SIC) (Lal 2004). The SOC represent a dynamic balance of gain and loss. Different forms of carbon are processed in terrestrial ecosystem and released back to the atmosphere by different mechanism. For example, CO_2 exchange between terrestrial ecosystem and the atmosphere depends on the balance between photosynthesis, respiration and decomposition of soil organic matter (Trumbore 2006). Atmospheric carbon fixes through photosynthesing plants, photo and chemoautotroph microbes (Trumbore 2006). In return, carbon organic carbon-decomposers consume the organic material, use a proportional of that as their biomass and release back the rest to the atmosphere as CO_2. The terrestrial areas are mainly covered by unsaturated soils with aerobic conditions, therefore CO_2 is the main respiration flux. Different mechanisms control CH_4 exchange between terrestrial ecosystem and the atmosphere, which relies on the equilibrium between oxidation and production rate of CH_4. The oxidation of CH_4 is an aerobic process regulated by methanotrophic activity and substrate CH_4 and O_2 availability; whereas, CH_4 production modulated by methanogenic archaea is the terminal step during microbial decomposition of soil organic matter in anaerobic environments such as natural wetlands, lake sediments and flooded fields. In saturated condition of soils, oxygen as the main electron acceptor for aerobic microbial respiration get constrained, therefore methanogensise pathway would became dominate. These mechanisms play important roles not only in the spatial and temporal stability of ecosystems, but also in determining the emission rate of greenhouse gases to the atmosphere (van Bodegom and Scholten 2001).

The importance of carbon cycle is coming into consideration because of the rising atmospheric carbon sources as CO_2 and CH_4 which are the most potent greenhouse gases among the others in trapping heat at the atmosphere. Soil organic carbon (SOC) pool has an important role on in the ecosystem and acts as the main CO_2 sink and the main buffer in mitigating the global climate warming. Additionally, as a vital component, plays a key role in soil fertility, hydrology and nutrient supply leading to the improving plant growth rate and consequently increasing photosynthesis rate (Wang and Houlton 2009). What we should consider when studying the carbon cycle is that the main process of carbon cycle in the belowground ecosystems is driving by soil microorganisms (Gleixner 2013). In addition to that, the microbial communities fix carbon in the soil 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).

1.3 Soil Microbial community structure and their functions

Soil microorganisms are mainly heterotrophs (Klein and Tate 1985). They obtain their requisite carbon either from plant's exudates or from the other available resources such as plant residuals. Bacteria are the most abundant microorganisms in soil reaching 10^8 to 10^9 per gram of soil, followed by actinobacteria numbering 10^7 to 10^8 per gram of soil (Whitman *et al.* 1998). Fungi are less abundant microorganism counting as 10^5 to 10^6 per gram soil (Kloepper *et al.* 1989). The proportion of organic carbon assimilated by fungi and bacteria would have an important consequence on the SOC decomposability due to the difference in decomposition rate of bacterial and fungi. Having formed the stable soil aggregates by hyphae, the soil-fungal aggregates decompose slower than the bacterial aggregates (Tisdall and Oades 1982). Further studies indicated that soil microbial structure can be classified by their ecological behavior related to their acquisition of carbon and their abundance in different niche of the soil (Bahn *et al.* 2013, Kuzyakov and Blagodatskaya 2015). The gram-negative bacteria and fungi (including AMF) are mainly recognized as root related bacteria and rapidly absorber of plant-derived carbon. Gram-negative bacteria not only are able to obtain carbon directly on root or fungal exudates but also, they can recycle carbon which had previously been incorporated in roots and other microbial groups (Bahn *et al.* 2013). Arbuscular mycorrhiza and other fungi equipped with hyphae net colonizing the roots and have direct access to plant carbohydrates (Olsson

1999). Gram-positive and actinobacteria mainly assimilate on older source of carbon includes fungal necromass or dead root material. Therefore, they are less dependent on initial plant-derived carbon and are mainly expected to decompose soil organic matter (Bird *et al.* 2011). The cyclopropyl PLFAs (17:0cyc, 19:0cyc) are often considered as gram-negative bacteria marker (Zelles 1999) which under stress condition are converted from their precursor (16:1ω7, 18:1ω7) to cyclopropyl forms (Guckert *et al.* 1986).

1.4 Effect of soil microbial community on the growing plant

The soil function regarding to the plant growing is related to the several factors such as chemical and physical soil properties such as soil pH, soil moisture and nutrient availability (Wall and Virginia 2000). For example, soil water is an important resource for plant growth and microbial activity, therefore, change in soil moisture would affect the osmotic potential, resulting in limiting the nutrient and energy transportation, as well as competition between microbial species to gain that limited resources (Williams and Rice 2007). Soil microbiota paly essential roles on determining soil properties and functions such as decomposing organic materials, nutrient cycling and enhancing soil fertility. Although the role of soil microbiota on the determining soil properties and functions should not be forgotten. They play an essential role in decomposing organic matter, cycling nutrients and fertilizing the soil. The life on Earth is highly dependent on the element cycles. Because the essential nutrients would rapidly be adsorbed by organisms and fixed in a form that cannot be used by others. The process of releasing fixed elements mainly happen through the chemical reaction in nature. But biochemical reactions, those mediated by organisms, also play an important part in the cycling of elements.

Soil microbes have a profound effect on a healthy soil structure (Sims 1990). For example, they produce some substances such as polysaccharides and mucilage that help to bond soil particles together. These aggregates are very stable against any physical and chemical weathering. Fungal filaments also stabilize soil structure by their hyphae network and aggregate the soil particles (Six *et al.* 2004). However, soil microorganisms account not only as the main mediator of soil organic carbon formation, but also as a component of that. Therefore, soil microorganisms may provide a significant effect on carbon stabilization in soil through enhancing soil fertility, water-retaining capacity and nutrient availability for plants (Condron *et al.* 2010).

1.5 Methods for studying the microbial community structure

In soils, it is crucial to understand how microorganisms govern the functioning of ecosystem processes, particularly belowground carbon cycling. Biochemical analyses of soils provide a useful tool to describe soil microbial community composition and microbial functions in soils. Among them stable carbon isotope technologies in combination with compound specific isotope measurements of biomarkers help track the flow of carbon in natural environments (Balesdent and Mariotti 1996, Gleixner 2013).

Stable isotope probing (SIP) helps linking the identity of uncultivated microorganisms to isotope labelled substrate utilization detected using a particular biomarker (Murrell and Whiteley 2011). Biomarkers are compounds which are produced by a limited group of organisms and thus represent this sub-population due to its specificity. Many different biomarkers have been tried and tested; each has its own advantages and disadvantages. Phospholipids, namely phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) are essential components of membranes of all living cells, and their fatty acid or ether-linked isoprenoid side chains allow a taxonomic differentiation within complex microbial communities (White *et al.* 1996, Zelles 1999). This approach is now well established in soil ecology (Frostegard and Baath 1996, Thoms and Gleixner 2013). Furthermore, differences in the chemical structure (saturation, chain length, branching) of individual PLFA markers and their relative abundances specify differently to the microbial groups (Steer and Harris 2000); in general saturated carbon chain in PLFA structure are found in all microorganisms; branched saturated PLFA (iso and anteiso saturation) are specific for gram-positive bacteria (Frostegard and Baath 1996); actinobacteria, which are a subgroup of gram-positive bacteria, have distinctively higher proportions of methylated PLFAs; monounsaturated compounds are distinctive for G-bacteria (Frostegard and Baath 1996, Zelles 1997) and polyunsaturated PLFA markers represent fungi and higher organisms (Zelles 1997). Thus, PLFA analysis is a useful technique for determination the soil microbial community composition. However, the specificity of PLFA depends greatly on the type of habitat and environmental conditions, therefore the assignment of PLFA markers to different microbial groups must be done with caution. NLFA are energy storage compounds in eukaryotes (Olsson and Johnson 2005), since most prokaryotes 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 carbon cycling belowground (Drigo *et al.* 2010).

1.6 Tracing C source via a natural isotopic experimental study

Since most of SOM is originally plant-derived, which has been either directly transferred to the microbial community or added to the soil as plant detritus and later decompose by the microbes, insights into the microbially turnover of soil organic carbon would help gain a better understanding of carbon cycling in soils. Stable isotope analysis provides a useful tool to track the flow of carbon in environments. Soils that have undergone vegetation change from C3 to C4 or vice versa are ideally suited to track carbon flow into different soil compartments and plant - soil – microbe route at natural isotope abundance levels (Balesdent and Mariotti 1996, Kramer and Gleixner 2006). In a C3-C4 vegetation change experiment, the soil has sown with former C3 plants with $\delta^{13}C$ value of -25 to -30 ‰ are replaced with C4 plants, which naturally have a richer isotope value (~ -12 ‰). Therefore, recent C4 plant carbon can be traced into the microbial biomass and be differentiated from SOM-derived carbon which has a C3 plant signature (Kramer and Gleixner 2008, Blagodatskaya *et al.* 2011). Later, the carbon assimilated by microbial community and their structure will be identified via lipid biomarker analysis in combination with stable isotope analysis.

1.7 Aim of study

The overall goal of this thesis was to gain more insight into the microbial community structure and microbial assimilation of plant-derived carbon in rhizosphere and non-rhizosphere soil in the course of the growing season. This study was designed mainly to answer following question:

- What is the difference of soil microorganism community structure in rhizosphere versus non-rhizosphere soil, and how is its ability in obtaining more carbon from rhizosphere soil where an access to the plant-derived carbon might be different?
- Are there any differences among various microbial groups in terms of their biomass and the portion of plant-derived carbon assimilated by them, and are they influenced by being either in the vicinity or in distance from plant roots? We hypothesize that root related microorganisms such as gram-negative bacteria and AMF receive more freshly plant-derived carbon, while gram-positive bacteria and saprotrophic fungi are more active in the non-rhizosphere soil.
- How would growing season affect the various microbial groups? We hypothesize that i) there is a seasonal pattern in microbial community structure, that root-related microbial biomass would increase along with extending plant root in soil; ii) these microorganisms assimilate a higher portion of plant-derived carbon when plants are developed enough compared to those are not dependent on direct plant-derived carbon.

2 Material and Methods

2.1 Site description

Experimental vegetation change plots were placed at the Max Planck Institute for Biogeochemistry in Jena-Germany. The experimental site was established in 2002 composed of two different plots, each 24 m^2. The sandy plot consisted of soil that originally was derived from a forest A-horizon and was included 50% sand, 44 % silt and 6 % clay (pH = 6.9) and the clayey plot consisted of soil originally from the B-horizon of a calcareous soil consist of 9 % sand, 75 % silt and 16 % clay (pH = 7.8). Both of soils were cultivated from 2002 with C3 plants. In 2006, the plots were split into four subplots. One of the four subplots were continuously cultivated with C4 plants since 2006 and the formers were maintained in C3 cultivation as the reference plot for comparison of $\delta^{13}C$ values until 2013. In 2013, the second C4 subplot was created to study the rate of diffusion of newly plant derived carbon (Fig 2-1).

Fig 2-1: sampling site design in MPI-experimental field. (a). the clayey soil and (b). the sandy soil

2.2 Soil sampling

In the summer 2016, soil samples were collected four times during plant growing season at every subplot with difference in plant type and in establishing plot year within

two main plots. Soil sampling started at beginning of the growing plant in June and continued with a monthly interval until the harvest time. At every sampling time, three soil cores contain plant roots were collected from each subplot and directly were transferred to the soil preparation room in order to continue further process in a stable temperature. Furthermore, the soil samples were separated into two different subsamples, the soils by direct contact to plant roots and soils which they had distance from plant-roots, from now on name as "Rhizosphere soil" and "Non- rhizosphere soil" respectively (Fig 2-1). Finally, soil samples collected from each point of the plot were mixed thoroughly and submitted as a single, composite sample for a single laboratory determination. Then samples were sieved using a 2-mm mesh and all plant debris was manually removed using a twiser. At the end, soil samples were stored in -20 °C until laboratory analysis.

Fig 2-2: separating of the rhizosphere (a) and non-rhizosphere (b) soil during soil sampling

2.3 Neutral and phospholipid fatty acid extraction

5 g of frozen soil were extracted according to the method of Bligh and Dyer (1959), modified by Kramer and Gleixner (2006). Total lipid extract (TLE) were obtained using pressurized solvent extraction (Speed extractor E-916, Büchi Labortechnik AG, Flawil, Switzerland) by a mixture of methanol, chloroform and 0.05 M K_2HPO_4 buffer (2:1:0.8, by volume; pH= 7.4). The soil samples in a combination with pre-combusted quartz sand were transferred to the 40 ml stainless steel extraction cells. Using a glass

syringe 10 µl of C19:0 phospholipid (1,2- Dinonadecanoyl-sn-Glycero-3-Phosphatiylchline; Larodan Fine Chemical AB, Malmö, Sweden) dissolved in extraction buffer with a concentration of 5 mg/ml was added on top of each cell to determine the extraction efficiency. The extraction was performed in 3 cycles with 10 min holding time at 70 °C and 120 - bars pressure. Then, the total lipid extract obtaining from chloroform phase were separated into phospholipid, glycolipid and neutral lipid using a silica-filled solid phase extraction (SPE) column (Chromabond, SiO, 2 g, 15 ml, ml, Macherey-Nagel GmbH & Co. KG, Düren, Germany) and eluting by chloroform, acetone and methanol respectively. The phospholipid and neutral fractions were hydrolyzed and methylated with methanolic KOH solution and resulting fatty acid methyl ester (FAME). The FAME was purified using an aminopropyl-modified SPE column (Chromabond NH4, 0.5 g, 3 ml, Macherey-Nagel GmbH & Co. KG, Düren, Germany). Finally, FAME C13:0 (Sigma-Aldrich Chemie GmbH, Munich, Germany) was added to all samples as internal standard.

2.4 Gas chromatography

PLFA/NLFA fractions were quantified on a GC-FID HP6890 with constant injector temperature (280 °C), using a DN-1MS column (50 cm × 0.32 mm internal diameter × 0.52 µm film thickness, Agilent Technologies, Palo Alto, USA) and helium gas as carrier gas (2 ml/min). The temperature program started at 140 °C for 1 min, following by the first ramp, the temperature raised up to 270 °C by 2°C/min and held for 6 min. In the second ramp the temperature was raised to 340 °C by 30°C/min and was kept constant for 5 min. Identification of fatty-acid methyl ester was accomplished by comparing retention times to those obtained for standard fatty-acids mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich Chemie GmbH, Munich, Germany) and in house data base (Kramer and Gleixner 2006, Thoms *et al.* 2010, Mellado-Vazquez *et al.* 2016).

Overall, among 35 PLFAs were identified, only the PLFAs with a concentration higher than 0.5 µg g^{-1} (soil dry weight) were taken into account. We considered the sum of PLFAs to represent different groups of bacteria including gram-negative bacteria (C16:1ω5, C16:1ω7, C17:1, C18:1ω7, C18:1ω9), Gram-negative (cyclo) bacteria (17:0cy and 19:0cy) (Zelles 1997), gram-positive bacteria (C15:0a, C15:0i, C16:0i, C17:0a, C17:0i), gram-positive actinobacteria (10Me16:0 and 10Me18:0) and non-specific markers (C15:0n, C16:0n, C17:0n, C18:1ω5). The PLFA C18:2ω6,9 was assigned as saprotrophic fungi marker (Frostegard and Baath 1996) and the NLFA C16:1ω5 as a specific marker for

arbuscular mycorrhiza (AM) fungi (Olsson 1999). PLFA C16:1ω5 was assigned as a gram-negative marker after recent study found that PLFA C16:1ω5 is more related to the gram-negative bacteria (Mellado-Vazquez *et al.* 2016).

2.5 PLFA/NLFA compound specific $\delta^{13}C$ measurement

Compound specific stable carbon isotope of PLFA and NLFA were analyzed by a GC-IRMS system (HP5890 GC, Agilent Technologies, Palo Alto USA; IRMS: Deltaplus XL, Finnigan MAT, Bremen, Germany). A 60 m HP Ultra column (0.25 mm internal diameter × 0.25 µm film thicknesses, Agilent Technologies, Palo Alto, USA) was installed in the GC-IRMS and helium was used as carrier gas at flow rate 1.8 ml/min. Volumes of 1 µl were injected in splitless mode (splitless time: 1 min). By first ramp, the temperature of GC column was programmed for an initial temperature of 45 °C and increased to 140 °C, then held constant for 1 min. By second ramp, the temperature was raised to 260 °C by 4 °C/min, followed by the third ramp of further heating to 340 °C by 30 °C/min and keeping constant for 5 min. Each sample measured three times. For the isotope value calibration, a mixture of FAMEs was injected before and after each triplicate.

Isotope ratios are expressed as $\delta^{13}C$ value in per-mille [‰] relative to the international reference standard v-PDB (1) using NBS 19 (Werner and Brand 2001).

Equation 1:

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

Where $(^{13}C\ /^{12}C)_{sa}$ is the $^{13}C/^{12}C$ ratio of the sample and $(^{13}C\ /^{12}C)_{std}$ is the $^{13}C/^{12}C$ ratio of the reference standard (NBS 19). The concentration of identified FAMEs was corrected for the ^{13}C of methyl group added to the FAME during derivatization (Kramer and Gleixner 2006).

Equation 2:

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

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

To estimate the contribution of fresh plant-derived carbon to rhizosphere and bulk soil microbes, we used the Equation 3 introduced by Kramer and Gleixner, 2006 and modified by Malik *et al.* (2013). In the modified equation, the change in $\delta^{13}C$ value of Soil organic matters as a result of the slow incorporation of C4 plant carbon into the SOM also takes into account:

Equation 3:

$$Plant-derived\ carbon(\%) = \frac{((\delta^{13}C_{C4-PLFA} - \delta^{13}C_{C3-PLFA}) - (\delta^{13}C_{C3-SOM} - \delta^{13}C_{C3-SOM}))}{((\delta^{13}C_{C4-plant} - \delta^{13}C_{C3-plant}) - (\delta^{13}C_{C3-SOM} - \delta^{13}C_{C3-SOM}))}$$

Where $\delta^{13}C_{C4\text{-}PLFA}$ and $\delta^{13}C_{C3\text{-}PLFA}$ are the $\delta^{13}C$ values of individual PLFA extracting from C4 and C3 soil, $\delta^{13}C_{C4\text{-}SOM}$ and $\delta^{13}C_{C3\text{-}SOM}$ are the $\delta^{13}C$ values of bulk SOM and $\delta^{13}C_{C4\text{-}plant}$ and $\delta^{13}C_{C3\text{-}plant}$ are the $\delta^{13}C$ values of plant material from the experiment plot with C4 vegetation change and control C3 plot, respectively.

2.6 Explanatory variables

Soil moisture [%] was measured gravimetrically according to the method introduced by Black (1965) from 5 g of soil (wet weight) that were collected as subsamples from the soil samples taken for PLFA analysis. For SOC (%) measurements, soils were dried at 40 °C until constant weight and homogenized by grinding in a ball mill. Concentration of SOC was calculated from the difference between total carbon and

inorganic carbon. Total carbon and inorganic carbon were measured by elemental analysis at 1150 °C (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany), inorganic carbon was obtained after burning organic carbon for 16 h at 450 °C in a muffle furnace (Steinbeiss *et al.* 2008). The $\delta^{13}C$ values of SOC ($\delta^{13}C$-SOC) were determined on 3 mg of air-dried and ball-milled soil. Soil carbonates were removed with 120 µl H2SO3 (5 – 6 % SO_2, Merck, Darmstadt, Germany) (Steinbeiss *et al.* 2008). Subsequently, the samples were dried at 40 ºC and $\delta^{13}C$-SOC values were measured repeatedly (standard deviation < 0.3‰) using EA-IRMS. The system was calibrated versus V-PDB using CO_2 as the reference gas (Werner and Brand 2001).

2.7 Statistical Analysis

All statistical analysis was performed under software R (R Core Team, 2016). Linear mixed-effects analysis applying *lme* function in the R library *nlme* (Pinheiro J 2018) were conducted to test the effect of environmental factors and time on the all response variables including microbial biomass, carbon isotope signature and plant-derived carbon at both community and species level in a C3/C4 vegetation change experiment. Total microbial biomass was calculated as a sum of bacterial and fungal PLFA marker. The carbon isotope and plant derived carbon was calculated as weighted mean per plot using equation 4 and 5.

Equation 4:

$$Weighted\ mean\ \delta^{13}C_{PLFA} = \frac{\sum_{i=1} \delta^{13}C_{PLFAi} \times \sum_{i=1} PLFAi_{nmol/g}}{\sum_{i=1} PLFAi_{nmol/g}}$$

Equation 5

$$Plant-derived\ carbon\ weighted\ mean = \frac{\sum_{i=1} Plant\ derived\ carbon_{PLFAi} \times \sum_{i=1} PLFAi_{nmol/g}}{\sum_{i=1} PLFAi_{nmol/g}}$$

In the models applied for total microbial biomass and weighted mean of plant-derived carbon plot identity by microbial groups were assigned as random factors. To

determine which factors has a significant effect on the response variables comprising microbial community composition, their carbon isotope and the plant-derived C, we started from a null model include just the intercepts following by gradually adding fixed factors and their interactions to the models. The model was fitted by maximum likelihood to eliminate non-significant fixed effects. Finally, in the case that significant differences were detected, Tukey post-hoc test in R library *mulcomp* (Hothorn *et al.* 2017) was conducted for multiple comparisons between microbial groups and sampling time. PLFA profiles were analyzed using CANOCO software (version 5, Microcomputer Power, Inc., Ithaca, NY). The relative amounts of individual PLFA were subjected to principal component analysis (PCA), where mol % of fatty acids was centered. PCA as an indirect gradient analysis was used to extract the major patterns from the variation in PLFA data. SOC and soil moisture was added to the PCA as supplementary variable to find the effect of these environmental variables on PLFA distribution.

3 Results:

3.1 Microbial biomass

Among phospholipids fatty acids identified, the components with concentration less than 1 nmol. g^{-1} were excluded from further calculation. The grouping of different soil microorganisms was performed based on their PLFA structure, employing principle component analysis and reviewing literature (Zelles 1997, Olsson 1999, Zelles 1999, Williams and Rice 2007, Mellado-Vazquez *et al.* 2016). Because of high variation in microbial behaviour in two different soil texture (clayey and sandy soil) we performed the analysis separately (Fig 3-1). The PCA analysis revealed that the PLFAs assigned to the gram-negative bacteria (C16:1ω5, C16:1ω7, C17:1, C18:1ω7, C18:1ω9) are highly correlated with rhizosphere soil, while the gram-positive bacteria marker (C15:0a, C15:0i, C16:0i, C17:0a, C17:0i) are more related to the non-rhizosphere soil. The results show that our assumption of grouping was correlated with the other previous studies.

The total microbial biomass (measured as total PLFA concentration) was significantly affected by soil texture, soil type, plant-growing season. Higher amount of microbial biomass was found in the clayey soil (mean = 191.00 nmol. g^{-1}, SD = 63.77) comparing to the sandy soil (mean = 148.60 nmol. g^{-1}, SD= 32.99). Significantly higher concentration of total microbial biomass and all individual PLFAs was identified in the rhizosphere soil (mean = 190.02 nmol. g^{-1}, SD = 60.92) compare to the corresponding non-rhizosphere soil (mean = 149.59 nmol. g^{-1}, SD = 39.08).

The total microbial biomass fluctuated during the plant-growing season, with the highest peak in July and the minimum in the September. Considering to the soil moisture it is observed that total microbial biomass is mainly driven by soil water content (Fig 3-3). The photosynthetic pathway caused no differences in total microbial biomass and microbial composition. Overall, the rhizosphere-clayey soil has held highest proportion of microbial biomass (Fig 3-3) followed by the non-rhizosphere-clayey soil. In contrast to the PLFA concentration, there were no significant differences of NLFA concentration (Arbuscular Mycorrhizal fungi marker) in the rhizosphere soil and the non-rhizosphere soil but, it changed marginally with differences in soil texture.

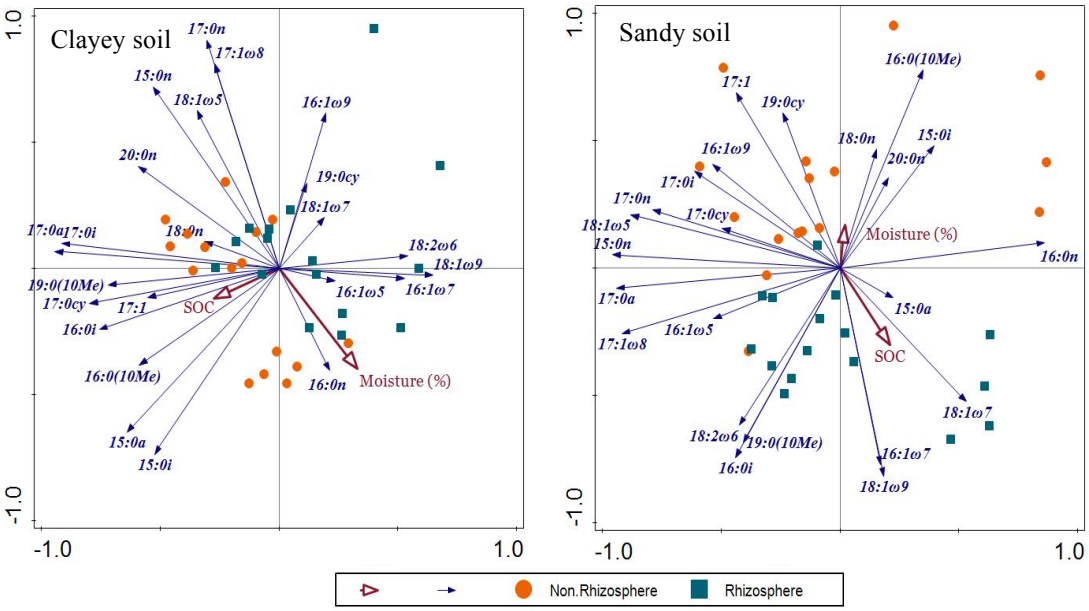

Fig 3-1: Principle component analysis of the mean relative proportion (mol %) of individual phospholipid fatty acids (PLFAs) in clayey soil and sandy soil sown with C3 and C4 plants. soil organic carbon (SOC) and soil moisture were set as explanatory variables.

The Arbuscular Mycorrhizal 16:1ω5-NLFA marker was changed significantly with growing season but in contrast to the PLFA markers, it was observed in the highest level in September and the minimum in July in both rhizosphere and non-rhizosphere soil (Fig 3-2). Linear mixed-effects models at microbial structure level with plots and groups as random effect used to test the effects of rhizosphere soil, seasonality, soil texture and plant type microbial structure on microbial biomass and its composition (Table. 2). In general, the absolute concentrations of PLFAs grouped into different microbial groups were relatively higher in the rhizosphere soil. However, the gram-negative bacteria were significantly more abundant along the other microbes at rhizosphere soil. The gram-negative bacteria change significantly over the growing season whereas the other bacteria did not show any significant change. They were more abundant in July and the amount of soil microbial biomass decreased dramatically in September (Fig 3-2).

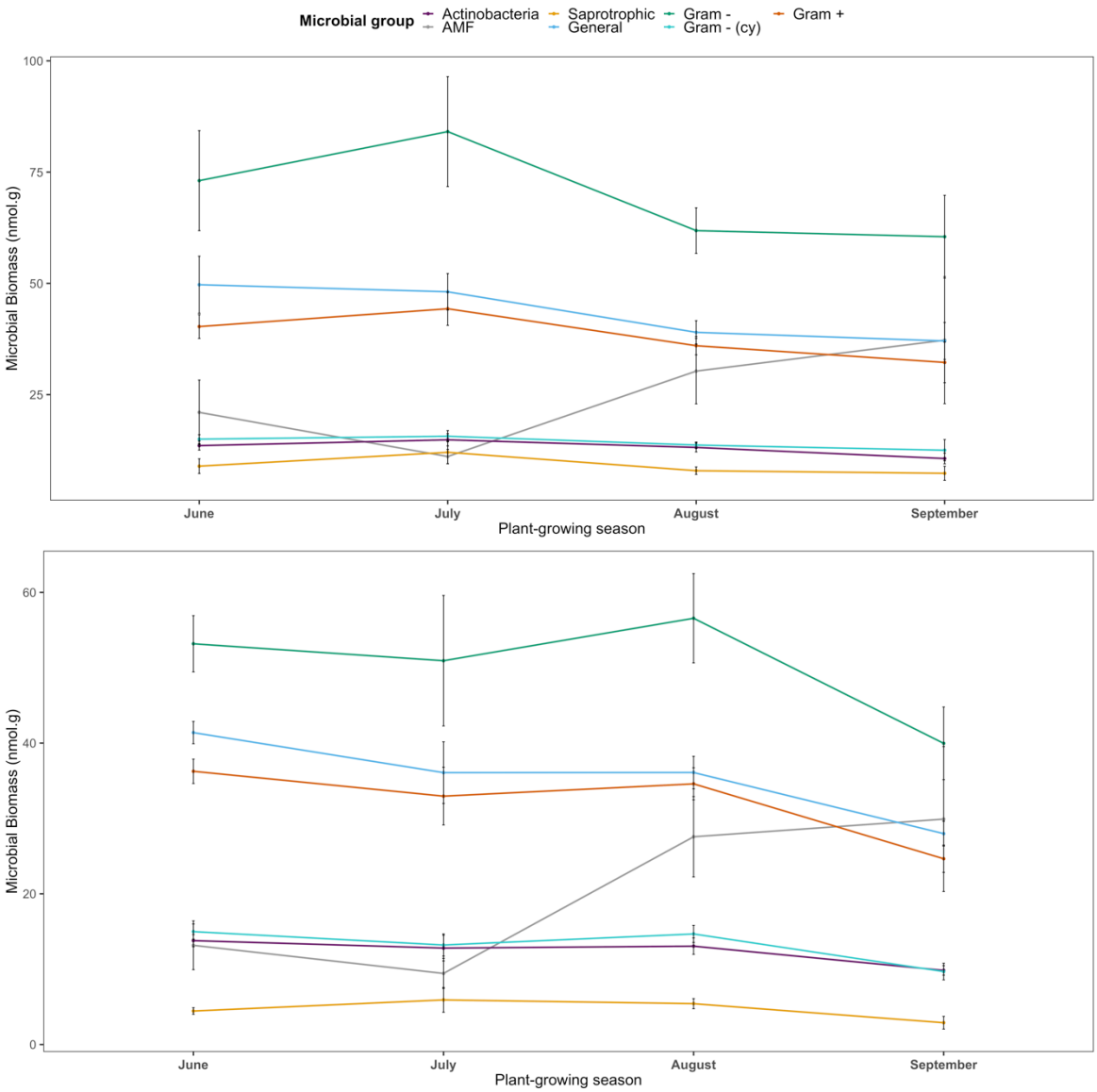

Fig 3-2:seasonal pattern of various microbial groups during plant growing season in two different soil type, rhizosphere and non-rhizosphere soil. Error bars represent standard deviation.

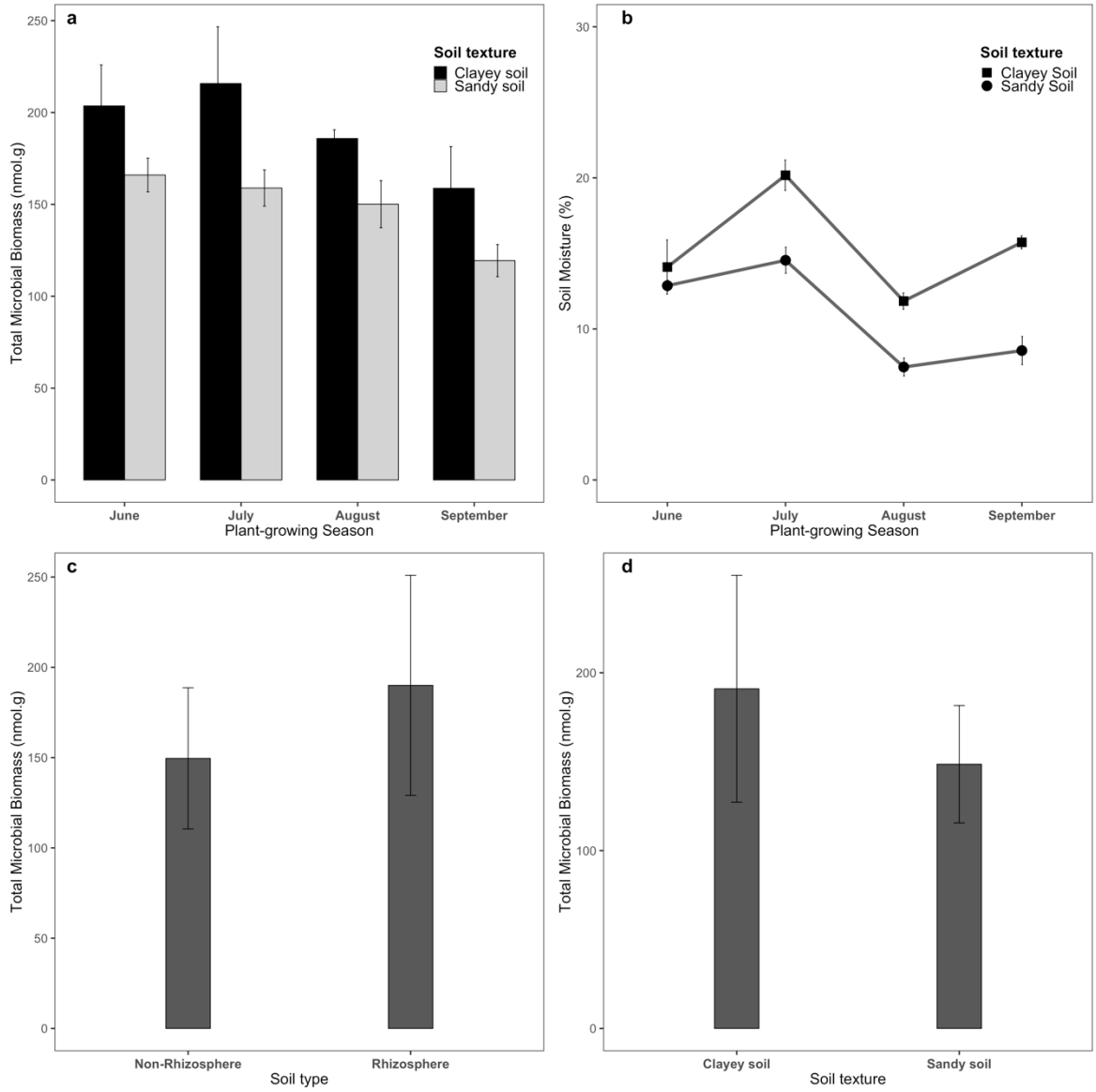

Fig 3-3: (a) the rate of change during the plant growing season in two different soil texture, (b) the rate of change of soil moisture in two different soil texture, (c) difference between total microbial biomass in rhizosphere soil vs. non-rhizosphere soil and (d) in two different soil texture. Error bars represent the standard deviation.

Table 3-1: summary of mixed-effect model analyses for total microbial biomass, δ13C as weighted average and plant derived carbon as weighted average.

Summary of mixed-effect model analysis									
Source of variation	Total microbial biomass (error: plots)			$\delta^{13}C$ (weighted average) (error: plots)			Plant-derived C (weighted average) (error: plots)		
	L-ratio	P-value	R^2	L-ratio	P-value	R^2	L-ratio	P-value	R^2
Rhizosphere soil	11.826	0.001	**0.141**	3.063	0.080	0.007	18.126	<.0001	**0.386**
Season	13.397	0.004	**0.127**	22.320	0.000	**0.042**	6.527	0.089	0.087
Soil texture	6.611	0.010	**0.153**	0.001	0.973	0.000	0.030	0.864	0.002
Plant type	3.089	0.079	0.038	21.701	<.0001	**0.810**			
Rhizosphere soil × Season	4.335	0.228	0.035	1.339	0.720	0.002	1.460	0.692	0.017
Rhizosphere soil × Soil texture	3.365	0.067	0.025	0.493	0.483	0.001	0.663	0.416	0.007
Rhizosphere soil × Plant type	0.189	0.664	0.001	20.213	<.0001	**0.025**			
Season × Soil texture	0.848	0.838	0.006	9.953	0.019	**0.009**	5.813	0.121	0.057
Season × Plant type	14.133	0.003	**0.088**	7.617	0.055	0.006			

Models were fitted by stepwise inclusion of fixed effects. Shown are the results of likelihood ratio tests (χ2) that were applied to assess model improvement and the statistical significance of the fixed effects (P values). Note that factors shown in indented lines were fitted in separate models. Bold values show significant affect of the source of variation.

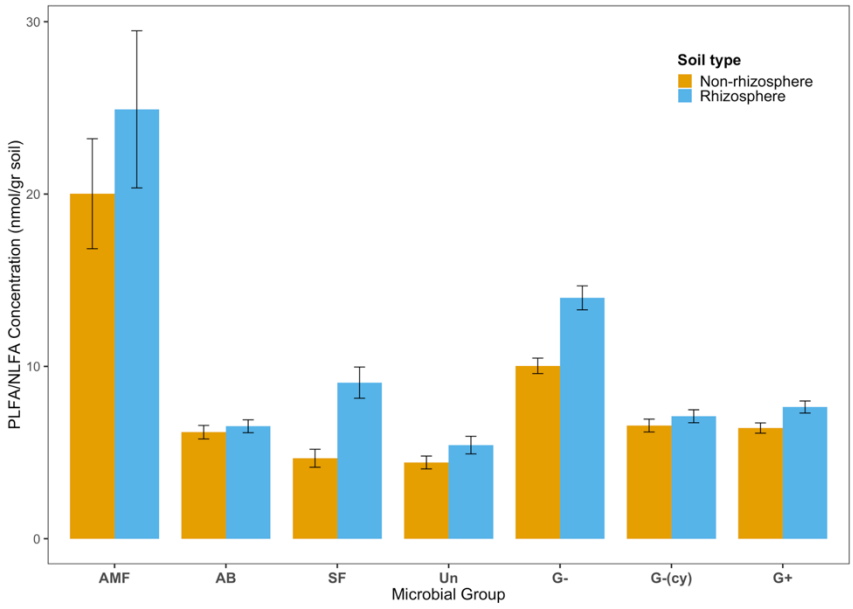

Fig 3-4: The difference of microbial group concentration in rhizosphere soil and non-rhizosphere soil. Abbreviations of microbial group: AMF = Arbuscular Mycorrhiza fungi, AB = Actinobacteria, SF = Saprotrophic fungi, Un = universal marker, G- = gram-negative bacteria, G-(cy) = gram-negative (cyclo) and G+ = gram-positive bacteria.

3.2 Microbial composition

The microbial community composition were defined based on the relative abundance of mirobial phosholipid fatty acids [mol %] assigned to the microbial groups. PCA was conducted to estimate how environmental variables influence individual PLFAs. The results indicated a strong variation in microbial community structure between rhizosphere soil and non-rhizosphere soil (30.0 % explanation) (Fig 3-1). The comparison of microbial structure in rhizosphere and non-rhizosphere soil revealed that gram-negative bacteria (PLFAs 16:1ω5, 16:1ω7, 17:1, 18:1ω7, 18:1ω7), and saprotrophic fungi (PLFA 18:2ω6) are relatively higher in rhizosphere soil, whereas, the gram-posetive bacteria (PLFAs 15:0a, 15:0i, 16:0i, 17:0a, 17:0i) were relatively more abundant in non-rhizosphere soil (Fig 3-3, Fig 3-4). The actinobacteria (PLFAs 10Me17:0, 10Me19:0) and the cyclic gram-negative bacteria (PLFAs 17:0cy, 19:0cy) showed different distribution among clayey soil and sandy soils (Fig 3-3). The quantity of the mol-percentage of microbial groups were almost constant with a slight changes during plant growing season excepting saprotrofic fungi. The fungies population were relatively higher in July and they show up in lower amounts in August and September.

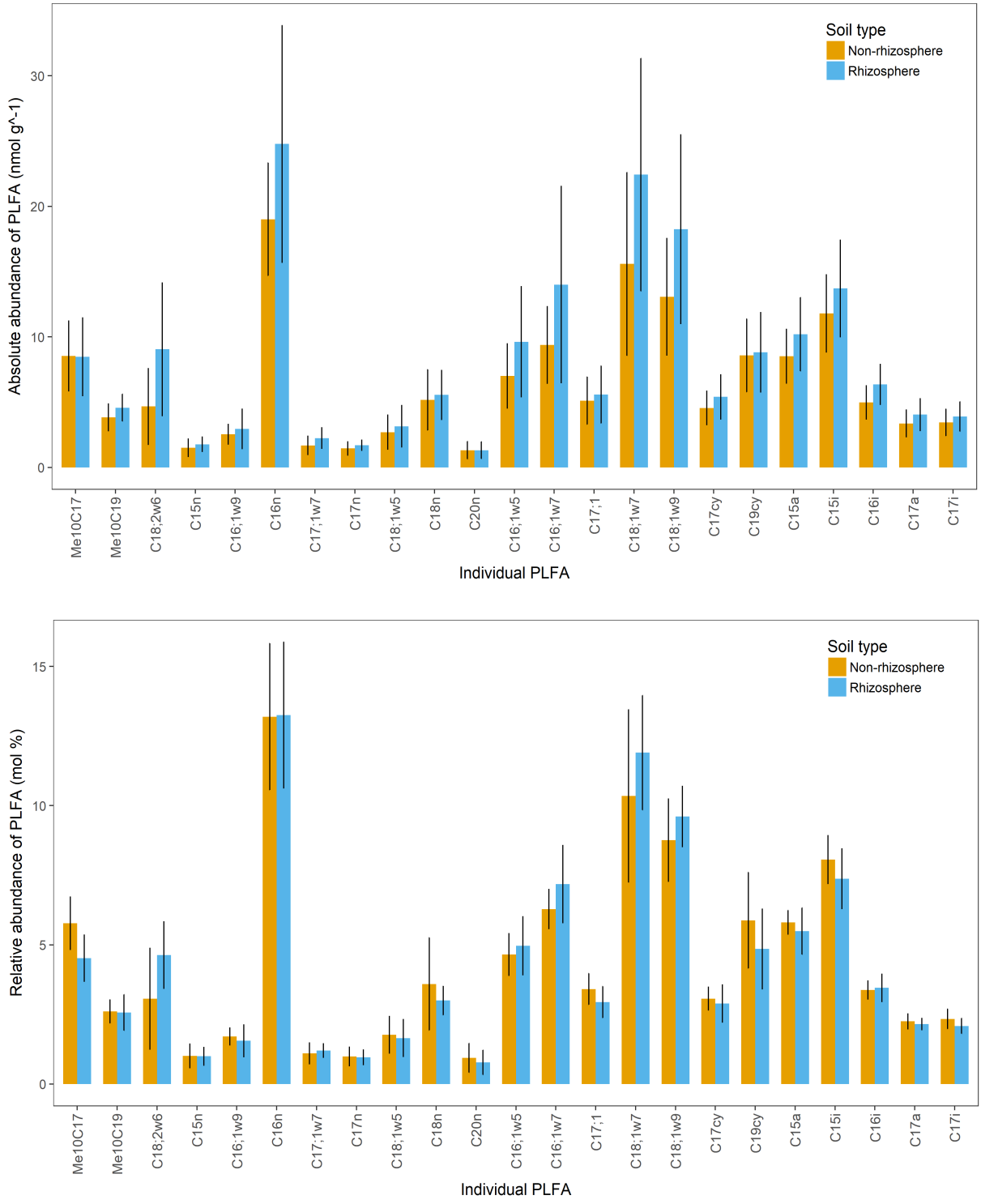

Fig 3-5: the absolute amount of PLFAs and the relative proportion (mol %) of them in rhizosphere soil and non-rhizosphere soil. The error bars indicate standard deviation.

3.3 Isotopic labeling of microbial carbon

Large isotopic differences between PLFA analyzed from soils planting by C3 (mean = -28.25 ‰, SD = 1.32) and C4 (mean = -20.65 ‰, SD = 2.26) indicated that the change in isotopic signatures controlled mainly by plant type. The linear mixed effect models revealed that there was not a significant difference in $\delta^{13}C$ value between rhizosphere and non-rhizosphere soil in C3 plots, in contrast, in the C4 plots there was a striking difference and C4 rhizosphere soil was more enriched in carbon isotopes (Table 3-1). Since the establishing of the first vegetation change experiment field in 2006 until our sampling, $\delta^{13}C$ of PLFAs has been decreased by 9.7 and 7.8 ‰ in rhizosphere soil and non-rhizosphere soil respectively. At the other hand, in the field established in 2013 carbon isotope of PLFAs were enriched by 7.9 and 4.5 ‰ in rhizosphere soil and the corresponding non-rhizosphere soil respectively.

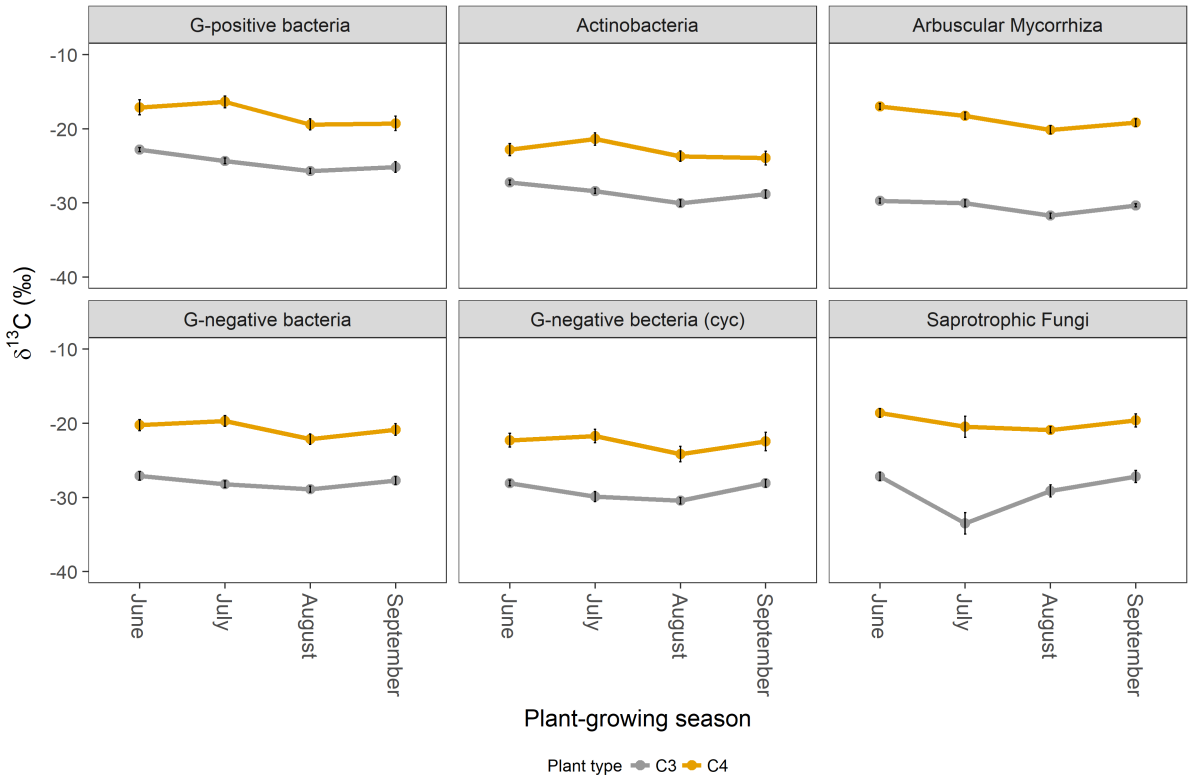

Fig 3-6: Stable isotope variation in different stage of plant growing. Error bars means standard deviation.

soils cultivated with C4 plant increased by nearly 20 % more in rhizosphere soil comparing to the non-rhizosphere soil (Fig 3-6). In addition, the $\delta^{13}C$ value of AMF increases by 64 % in both rhizosphere and non-rhizosphere soil.

3.4 Carbon source of different microbial groups

The amount of plant-derived carbon used as microbial carbon source was calculated using the ^{13}C isotopic shift between PLFA from C3 and C4 cultivated soils at both study sites and their subplots as well. The plant-derived carbon was mainly impacted by rhizosphere soil by 38.6 % and the season by 8 %. No significant differences in microbial plant-derived carbon were detected between plots with different soil textures (clayey and sandy soil). In contrast, the subplots with different establishing year displayed noticeable differences not only in total amount of plant-derived carbon but also within their rhizosphere soil and the corresponding non-rhizosphere soil.

The Tukey's honest test under linear-mixed effect model revealed that the differences between the rhizosphere soil and the corresponding non-rhizosphere soil in subplot 2013 is highly significant (p-value = 0.004) whilst, less significant differences were observed for subplot established in 2006 (p-value = 0.020). At the other hand, in the line with the total microbial biomass, plant-derived carbon fluctuated over the plant-growing season. Besides that, the peak was observed in second sampling time in July, in contrast, in August and September the amount of plant-derived carbon dramatically decreased along with decreasing moisture.

Table 3-2:The amount of plant derived carbon received by different microbial groups in two different soil type. SD represent for standard deviation.

Microbial group	Soil type			
	Rhizosphere		Non-rhizosphere	
	Mean (%)	SD	Mean (%)	SD
Actinobacteria	37.41	17.73	18.43	14.98
Gram positive bacteria	47.84	17.28	25.03	16.35
Gram negative bacteria	51.87	14.98	26.27	11.61
Cyclic gram neg. bacteria	46.51	20.13	22.51	15.47
Saprotrophic fungi	64.51	20.89	46.30	26.27
Arbuscular Mycorrhiza	77.49	11.42	76.35	10.22
Bacteria	47.07	19.75	24.07	13.46

Among all the microbial groups, arbuscular mycorrhiza fungi assimilated on average 77 % of its requisite carbon directly from plants following by saprotrophic fungi which obtain on average 55 % of their carbon from freshly photosynthetic carbon by plants. Plant contribution to the bacteria was estimated at 39.1 ± 3.2 % by gram-negative bacteria, 36.4 ± 3.6 % by gram-positive bacteria, 34.5 ± 3.8 % by cyclic gram-negative bacteria and 27.9 ± 3.3 % by actinobacteria. In the rhizosphere soil all bacteria obtained their carbon almost two-fold more than in non-rhizosphere soil. The saprotrophic fungi received by 18 % more carbon in rhizosphere. On the contrary, arbuscular mycorrhiza obtained the same proportion of carbon in both rhizosphere and non-rhizosphere soil (Table 3-2). The uptake of plant-derived C of saprotrophic fungi, gram-negative and gram-positive bacteria increased slightly in September, whereas, at the same time these microorganisms the proportion of received C in the non-rhizosphere soil declined (Fig 3-7).

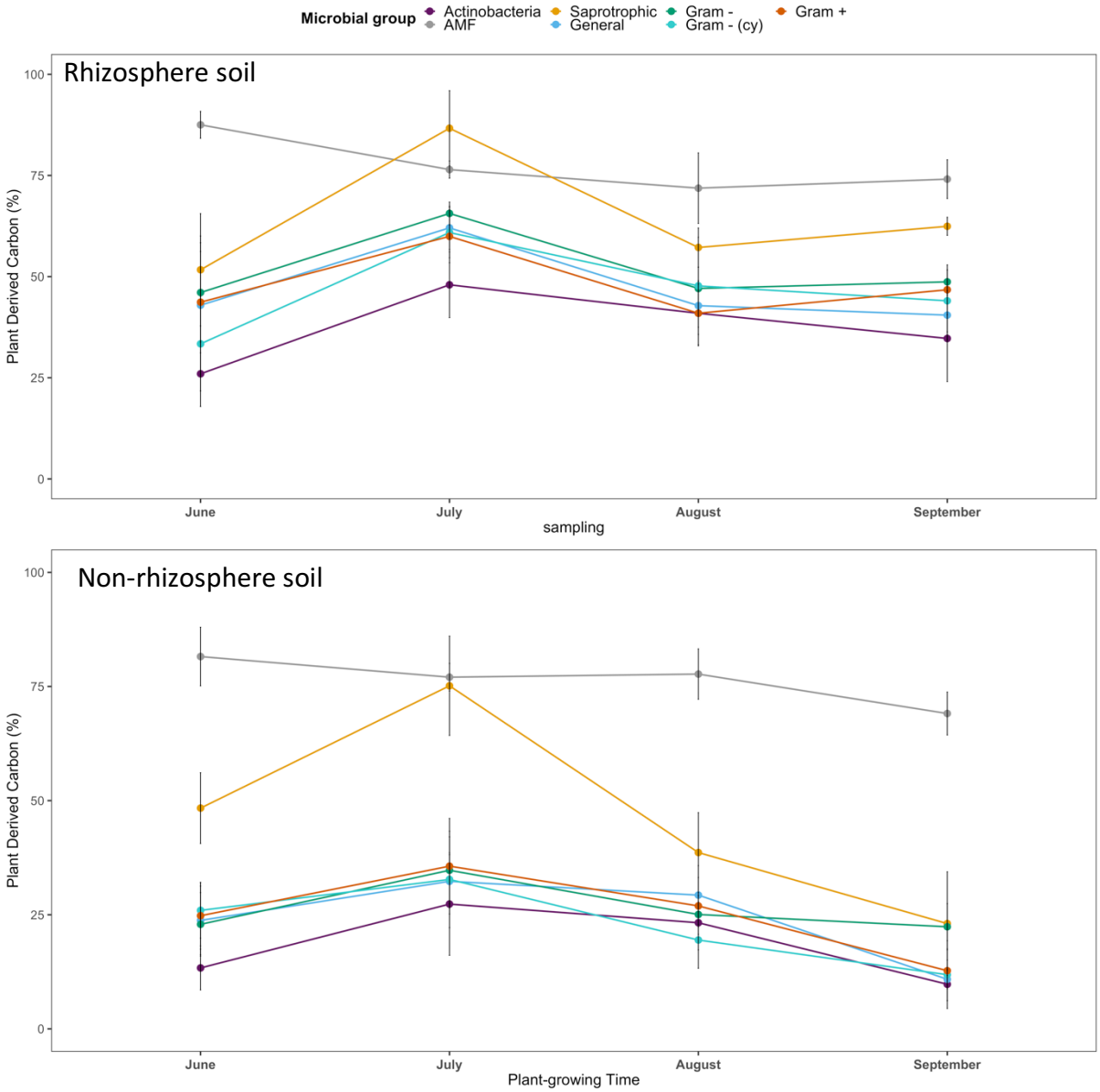

Fig 3-7: the deviation of plant deriving carbon during plant growing season in two different soil type, rhizosphere and non-rhizosphere soil. Error bars represent standard deviation.

4 Discussion

4.1 Microbial biomass and carbon turnover in rhizosphere and non-rhizosphere soil

In this study, we investigated the microbial community distribution pattern and the utilization of plant-derived carbon in the rhizosphere soil and non-rhizosphere soil in a period of plant-growing season. Our results show higher amount of microbial biomass in the rhizosphere soil. The rhizosphere soil is the most vital interface in terms of plant-microbes interaction and the abundance of root-associated microorganism including gram-negative bacteria and saprotrophic fungi in rhizosphere soil is strongly related to the amount of carbon providing by roots (Berg 2010). We found a highly dynamic niche in the rhizosphere soil with microbial community; confirm that there is a close connection between plant photosynthesis carbon and its assimilation by microbes (Bahn *et al.* 2013).

Most of PLFAs on our study plots, appeared in higher quantity in the rhizosphere soil comparing to the corresponding non-rhizosphere soil, whereas the relative amount of them followed different pattern. The structure of the microbial community was well separated based on their dependency to the root exudates. The PLFAs assigned to the gram-negative bacteria and saprotrophic fungi, as they are considered to live in the rhizosphere and depend on fresh plant derived carbon like exudates, except C17:1 were presented in higher proportion comparing to the rest of PLFAs as assigned for non-associated microbes includes gram-positive and actinobacteria. Our results confirm the results of Blagodatskaya *et al.* (2014) using respiration technique and DNA content that found 14 – 31% higher of total microbial biomass and 45 – 83 % higher growing (active) part of microbial biomass in the rhizosphere compare to that in the root free soil.

All microbial groups had significantly more enriched $\delta^{13}C$ values when they are in vicinity of the plant roots. Long-term C3/C4 vegetation change experiment notably change the composition of carbon isotopes in soils (Kramer and Gleixner 2006), therefore, especially in the rhizosphere soil microorganisms assimilating carbon either from soil pools or directly from plant exudates are more enriched in carbon isotopes. The carbon isotope value decreased from the rhizosphere soil to the non-rhizosphere soil by 3.8 ‰ in saprotrophic fungi and by 2.8 ‰ in bacterial groups related to C4 plants. Nonetheless, the $\delta^{13}C$ value of

AMF was relatively similar in both rhizosphere and non-rhizosphere soil. It is clear that, AMF colonizing the roots, regardless of living in vicinity of root or in a root free soil has ability to assimilate carbon directly from freshly plant fixed carbon, therefore, there is not a considerable difference between rhizosphere and non-rhizosphere soil (Fig 3-7).

As we hypothesized, our results demonstrated that the assimilation of plant-derived carbon by microbial communities is more considerable when they are in a direct contact with the plant root except for the AMF. The bacterial communities (gram-negative, cyclic gram-negative, gram-positive and actinobacteria) in non-rhizosphere soil assimilate on average 23.3 % of their requisite carbon from recently photo-assimilated carbon. By contrast, this amount in rhizosphere soil is twice as large in non-rhizosphere soil (mean = 46.2 %). The incorporation of plant-derived carbon into fungal groups was much higher than bacterial groups. Saprotrophic fungi and arbuscular mycorrhiza received respectively 55.4 % and 76.0 % of the carbon from plant pools, confirming the fact that saprotrophic fungi as primary consumers of root exudates are more dependent on plant-derived carbon than the other sources (Ballhausen and de Boer 2016). There was not considerable difference of the carbon utilization rate by arbuscular mycorrhiza in rhizosphere and non-rhizosphere soil and the saprotrophic fungi just had a 39.0 % increase in rhizosphere soil versus non-rhizosphere soil. It is clear that, AMF equipped with hyphae network, regardless of living in rhizosphere or non-rhizosphere soil has ability to assimilate carbon directly from freshly plant fixed carbon, therefore, was not observed a considerable variation between rhizosphere and non-rhizosphere soil. Growing season statistically has a significant effect on carbon isotope value of microbial groups with depletion in August and September (Fig 3-6).

We additionally observed that there is almost no difference in the portion of plant-derived carbon among the bacterial groups either in rhizosphere or in non-rhizosphere soil especially between root-associated gram-negative bacteria and soil-associated gram-positive bacteria. It shows that, despite of the fact that soil microorganisms are functionally different, but they are all more active in vicinity of plant roots and dependent on carbon resource supply by plants.

4.2 The effect of growing seasonon the microbial behavior

Many environmental factors affect microbial biomass growing a plant such as soil moisture. Root exudates, as the main source of carbon for soil microorganism, undoubtedly caused a

significant cost to the plants (Marschner 2012), and the amount of photosynthetic components providing by plant differs with the type of soil, plant's age and its physiological state, nutrient and water availability (Bais *et al.* 2006). In current study, we started our sampling from the early stage of growing plant until their senescence. Overall, the total microbial biomass represents an increasing pattern during vegetative and budding stage and start inclining as plant started flowering stage until senescence. This trend was not observed in all the microbial groups. The actinobacteria and cyclic gram-negative bacteria stayed relatively constant during all period of plant growth. Additionally, the variation in PLFA concentration in rhizosphere soil was greater than the variation of those in the corresponding non-rhizosphere soil. The magnitude of total microbial biomass during earlier stage of growing season(sampling June and July) in the rhizosphere soil showed a considerable shift from those of the respective non-rhizosphere soil. These results are in line with previous finding and supported the fact that in the early stage of vegetating, plants need more nutrients and therefore they try to assemble a higher community by providing more root exudates (Jacoby *et al.* 2017). Additionally, with declining plant activity due to high temperature and less water supply plants started reducing their exudates, therefore microbial community mainly in vicinity of this limited carbon pool are able to obtain the efficient carbon. The Arbuscular Mycorrhizal 16:1ω5-NLFA marker was changed significantly with growing season but in contrast to the PLFA markers, it was observed in the highest level in September and the minimum in July in both rhizosphere and non-rhizosphere soil. In fact, NLFA is the storage source of energy for microorganisms and the NLFA16:1ω5 appears to be a reliable marker for AM fungal storage lipids such as spores (Ngosong *et al.* 2012) and the magnitude of NLFAs is closely correlated to the number of AM spores formed (Olsson 1999). from this finding we can conclude that, the amount of carbon stored in soil would increase in late growing season stages.

In addition, growing season has a significant effect on $\delta^{13}C$ value in microbial groups but this effect is just a trend in line with soil moisture and probably plant activity or another seasonal factor. It means that, in August and September, with a decrease in soil moisture, carbon isotope of microbial PLFAs got more depleted. Because of limited soil moisture and probably nutrient mobilization in soil, plants decreased their activity such as root grow and carbon-nutrient exchange. Therefore, microbial activity will we decreased affected by limited resources derived from plant roots. Furthermore, the differences in isotopic abundances between the rhizosphere soil and the corresponding non-rhizosphere soil in subplot 2013 is

highly significant whilst, a small difference were observed for subplot established in 2006. It shows that in the former C4 cultivated subplot the plant-derived carbon had enough time to get defused in soil after 10 years C4 plant cultivation but newly established subplot still contains higher percentage of C3 plant legacy carbon (Balesdent and Mariotti 1996). At the other hand, plant-derived carbon fluctuated over the growing season, in the line with the total microbial biomass and plant activity. On top of that, the peak was observed in the second sampling time in July when the soils contained highest amount of water; in contrast, in August and September the amount of plant-derived carbon dramatically decrease. Specificall, plants decrease their energy cost in a limited environment by decreasing their exudation rate which cause limitation in microbial activity.

4.3 The effect of soil texture and photosynthetic pathway on microbial structure

The total microbial biomass was highly affected by soil texture and they were highly abundant in clay soil. Among the microbial groups, Gram-negative bacteria, saprotrophic fungi and actinobacteria were more affected by soil texture than the others. In both soils, there was a significant different between rhizosphere and non-rhizosphere soil. But this difference was more obvious in the clay soil, explained this fact that, having finer structure and more soil organic carbon in addition to its capability to hold more water and nutrients (Marschner 2012) is an easier niche for microbial grows. Previous studies indicated that the microbial community impacted by abiotic and biotic factors such as soil texture (de Vries *et al.* 2012, Mellado-Vazquez *et al.* in press), soil pH (Thoms *et al.* 2010), soil moisture (Williams and Rice 2007, Lange *et al.* 2014) and season (Mellado-Vazquez *et al.* in press).

5 Conclusion

Despite the large number of studies about the effect of various environmental factors on microbial behavior in soil, there is limited study focusing on these factors on soil microorganism structure and the rate of carbon flows from plants to the microbial community. In addition, the respond of soil microorganism to these changes and their interaction with plant root in different stage of growing a plant still needs to be investigated. Thanks to the new methods of microbial analysis coupled with spectrometry approaches, we are able to trace the elements such as carbon via isotopic labeling. Therefore, we are able to chase the carbon in a very diverse environment such as soil from its main photosynthetic source to the soil microorganisms and soil organic carbons. In our study, tracing carbon from root to the soil microbial groups and comparing them in the rhizosphere and non-rhizosphere soil, using natural labeling we found that soil microorganisms exert themselves establish their community in vicinity of plant roots. Because they are highly dependent on the carbon derived from plant. Besides that, any change in the plant phenology during the different level of plant growing, may directly affect the microbial biomass and their activity. In fact, the amount of plant derived carbon in a growing season, depends on the soil moisture availability and probably nutrient mobility in soil. Therefore, soil microorganisms which their activity and biomass rely on the rate and carbon derived from plant. In the current experimental study, it was clearly shown that rhizosphere soil hold more abundant microbial community and the soil microorganism activity is much higher when plant growing faster. Among various environmental factors that we examined, the main deriver was the soil texture. It has been revealed that soil texture has a profound affect at many other soil properties such as soil moisture, oxygen level and nutrient availability and cation exchange capacity to name but a few. Therefore, it would change the quality and quantity of carbon-nutrient exchange between root and soil microorganisms.

6 Summary

Soil microorganisms play an important role in soil ecosystem functioning by mediating carbon - nutrient exchange between terrestrial ecosystem and atmosphere which consequently leads to formation and accumulation of soil organic carbon in soil. On the one hand, the microbial soil functions are very sensitive to the change in ecosystem properties such as plant species and plant biodiversity, soil type, soil moisture and season. On the other hand, plants act differently in terms of nutrient exchange with soil and microbial community during their growing season. This raises the question whether plants supervise the microbial community structure and abundance in the vicinity of their root by supplying them with different amount of photosynthetic carbon during their growing season. To address this question, a C3-C4 vegetation change experiment couple with phospholipid fatty acid analyses was employed to trace and compare the quantity and the flow of plant carbon into different microbial compounds over a growing season in annual plants. Phospholipids are essential membrane components of all living cells. They are not found in the storage products or in the dead cells. Therefore, rapid change in the microbial community structure can be detected by changes in the carbon stable isotope ratios in phospholipids.

soil samples were collected from June to the end of September with monthly intervals from the MPI field – Jena, Germany. The field which has been under vegetation change experiment since 2006, is separated in two different soil type plots with subplots sowing with C3 and C4 plants. Tracer carbon was chased into rhizosphere and non-rhizosphere soils using mass spectrometry approaches. The abundance of total microbial biomass and microbial groups was determined using measuring PLFA/NLFA concentration by GC-FID. Additionally, the compound specific $\delta^{13}C$ was determined by GC-IRMS to estimate the soil microbial composition and the flow of plant-derived carbon to the soil microorganisms.

Having analyzed data using linear-mixed effect models' method, we observed that total microbial biomass in both rhizosphere and non-rhizosphere soil vary during the season. In the rhizosphere soil, total microbial biomass fluctuated with the highest peak in July and the lowest at September, while in non-rhizosphere soil it decreased gradually from June to September. The result of compound specific analysis in two different plot sowing with C3 and C4 plants revealed that different microbial groups received various proportion of carbon from plants. Among them, mycorrhiza fungi and saprotrophic fungi in both soil types

obtained almost 80 % and 50 % of plant-derived carbon respectively. The bacterial community in rhizosphere soil were significantly incompatible with those in non-rhizosphere soil. In the rhizosphere soil, bacterial community gained approximately 45 % of their requisite carbon from plants whereas in the non-rhizosphere only 20 % of their assimilated carbon have plant origin. Besides that, microbial biomass varied by soil texture and season while the other factors such as photosynthetic pathway has no effect. Statistically speaking, season has not a significant effect on the plant derived carbon neither in fungi community nor in bacterial groups. Our study revealed that, microbial community are highly active in the vicinity of plant root and obtain considerable proportion of their requisite carbon directly from freshly plant substrates.

7 Zusammenfassung

Mikrobielle Gemeinschaften sind ein wesentlicher Bestandteil des Kohlenstoffs (C)-Kreislaufs zwischen dem terrestrischen Ökosystem und der Atmosphäre und folglich die Bildung und Akkumulation von organischem Kohlenstoff im Boden vermitteln. Auf der einen Seite, reagieren die mikrobiellen Bodenfunktionen sehr empfindlich auf die Veränderung der Ökosystemeigenschaften wie Pflanzenarten und Pflanzenvielfalt, Bodentyp, Bodenfeuchte und Jahreszeit. Auf der anderen Seite, agieren Pflanzen in Bezug auf den Nährstoffaustausch mit der Boden- und Mikrobengemeinschaft während ihrer Wachstumsperiode unterschiedlich. Dies wirft die Frage auf, ob Pflanzen die mikrobielle Gemeinschaftsstruktur und -häufigkeit in der Nähe ihrer Wurzel überwachen, indem sie ihnen während ihrer Wachstumsperiode eine unterschiedliche Menge an photosynthetischem Kohlenstoff zuführen. Um diese Frage zu beantworten, wurde ein C3-C4-Vegetationsänderungsexperiment mit Phospholipid-Fettsäure-Analysen verwendet, um die Menge und den Fluss des Pflanzenkohlenstoffs in verschiedenen mikrobiellen Verbindungen über eine Vegetationsperiode in einjährigen Pflanzen nachzuverfolgen und zu vergleichen. Phospholipide sind essentielle Membrankomponenten aller lebenden Zellen. Sie werden nicht in den Speicherprodukten oder in den toten Zellen gefunden. Daher kann eine schnelle Veränderung der mikrobiellen Gemeinschaftsstruktur durch Änderungen der Kohlenstoff-stabilen Isotopenverhältnisse in Phospholipiden nachgewiesen werden.

Wir sammelten Bodenproben von Juni bis Ende September in monatlichen Abständen vom MPI-Feld - Jena, Deutschland. Das Feld, das seit 2006 unter einem

Vegetationsänderungsversuch steht, ist in zwei verschiedene Bodenartparzellen mit Nebenpflanzen mit C3- und C4-Pflanzen aufgeteilt. Tracer-Kohlenstoff wurde mit Hilfe von Massenspektrometrieansätzen in Rhizosphären- und Nicht-Rhizosphäre-Böden verfolgt. Die Häufigkeit der gesamten mikrobiellen Biomasse und der mikrobiellen Gruppen wurde unter Verwendung der Messung der PLFA / NLFA-Konzentration durch GC-FID bestimmt. Zusätzlich wurde das verbindungsspezifische δ13C durch GC-IRMS bestimmt, um die mikrobielle Zusammensetzung des Bodens und den Fluss von pflanzlichem Kohlenstoff zu den Bodenmikroorganismen abzuschätzen.

Nach Analyse der Daten mit Hilfe von linearen gemischten Effektmodellen beobachteten wir, dass die gesamte mikrobielle Biomasse sowohl in der Rhizosphäre als auch in der Nicht-Rhizosphäre während der Saison variiert. Im Rhizosphärenboden schwankte die gesamte mikrobielle Biomasse mit der höchsten Ernte im Juli und der niedrigsten im September, während sie im nicht-rhizosphärischen Boden von Juni bis September allmählich abnahm. Das Ergebnis der verbindungsspezifischen Analyse in zwei unterschiedlichen Parzellen, die mit C3- und C4-Pflanzen gesät wurden, zeigte, dass verschiedene mikrobielle Gruppen verschiedene Anteile von Kohlenstoff aus Pflanzen erhielten. Unter ihnen erhielten Mykorrhizapilze und saprotrophe Pilze in beiden Bodentypen fast 80% bzw. 50% des pflanzlichen Kohlenstoffs. Die Bakteriengemeinschaft in Rhizosphärenboden war mit denen in nicht-rhizosphärischem Boden signifikant inkompatibel. In der Rhizosphärenerde hat die Bakteriengemeinschaft etwa 45% ihres erforderlichen Kohlenstoffs aus Pflanzen gewonnen, während in der Nicht-Rhizosphäre nur 20% ihres assimilierten Kohlenstoffs pflanzlichen Ursprungs sind. Darüber hinaus variiert die mikrobielle Biomasse je nach Bodenbeschaffenheit und Jahreszeit, während die anderen Faktoren wie der Photosyntheseweg darauf keinen Einfluss haben. Statistisch gesehen hat die Jahreszeit weder in der Pilzgemeinschaft noch in bakteriellen Gruppen einen signifikanten Effekt auf den pflanzlichen Kohlenstoff. Unsere Studie hat gezeigt, dass die mikrobielle Gemeinschaft in der Nähe der Pflanzenwurzel sehr aktiv ist und einen beträchtlichen Anteil ihres erforderlichen Kohlenstoffs direkt von den frischen Pflanzensubstraten bezieht.

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Appendix:

Table A-0-1: Sum of PLFA concentration (nmol/g) and mean isotope C values (δ13C [‰]) of individual phospholipid fatty acids (PLFAs) in rhizosphere and non-rhizosphere soil sown with C3 and C4 plants.

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C
			nmol/g		nmol/g		nmol/g		nmol/g	
Gram-negative bacteria	**16:1ω5**									
		June	25.72	-25.02	33.38	-18.92	36.07	-26.12	46.22	-16.59
		July	20.26	-25.05	38.46	-17.24	36.45	-25.95	51.06	-15.43
		August	32.61	-25.74	29.71	-19.94	35.34	-24.14	33.75	-17.98
		September	21.22	-24.68	22.93	-17.82	21.92	-24.64	47.10	-16.60
Gram-negative bacteria	**16:1ω7**									
		June	38.99	-27.19	41.34	-21.63	75.97	-28.63	57.01	-19.46
		July	28.06	-27.61	45.80	-21.15	52.03	-28.97	73.85	-19.08
		August	45.90	-28.68	40.14	-24.31	50.26	-29.59	43.49	-21.98
		September	30.42	-27.18	29.51	-22.71	39.63	-26.99	55.96	-18.85
Gram-negative bacteria	**17:1**									
		June	21.77	-25.04	23.96	-20.81	21.62	-23.05	23.36	-18.05
		July	16.25	-26.81	26.48	-19.36	21.48	-28.15	30.33	-18.21
		August	21.52	-28.50	22.08	-24.27	21.78	-29.33	21.22	-21.92
		September	15.16	-26.67	16.52	-23.97	15.45	-27.40	23.28	-20.12
Gram-negative bacteria	**18:1ω7**									
		June	66.26	-28.27	68.61	-23.72	88.37	-28.93	92.63	-20.88
		July	29.87	-28.98	88.61	-22.47	94.28	-30.24	132.74	-19.88
		August	77.79	-29.36	66.48	-25.45	85.58	-30.41	73.97	-22.39
		September	59.19	-29.61	42.01	-24.28	62.82	-28.97	87.49	-20.69

Table A-1: Continuum

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C
Gram-negative bacteria	**18:1ω9**									
		June	51.26	-25.57	54.14	-20.06	71.50	-26.32	71.97	-18.54
		July	45.23	-27.06	68.41	-20.91	75.82	-29.49	104.83	-18.49
		August	63.10	-28.29	53.22	-24.05	70.01	-30.50	59.50	-21.21
		September	42.66	-27.37	40.08	-22.38	56.08	-27.83	74.24	-19.29
Gram-negative bacteria (Cyclo)	**17:0(cy)**									
		June	20.73	-25.34	22.08	-19.38	23.41	-24.39	24.86	-18.42
		July	13.89	-27.38	21.86	-19.22	20.62	-28.48	29.11	-17.55
		August	20.82	-27.52	19.72	-23.25	22.29	-29.14	20.61	-20.65
		September	12.49	-25.63	14.26	-22.12	11.23	-25.66	20.75	-17.39
Gram-negative bacteria (Cyclo)	**19:0(cy)**									
		June	37.24	-29.86	39.85	-25.26	34.55	-28.69	37.20	-23.45
		July	27.93	-30.18	41.95	-24.68	32.18	-31.87	43.45	-22.83
		August	33.76	-31.12	43.08	-27.08	33.83	-32.46	32.74	-24.21
		September	19.62	-29.46	31.08	-26.11	33.4	-28.69	34.77	-22.48
Gram-positive bacteria	**15:0a**									
		June	36.64	-26.14	39.29	-22.01	39.69	-25.92	44.00	-18.87
		July	26.77	-25.63	42.66	-19.53	41.53	-25.64	54.31	-16.58
		August	37.22	-27.99	35.59	-23.83	41.08	-28.46	35.66	-21.25
		September	25.73	-27.46	28.63	-23.53	30.56	-25.38	39.71	-19.51

Table A-1: continuum

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C
			nmol/g		nmol/g		nmol/g		nmol/g	
Gram-positive bacteria	**15:0i**									
		June	50.68	-25.00	54.99	-19.36	54.22	-24.17	61.16	-17.43
		July	37.96	-26.11	60.83	-19.49	55.85	-27.03	73.80	-17.61
		August	49.46	-27.50	49.51	-23.20	52.04	-28.40	48.10	-20.89
		September	34.00	-26.38	39.90	-22.75	37.27	-26.90	56.3	-18.66
Gram-positive bacteria	**16:0i**									
		June	22.33	-24.64	23.4	-18.97	26.71	-23.64	26.51	-17.49
		July	15.63	-26.13	24.4	-19.20	25.77	-27.54	32.54	-17.31
		August	23.14	-27.78	21.33	-23.39	26.27	-29.01	22.67	-20.84
		September	14.23	-26.65	14.99	-22.62	18.65	-27.47	24.67	-19.54
Gram-positive bacteria	**17:0a**									
		June	15.32	-23.73	16.35	-18.17	17.24	-21.43	17.73	-15.32
		July	10.40	-23.53	16.43	-16.42	15.47	-25.35	20.89	-14.20
		August	15.35	-25.79	14.67	-21.48	16.62	-26.57	15.18	-19.02
		September	9.29	-23.66	10.04	-20.85	11.22	-24.16	14.99	-16.95
Gram-positive bacteria	**17:0i**									
		June	16.44	-22.91	14.61	-17.45	16.87	-21.00	18.31	-15.35
		July	11.09	-24.77	17.52	-17.69	14.23	-26.08	20.11	-15.81
		August	14.82	-27.02	15.66	-23.07	15.19	-27.57	15.20	-20.75
		September	9.18	-25.99	11.24	-23.22	9.41	-26.02	15.17	-19.20

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C	PLFA Conc. nmol/g	δ13C
Actinobacteria	**16:0(Me10)**									
		June	35.37	-27.77	39.01	-24.22	34.75	-26.68	35.20	-22.03
		July	27.73	-28.05	43.64	-22.53	32.71	-28.58	44.63	-20.48
		August	34.50	-29.57	36.78	-25.97	35.75	-30.38	32.52	-23.65
		September	26.93	-29.31	29.22	-25.79	25.41	-28.92	30.26	-22.58
Actinobacteria	**19:0(Me10)**									
		June	17.91	-27.08	18.01	-23.12	20.04	-26.47	18.46	-21.05
		July	12.49	-27.56	18.54	-21.35	18.71	-29.21	22.84	-20.93
		August	17.60	-29.13	15.58	-25.27	20.08	-30.76	16.82	-23.04
		September	11.18	-26.51	11.34	-25.58	13.00	-28.33	16.52	-21.56
Saprotrophic Fungi	**18:2ω6**									
		June	16.55	-26.95	19.01	-18.63	31.88	-27.38	39.58	-18.63
		July	22.50	-33.03	24.89	-24.19	33.74	-33.96	62.68	-19.79
		August	25.48	-28.58	17.91	-23.54	35.05	-29.65	28.29	-20.26
		September	8.87	-26.42	14.25	-21.34	25.13	-27.95	33.48	-17.93
Universal marker	**15:0n**									
		June	8.21	-25.18	8.69	-23.87	9.04	-22.37	8.79	-19.48
		July	4.09	-23.63	5.54	-21.94	5.23	-27.05	7.29	-17.35
		August	7.69	-27.60	6.85	-22.56	8.41	-29.22	7.25	-20.73
		September	3.00	-25.33	4.18	-23.41	4.90	-26.66	5.78	-17.40

Table A-1: Continuum

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C
			nmol/g		nmol/g		nmol/g		nmol/g	
Universal marker	**16:1ω9**									
		June	11.49	-23.09	12.13	-15.66	10.68	-22.47	14.5	-17.33
		July	7.88	-22.13	12.07	-16.71	10.86	-22.50	13.48	-13.59
		August	12.63	-22.84	10.59	-19.17	10.67	-23.89	11.04	-16.58
		September	6.52	-21.33	8.18	-17.68	8.56	-19.72	14.65	-18.83
Universal marker	**16:0n**									
		June	86.68	-26.95	84.19	-21.42	126.08	-27.77	105.08	-19.82
		July	70.06	-27.79	90.44	-21.19	96.65	-29.07	129.89	-18.79
		August	78.88	-28.98	69.65	-25.10	93.63	-29.96	73.91	-22.16
		September	65.5	-27.61	63	-23.77	76.38	-27.42	91.29	-20.14
Universal marker	**17:1ω7**									
		June	7.91	-29.78	8.39	-22.52	9.64	-29.77	9.32	-23.42
		July	5.03	-26.98	7.32	-19.87	8.07	-28.50	11.2	-18.04
		August	9.25	-29.05	7.52	-24.24	10.04	-28.97	8.38	-22.16
		September	4.08	-25.27	4.36	-20.76	6.35	-27.35	8.94	-19.54
Universal marker	**17:0n**									
		June	7.08	-24.87	7.69	-21.17	7.55	-23.17	7.84	-20.33
		July	4.14	-25.83	5.92	-21.73	4.80	-27.32	7.79	-15.71
		August	6.82	-27.77	6.27	-22.75	7.40	-27.08	6.58	-22.23
		September	3.44	-25.88	5.12	-24.22	5.43	-25.04	6.97	-19.33

Table A-1: Continuum

Microbial group	Marker	Season	Non-rhizosphere soil				Rhizosphere soil			
			C3		C4		C3		C4	
			PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C	PLFA Conc.	δ13C
			nmol/g		nmol/g		nmol/g		nmol/g	
Universal marker	**18:1ω5**									
		June	11.85	-27.79	12.54	-21.86	13.30	-27.54	13.43	-20.60
		July	8.18	-25.90	12.72	-19.79	11.36	-24.69	15.75	-15.67
		August	12.46	-23.48	13.11	-19.32	12.92	-27.44	12.71	-17.83
		September	7.70	-28.82	7.72	-20.25	9.07	-21.82	12.22	-18.75
Universal marker	**18:0n**									
		June	27.08	-25.06	22.91	-20.13	25.30	-23.18	23.58	-18.88
		July	25.99	-27.05	22.87	-21.11	21.64	-28.63	34.23	-18.03
		August	18.86	-28.30	17.08	-24.92	20.02	-29.37	17.51	-22.67
		September	15.07	-26.56	15.67	-24.17	15.93	-27.06	19.62	-20.36
Universal marker	**20:0n**									
		June	7.02	-28.33	7.27	-28.54	7.01	-28.21	6.49	-27.17
		July	3.01	-31.88	3.35	-26.20	3.29	-31.40	3.45	-25.73
		August	5.72	-28.93	5.36	-28.40	6.12	-32.28	5.46	-25.62
		September	4.82	-30.04	5.49	-28.94	4.81	-30.89	5.66	-24.78

8 Declaration of independent work

I hereby declare that this thesis was written by me under the supervision of Prof. Gerd Gleixner and whatever, personal or literature help was taken for the preparation of thesis is mentioned in the acknowledgements and references sections. Neither this, nor a similar work, has been published or presented to an examination committee before to obtain an academic degree.

Jena, July 2018

Mina Azizi Rad