FRIEDRICH-SCHILLER-UNIVERSITÄT JENA

Stabilization and dynamics of soil organic matter
in response to long-term mineral and organic fertilization

Dissertation

(kumulativ)

zur Erlangung des akademischen Grades doctor rerum naturalium

(Dr. rer. nat.)

vorgelegt dem Rat der Chemisch-Geowissenschaftlichen Fakultät

der Friedrich-Schiller-Universität Jena

von Dipl.-Biologin Jana Schmidt

geboren am 20.03.1977 in Suhl

Gutachter 1: Prof. Dr. Beate Michalzik

Friedrich-Schiller-Universität Jena, Institut für Geografie,

Löbdergraben 32, 07743 Jena

Gutachter 2: Prof. Dr. François Buscot

UFZ - Helmholtz Zentrum für Umweltforschung, Department Bodenökologie

Theodor-Lieser-Str. 4, 06120 Halle

Acknowledgements

After this long period of work, today is the day on which I can give my dissertation the final touch by writing this note. The completion of this work would not have been possible without the support of the many people whom I would like to thank in the following.

First of all, I would like to thank Prof. Dr. François Buscot as head of this Department for providing excellent working facilities but moreover, for his support in many different ways. The same applies to my supervisors Dr. Elke Schulz, Dr. Jessica Gutknecht, and Prof. Dr. Beate Michalzik, who I would like to thank for their patience and encouragement as well as their reviews of my manuscripts, and constructive criticisms improving this work.

I was lucky to have had really nice colleagues at the UfZ Halle, who helped me a lot not only in the scientific arena, but also on a personal level: first of all, Julia Sandow – the good soul of the department who was always helpful with administrative problems, Yvonne Eckstein, who became a good friend to me, Stephan König, Beatrice Schnabel, Gabriele Henning, Jaqueline Rose, Renate Rudloff, Dr. Björn Hoppe, Prof. Dr. Ingolf Kühn, and Dr. Martin Schädler, who supported me in the lab or gave me assistance in scientific and statistical questions, and all other members of the Soil Ecology Department for having a great time together.

Special thanks go to Dr. Ines Merbach as technical coordinator of the Experimental research station Bad Lauchstädt.

Ohne Eure Unterstützung, Friederike, Christiane, Fanny und Anne hätte ich es wahrscheinlich nicht geschafft, die Kraft aufzubringen, um diese Arbeit zu beenden. Vielen Dank dafür!

Ganz besonders möchte ich meiner Familie danken. Meinen beiden großartigen Mädels, die mich allein dadurch angespornt haben ihnen ein Vorbild sein zu wollen, die aber auch oft zurückstecken mussten, Conny und Holger, meiner Schwester, meinen Großeltern und Eltern, die alle fest an mich geglaubt und mir so oft durch finanzielle Unterstützung, besonders in den ersten drei Jahren meiner Doktorarbeit den Rücken freigehalten haben. Mama, ich weiß, Du wärst sehr stolz auf mich gewesen.

Thank you very much, everyone!

Table of Contents

List of Tables

List of Figures

Figure 8 NMDS ordination of total phospholipid profiles based on relative abundances of all determined PLFAs from the investigated treatments on both rotation types. Fitted environmental variables: pH; CWC = cold water extractable carbon (mg kg^{-1}); CWN = cold water extractable nitrogen (mg kg^{-1}); NH_4^+ = ammonia (mg kg^{-1}); NO_3^- = nitrate (mg kg^{-1}). Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t

List of Abbreviations

AA	amino acids
actino	actinomycetes
AM, AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
Ara	Arabinose
AS	amino sugars
bac	Bacterial
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
CF1	clay fraction <1 µm
CF2	clay fraction 1-2 µm
CWC	cold water extractable carbon
CWE	cold water extracts
CWN	cold water extractable nitrogen
DAP	meso-Diaminopimelic acid
DFG	German Science Foundation
DOM	dissolved organic matter
FA	fatty acid
FAO	Food and Agriculture Organization of the United Nations
Fuc	Fucose
fung	Fungal
FYM	farmyard manure
Gal	Galactose
GalN	Galactosamine
GC/MS	Gas chromatography mass spectrometry
GF	general fungi
GHG	greenhouse gas
GluN	Glucosamine
Gm$^-$	Gram-negative bacteria
Gm$^+$	Gram-positive bacteria

HWC	hot water extractable carbon
HWE	hot water extracts
HWN	hot water extractable nitrogen
IPCC	Intergovernmental Panel on Climate Change
LF1	light fraction <1.8 g cm^{-3}
LF2	light fraction 1.8-2.0 g cm^{-3}
Man	Mannose
ManN	Mannosamine
MS	Monosaccharides
MurA	muramic acid
NLFA	neutrallipid fatty acids
NMDS	Nonmetric Multidimensional Scaling
OBS	organische Bodensubstanz
OM	organic matter
PLFA	phospholipid fatty acids
POM	particulate organic matter
Py-FIMS	pyrolysis-field ionization mass spectrometry
Py-GC/MS	pyrolysis-gas chromatography mass spectrometry
Rham	Rhamnose
SFEBL	Static Fertilization Experiment Bad Lauchstädt
SOC	soil organic carbon
SOM	soil organic matter
TC	total carbon
t-FA	total fatty acids
USDA	United States Department of Agriculture
Xyl	Xylose

1. General introduction

Around 10.000 years BC, agriculture commenced independently in different parts of the globe along with the rise of sedentary societies. The following centuries were marked by rapid growth of the human population, accompanied by an intensified conversion of natural into cultivated land for food production. Today over one third of land on earth is in agriculture (WorldBank 2015). Still 100 years ago, developments of agricultural techniques and practices focused mainly on the optimization of crop yields. In contrast, the todays understanding of a modern agriculture includes a sustainable use of the soil in order to ensure soil quality and thus, the ability of soil to perform a broad range of ecosystem services besides of food production. One of the soil functions being of global importance is the ability to act as a sink or source for atmospheric CO_2 and other greenhouse gases. According to the Intergovernmental Panel on Climate Change (IPCC), substantial reductions in anthropogenic carbon and greenhouse gas (GHG) emissions only will not be sufficient to ensure levels of atmospheric CO_2, low enough to mitigate the global climate change. In addition, further efforts are necessary to achieve a re-sequestration of previously emitted carbon following the intensification of land use (Smith et al. 2014).

It is undeniable that any conversion of natural into cultivated land leads first to a drastic decrease in soil organic carbon (SOC) stocks and a release of high amounts of CO_2 (Van Wesemael et al. 2010; Wei et al. 2014). However, once changed, even an agricultural soil bears great potential to act as carbon sink. According to Lal (2004) up to 0.8 Gtons C per year can be sequestered at a global scale in cropland soils, while there is a broad range of estimates across the literature depending on the management practice used (Conant et al. 2001; Jarecki and Lal 2003; Lal et al. 2003).

1.1. Carbon sequestration and stabilization of soil organic matter (SOM)

In principle, an increase of SOC-levels can be achieved either by increasing the amount of organic matter entering the soil and/or by reducing the microbial degradation of soil organic matter (SOM), which represents the total organic content of a soil. SOM is a heterogeneous mixture of simple as well as complex organic material, originating from different sources, such as plant root debris, root exudates, organic fertilizers, or soil dwelling organisms. Beside of this, SOM is also heterogeneously regarding its age, its degree of disintegration, and its bioavailability, which is standing, in turn, directly in relation to the mechanisms responsible for C-stabilization in soil. Lützow et al. (2006) defined three mechanisms of SOM stabilization in soil:

a) primary/secondary recalcitrance,

b) spatial inaccessibility

c) interactions with surfaces and metal ions.

Recalcitrance describes the stabilization of organic matter based on its intrinsic biochemical properties, whereas this does not mean that an organic compound is not decomposable at all. Recalcitrance is characterized by long turnover times, while in some cases degradation is even only possible in presence of specific degraders or under specific environmental conditions. Degradability can be hampered based on the molecular structure, for example by the presence of one or more aromatic rings, ether-bridges, quaternary C-atoms, halogen substituents or increased molecule size (Loonen et al. 1999; Lützow et al. 2006). Thus, organic polymers or molecules containing long chains of hydrophobic hydrocarbons show

longer turnover times than simple organic molecules. Besides of that, some organic compounds can resist degradation by inhibiting enzymatic activities due to complexation with degradation enzymes (Dungait et al. 2012). While Lützow et al. (2006) refer primary recalcitrance exclusively to the above- and belowground plant material, such as plant litter and rhizodeposits, this can be in principle extended on the original form of all organic matter, which is entering the soil, i.e. also on non-herbal ingredients of organic fertilizers or on pesticides. Once being in soil, organic material can be transformed via biological (synthesis of biomass) or chemical processes (f.ex. Maillard reaction, polycondensation) into new organic compounds, which can resist degradation due to the same reasons as mention above, possessing now secondary recalcitrance. It should be noted, that recalcitrance is increasingly considered to fail as a concept in understanding SOM stability, since it was shown that even simple and decomposable organic molecules contribute to very old SOM in soils, while on the other hand "recalcitrant" compounds, such as lignin, were found in fast cycling fractions of SOM (Derrien and Amelung 2011; Dungait et al. 2012; Jenkinson et al. 2008). Following Kleber (2010), recalcitrance is not more than a semantic convenience, since degradability is always based on the interaction between the organic compound and the microbial degrading enzyme, whereas this interaction is controlled by a number of abiotic factors, resulting in a wide range of possible turnover rates for one and the same organic compound under different environmental conditions.

Spatial inaccessibility comprises all cases where organic matter is protected from degradation due to physical barriers, preventing access of microbial enzymes to their substrates. Thereby, the spatial isolation of organic matter can be based on intercalation within phyllosilicates, its inclusion into organic macromolecules or its entrapment within

3

organo-mineral complexes in form of aggregates. Soil aggregation is assumed to follow an hierarchical order, starting with the formation of microaggregates by adherence of clay-particles and silt-aggregates (<20µm), which is strongly mediated by organic binding agents, such as fungal glomalin, microbial polysaccharides, or plant mucilage (Czarnes et al. 2000; Oades 1984; Spohn and Giani 2010). Besides of the mentioned binding agents, fungal hyphae or plant roots support further agglomeration of microaggregates, leading to the formation of macroaggregates (> 250µm), which provide only a minimal amount of physical protection and are susceptible against disturbances, such as tillage, than microaggregates (Six et al. 2004).

Interactions with surfaces and metal ions hamper the microbial degradation of organic compounds by reducing their bioavailability. OM can interact with mineral particles or metals as well as with other OM in several ways, which differ regarding reversibility and bonding force. In opposite to the temporary adsorption of OM via ionic interactions, hydrogen bonding, charge-transfer, ligand exchange, van-der-Waals forces, and hydrophobic bonding, the covalent bonding, resulting in ether, ester, or carbon–carbon linkages, is considered very stable and almost irreversible (Kästner et al. 2014; Senesi 1992). Beside of chemical catalysis and radical reactions, microbial enzymes can act as mediators of covalent bonding (Gevao et al. 2000; Senesi 1992).

1.2. Functional SOM pools and measurable SOM fractions

The decay of organic matter is generally assumed to follow first order kinetics, where the transformation rate is proportionally to the substrate concentration. However, turnover rates are altered by the different mechanisms of OM stabilization as mentioned above. Already the

fact that simple and easily degradable components, such as neutral sugars can be degraded in soil within of hours but can also resist mineralization for hundreds of years (Derrien et al. 2006; Fischer et al. 2010) implies that OM decomposition in soil cannot be described by only one equation or turnover rate. Thus, conceptual models describing SOM dynamics differentiate at least three functional SOM pools - a labile, intermediate, and passive SOM pool - each possessing an own turnover rate based on the underlying stabilization mechanisms (Six et al. 2002). In order to investigate SOM pool dynamics it is necessary to characterize size and composition of separated SOM fractions as proxy for functional SOM pools, which led to the development of a range of chemical and/or physical fractionation methods over the past decades. Chemical fractionation methods, such as mild oxidation, hydrolysis using weak acids as well as cold and hot-water extraction (CWE, HWE) are commonly used as proxy for labile SOM pools (Landgraf et al. 2006). Introduced in the 1980s, cold water-extraction methods deliver SOM which largely corresponds to the dissolved organic matter fraction (DOM) collected *in situ* (Kalbitz et al. 2007). Dissolved organic matter is defined as organic matter that pass through a filter size of <0.45 µm and is transported in soil solution (Kalbitz et al. 2000), consequently serving as important energy source for microorganisms even in the soil subsurface. Cold water extracted DOM comprises low as well as high-molecular weight compounds and is thermally more stable than DOM gained from a hot-water extraction. The hot water extracted fraction, in turn, is dominated by carbohydrates, lignin- and phenol-monomers and is considered to represent a pool of easily decomposable SOM (Landgraf et al. 2006; Schulz 2004).

While chemical fractionation methods are primarily useful to isolate mineral-particle free fractions of easily decomposable (Schulz and Körschens 1998) up to very old and stable

SOM (Helfrich et al. 2007), physical fractionation methods allow elucidating the effects of interactions between OM and the soil mineral matrix on SOM dynamics (Christensen 2001). Physical fractionation using density or size separation provide chemically less altered fractions, representing uncomplexed OM or organomineral associations of various sizes, respectively. Labile SOM can be assessed successfully by particulate organic matter (POM) fractions, densiometrically obtained using liquid mixtures based on polytungstates, iodates, or bromoform/ethanol mixtures with densities of $1.6 - 2.0$ g cm^{-3} (Gregorich et al. 2006; Lützow et al. 2006). POM represents uncomplexed, free or occluded and hence, physically protected SOM, which is not or only less associated with minerals. Whereas labile SOM in temperate arable soils is generally characterized by intermediate to high turnover rates, the stable/stabilized SOM pool is associated with half-live times from decades to centuries. The major part of SOM is present in the stable/stabilized pool, bound to clay- (50-75%) and silt-sized (20-40%) particles. Physical separation of the soil enables to measure stable SOM by providing primary and secondary complexed SOM, depending on the applied method and degree of dispersion. Thus, secondary complexed SOM can be obtained by sieving into micro- (< 250 μm) and macroaggregates (> 250 μm) (Denef et al. 2010). Complete dispersion of the soil, for example using ultrasonication, allows the separation of primary complexed SOM, comprising SOM associated with clay- and silt sized particles and smallest microaggregates (Lützow et al. 2006; Schulz 2004; Shaymukhametov et al. 1985).

In the present work, a size-density fractionation approach was used in order to investigate the labile, intermediate and stable SOM pool, respectively (Fig. 1). The applied method was devised by Shaymukhametov et al. (1985) and later modified by Schulz (2004). Two particle-size fractions were gained by repeated ultrasonic treatment followed by two

centrifugation steps: 1.) CF2 with a diameter of 1-2 µm; still containing OM entrapped within smallest microaggregates; 2.) CF1 with a diameter < 1 µm; representign OM bound on primary and secondary clay particles (Lützow et al. 2006; Totsche et al. 2018). By subsequent density-fractionation OM was separated, which was formerly occluded in small to large microaggregates (> 2-250 µm diameter) and released by the ultrasonic treatment. These so called "light" fractions were obtained using two density mixtures of bromoform (tribrommethane)/ethanol. The LF1, with a density of < 1.8 g cm^{-3}, thereby contains mineral free OM, whereas some of the OM within the LF2 (1.8-2.0 g cm^{-3}) is loosely associated with Fe- and Al-oxides or hydroxides.

1.3. Biomarkers as tools to assess the role of soil microorganisms as source of stabilized SOM and drivers of SOM turnover

Soil microorganisms play a key role in soil genesis and soil functioning. They are the main drivers of nutrient cycling as well as of energy and matter fluxes in soil. Besides, they are contributing to the formation of soil aggregates, which, in turn, alters soil physical conditions and promotes C-stabilization (Buscot and Varma 2005). The soil dwelling microbiota also influence plant growth by a range of plant-microbial relationships (e.g. symbiosis or parasitism) or simply by controlling SOM turnover and thus, the availability of mineral nutrients (Caravaca et al. 2015; Wardle et al. 2004). For a long time, microbial contribution to SOM was estimated to correspond to the amount of living microbial biomass C, making up around 1-5% of total SOC (Jenkinson and Ladd 1981). However, during the past two decades it became increasingly apparent that the proportion of microbial derived SOM must be much higher. Currently, it is estimated that up to 80% of the organic C in soils is of microbial

origin, representing transformed and non-transformed residues of the microbial biomass and metabolism (Liang et al. 2011; Miltner et al. 2011).

This finding would not have been made without the analysis of biomarkers, representing organic components of microbial cells and useful tools in order to elucidate the fate of organic C along the soil foodweb. First evidence for a significant contribution of microbial biomass C to the pool of stabilized SOM was gained from studies investigating the decomposition of ^{14}C-labeled environmental contaminants (e.g. phenanthrene, anthracene) (Kästner et al. 1999; Richnow et al. 1999). In these studies, a part of the C-label was found to be incorporated in fatty and amino acids extracted from the soil residue after performance of alkaline hydrolysis. Outgoing from this, Kindler et al. (2006) intended to elucidate the contribution of microbial residues to SOM using isotopically (^{13}C) and genetically (lux gene) labeled *E. coli* cells in a mesocosm experiment. Phospholipid fatty acids (PLFA) and total fatty acids (t-FA) were used as biomarkers in order to assess ^{13}C-enrichment within the living microbial biomass and C of non-living, stabilized SOM, respectively (Kindler et al. 2009). Besides, also the fate of microbial derived amino acids was investigated (Miltner et al. 2009). The results showed that only 56% of the introduced C was mineralized, while around one fourth of the remaining C was associated with the indigenous microbial biomass or stabilized with SOM, respectively.

Incorporation of plant derived organic material into bacterial biomass was also proven on amino sugars and muramic acid, which were found to accumulate with ongoing decomposition of plant litter (Amelung et al. 2001b). Since amino sugars are contained in plants only in negligible amounts and persist after microbial cell lysis, these biomarkers are suitable tools to investigate the fate of microbial necromass derived C and N. The fact that

muramic acid exclusively occurs in bacteria, additionally allows to roughly differentiate between bacterial and fungal derived C (Joergensen and Wichern 2008). Furthermore, the simultaneous analysis of the microbial biomass based on f.ex. chloroform-fumigation extraction, or PLFA-extraction, supported the hypothesis that organic matter derived from dead fungi or bacteria largely exceeds the proportion representing living microbial biomass. Thus, calculations by Appuhn and Joergensen (2006) suggested that around 50% of SOM in the rhizosphere soil of a pot experiment was dead matter of bacteria and fungi, while only 6% was attributed to the microbial biomass.

Finally, also discrimination between plant and bacterial SOC is possible using neutral sugars. The composition of plant and microbial saccharides in soil differs from one another regarding the contained monosaccharides. Hexoses and deoxy-hexoses are predominately synthesized by microorganisms, while pentoses are more frequent in plant material. The commonly observed shift from plant to microbial derived saccharides in soil along with decomposition of exogenous substrates, and the fact that fine fractions frequently show higher hexose-to-pentose ratios than coarse fractions also supports the transformation of plant into microbial SOM and its subsequent stabilization in soil (Kiem and Kögel-Knabner 2003; Murayama 1984).

Table 1 provides an overview about origin, interpretation, and relevance for SOM of the most frequently used biomarkers.

Table 1: Origin, interpretation, and relevance for SOM of frequently used biomarkers

Biomarker	Significance/origin/interpretation	References
1. Lipids	2-9% of SOC ~10% of microbial biomass (dry weight)	(Martens et al. 2004; Miltner et al. 2011)
PLFA (phospholipid fatty acids)	main components of biomembranes microbial biomass: PLFA ≤ C_{20} Gram-positive: terminal branched PLFA (iso, anteiso) Gram-negative: hydroxylated PLFA, monounsaturated Actinomycetes: methyl-branched General fungi: mono-/diunsaturated C_{18}-PLFA Arbuscular mycorrhizal fungi: 16:ω5c	(Balser 2005; Bligh and Dyer 1959; Vestal and White 1989; Zelles 1999)
NLFA (neutrallipid fatty acids)	Triacylglycerols, eukaryotic/fungal storage lipids, degradation products of PLFA NLFA/PLFA ratio = physiological state of fungi	(Bååth 2003)
2. Amino acids	1.2-2.5% of SOC, 20-51% of TN ~50% of microbial biomass (dry weight)	(Friedel and Scheller 2002; Miltner et al. 2011; Senwo and Tabatabai 1998)
D-glutamic acid* D-alanine*	Peptidoglycane layer of microbial cell walls D:L-ratio = relative proportion of bacterial amino acids to SOM	(Amelung and Zhang 2001; Schieber et al. 1999)
2,4-meso diaminopimelic acid	Peptidoglycane layer of microbial cell walls	
3. Ergosterol*	Predominant sterol in fungal cell membranes (Basidiomycetes, Ascomycetes, Zygomycetes)	(Joergensen and Wichern 2008; Peacock and Goosey 1989)

Biomarker	Significance/origin/interpretation	References
4. Amino sugars	3% of SOC, 0.9-6.3% of TN 0.1-7% of microbial biomass-C	(Amelung 2001; Glaser et al. 2004; Joergensen and Meyer 1990)
glucosamine (GluN) galactosamine (GalN) mannosamine (ManN) muramic acid (MurA)	Peptidoglycane, bacterial exopolysaccharides, chitine of fungal cell walls and arthropods, snail gelatine Peptidoglycane, bacterial exopolysaccharides, fungal cell wall Bacteria, fungi, actinomycetes Peptidoglycane layer of bacterial cell walls, actinomycetes GluN/GalN ratio = relative proportion of fungal amino sugars on SOM MurA/GalN ratio = relative proportion of bacterial amino sugars on SOM GalN/MurA ratio = relative proportion of bacterial amino sugars on SOM	(Zhang and Amelung 1996) (Glaser et al. 2004)
5. Neutral sugars	2-11% of SOC	*(Zhang et al. 2007)*
Xylose (Xyl) Arabinose (Ara) Galactose (Gal) Mannose (Man) Rhamnose (Rham) Fucose (Fuc) Uronic acids	C5-sugar, monomers of plant hemicelluloses (Xylane) C5-sugar, plant nucleosides, plant and mycobacterial arabinogalactanes C6-sugar, bacterial lipo- and exopolysaccharides, arabinogalactanes C6-sugar, bacterial lipopolisaccharides, plant polysaccharides deoxyC6-sugar, bacterial lipopolysaccharides, buckthorn growths (L-Rham) deoxyC6-sugar, bacterial lipopolysaccharides, glycoproteins C6:C5 ratio < 0.5 = plant polysaccharides C6:C5 ratio > 2.0 = microbial polysaccharides deoxyC6:C5 ratio high = microbial polysaccharides; low = plant polysaccharides bacterial exopolysaccharides, pectines	(Oades 1984) (Murayama 1984)

* not applied in this work

Concerning functional SOM pools and fractions (*cf. section 1.2.*), each biomarker extraction from soil can be considered as a kind of chemical fractionation, followed by subsequent separation and purification of the compounds of interest. Based on the applied extractants and procedures one or more SOM pools are grasped. Figure 1 illustrates the basic approaches of biomarker extractions, chemical and physical fractionation methods to separate fractions as representatives of SOM pools.
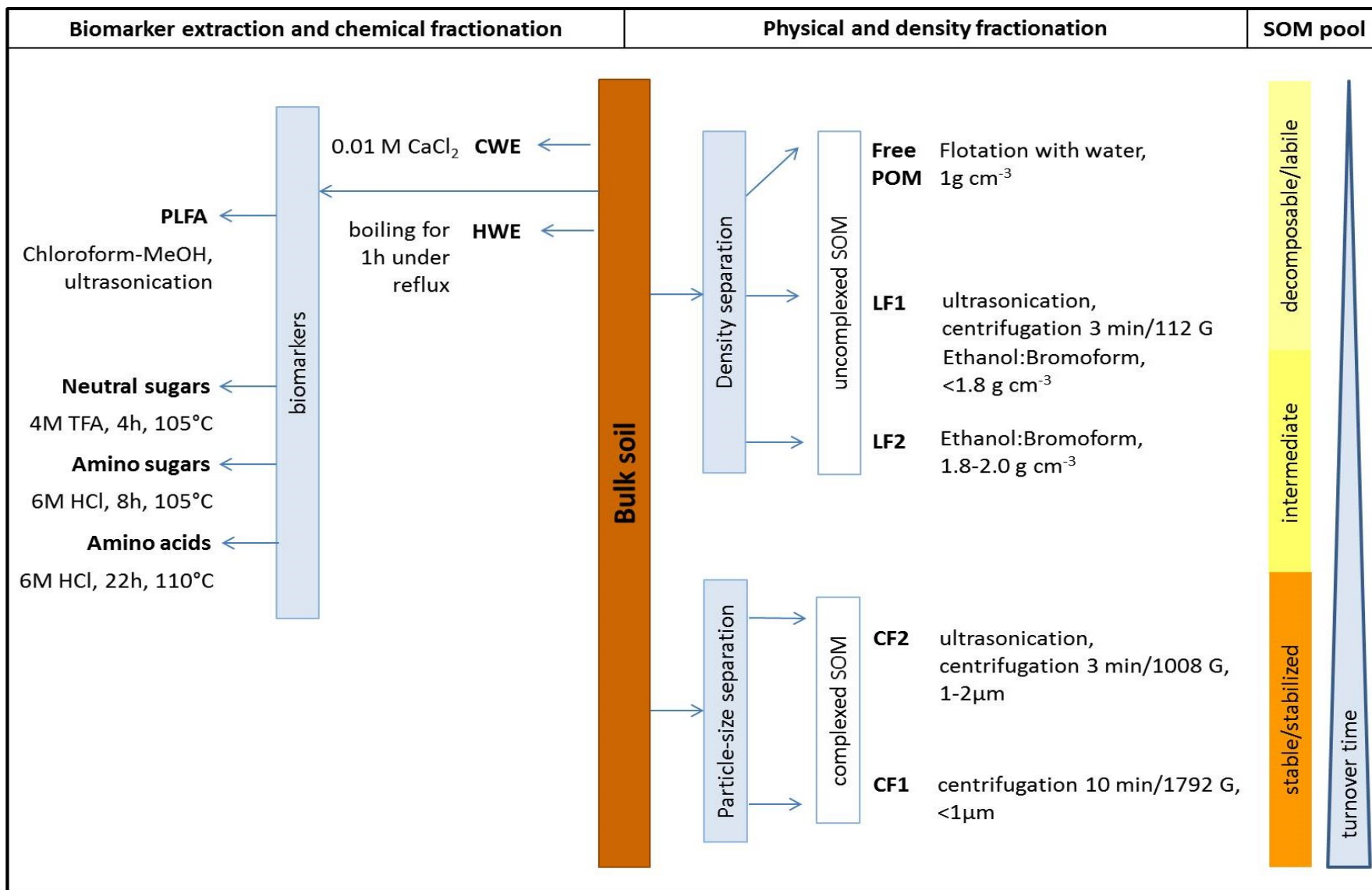
Figure 1: Overview of different soil fractions (chemical: CWE and HWE; physical: Free POM, LF1, LF2, CF1, CF2) as well as biomarkers (PLFA, neutral sugars, amino sugars, amino acids) extracted from bulk soil representing related SOM pools.

13

1.4. Study site – the Static Fertilization Experiment Bad Lauchstädt

Soil is not a homogenous system but highly variable regarding its chemical and physical properties over time and space. Since soil processes run very slowly, significant changes can be quantified often only after decades. In long-term experiments, the spatial heterogeneity gets reduced due to the constant treatment for many years, resulting in a dynamic equilibrium of formation and degradation of SOM. This, in turn, provides an ideal experimental background to study effects on SOM pools and their dynamics.

The Static Fertilization Experiment Bad Lauchstädt (SFEBL) is one of the ten oldest long-term field experiments in the world with duration of more than100 years. Established in 1902, the experiment serves as suitable platform to investigate the responses of soil quality and fertility to a differentiated organic and mineral fertilization remaining consistent over a long-term. The soil is classified as Haplic Chernozem (FAO), characterized by high natural soil fertility at an average SOM content of 3.5% (2.0% SOC). Situated in the climatic region of the Börde and Central German continental climate, the typical annual temperature of this area lies between 8.5 and 9.0°C on average. The average annual precipitation is 470-540 mm, reaching its maximum between June and August. The experiment comprises eighteen different fertilization treatments including the unfertilized control (Fig. 2). These treatments represent: Three levels of organic fertilization (without, 20t FYM ha^{-1} 2yrs^{-1} and 30t FYM ha^{-1} 2yrs^{-1}), six different treatments of mineral fertilizers (without, PK, N, NK, NP, NPK), and finally, the respective combination of each level of organic and mineral fertilization. The amount of added mineral N fertilization with calcium ammonium nitrate is corresponding to the requirements of the individual crop (Tab. 2). All fertilization treatments are established on six of the eight strips in total (S1, S2, S3, S6, S7, and S8). Therein, strip S1 is getting lime every 4th year since 1924, and on strip S8 legumes are replacing potato and winter wheat

every 7th and 8th year within the general crop rotation of sugar beet, spring barley, potato, and winter wheat. Since 1978, strips S4 and S5 were excluded from the original experiment and used for establishing a new separate experiment "Static Fertilization Experiment Bad Lauchstädt after extension of the experimental issue" where the treatments of organic and mineral fertilizer were turned to the opposite and fife levels of N fertilization were introduced.

Over an experimental area of 4 ha, extreme spans regarding individual soil conditions such as SOC, TN, pH, P and K have been manifested as a result of the long-term differentiated fertilization. For example, SOC and TN differ up to 0.9% and 0.09%, respectively, between the highest fertilization level (30t FYM ha^{-1} 2yrs^{-1} plus NPK) and the unfertilized control. Due to this, the SFEBL is of importance not only for agricultural but also for fundamental ecological research of soils and their ecosystem services in general.

Table 2: Properties of farmyard manure applied in 2010, and 2011

Year	Dry matter content %	N % of fresh matter	P % of dry matter	K % of dry matter	Ca % of dry matter	Mg % of dry matter	SOC %
2010	19.80	0.60	0.46	1.33	1.96	0.46	35.61
2011	19.60	0.83	0.66	3.61	2.55	0.68	71.80

Table 3: Mineral-N-fertilization (kg N ha^{-1} as calcium ammonium nitrate) since 1903

Year	Sugar beet with	Sugar beet without	Spring barley with	Spring barley without	Potatoes With	Potatoes without	Winter wheat with	Winter wheat without
	Farmyard manure							
1903-09	60	90	20	40	40	60	40	60
1910-25	60	90	20	40	20	40	20	40
1926-28	60	90	20	40	20	40	30	60
1929-51	90	120	20	40	20	40	30	60
1952-70	90	120	20	40	40	60	30	60
1971-77	200	240	30	50	120	160	40+40	60+40
1978	140	160	35	25+40	110	130	20+45	30+60
1979	150	180	25+25	35+35	110	130	30+25	40+40
1980	150	170	15+20	25+30	120	140	15+45	25+55
1981	150	170	15+15	25+25	120	140	15+45	25+55
1982	150	170	20+20	30+30	120	140	30+30	40+40
1983	150	170	20+20	30+30	120	140	20+20	30+30
1984	150	170	20	30	120	140	20+20	30+30
1985	150	170	20	30	120	140	40	60
1986	150	170	20+20	30+30	120	140	40+40	60+40
1987	150	170	30+30	40+40	120	140	60+20+20	70+30+30
1988	150	170	30+30	40+40	120	140	30+30	40+40
1989	150	170	20	30	120	140	30	40
1990	150	170	20	30	120	140	30+30	40+40
1991	150	170	20	30	120	140	30+30	40+40
1992	150	170	20	30	120	140	30	40
1993	150	170	30+30	30+30	120	140	40+30	40+30
1994	150	170	30+35	50+35	120	140	40+40	50+50
1995-2014	100+50	100+70	30+30	30+30	120	140	40+40	50+50

Table 4: Mineral-P-fertilization (kg P ha^{-1} as superphosphate) since 1902

Year	30t FYM ha^{-1} 2yrs^{-1}	20t FYM ha^{-1} 2yrs^{-1}	Without FYM
Root crops			
1902-80	43	43	43
Since 1981	12	28	60
Grain crops			
1902-81	22	22	22
Since 1982	0	0	0

Table 5: Mineral-K-fertilization (kg K ha^{-1} as 50er potash fertilizer since 1970) since 1902

Year	30t FYM ha^{-1} 2yrs^{-1}	20t FYM ha^{-1} 2yrs^{-1}	Without FYM	
	Root crops		Potatoes	Sugar beet
1902-28	100	100	100	100
1929-70	100	100	100	166
1971-75	100	100	166	166
1976	100	100	166	166
1977-78	100	100	166	166
1979-80	100	100	232*	166
Since 1981	50	110	230	230
	Grain crops			
1902-81	66	66	66	
Since 1982	0	0	0	

*alfalfa
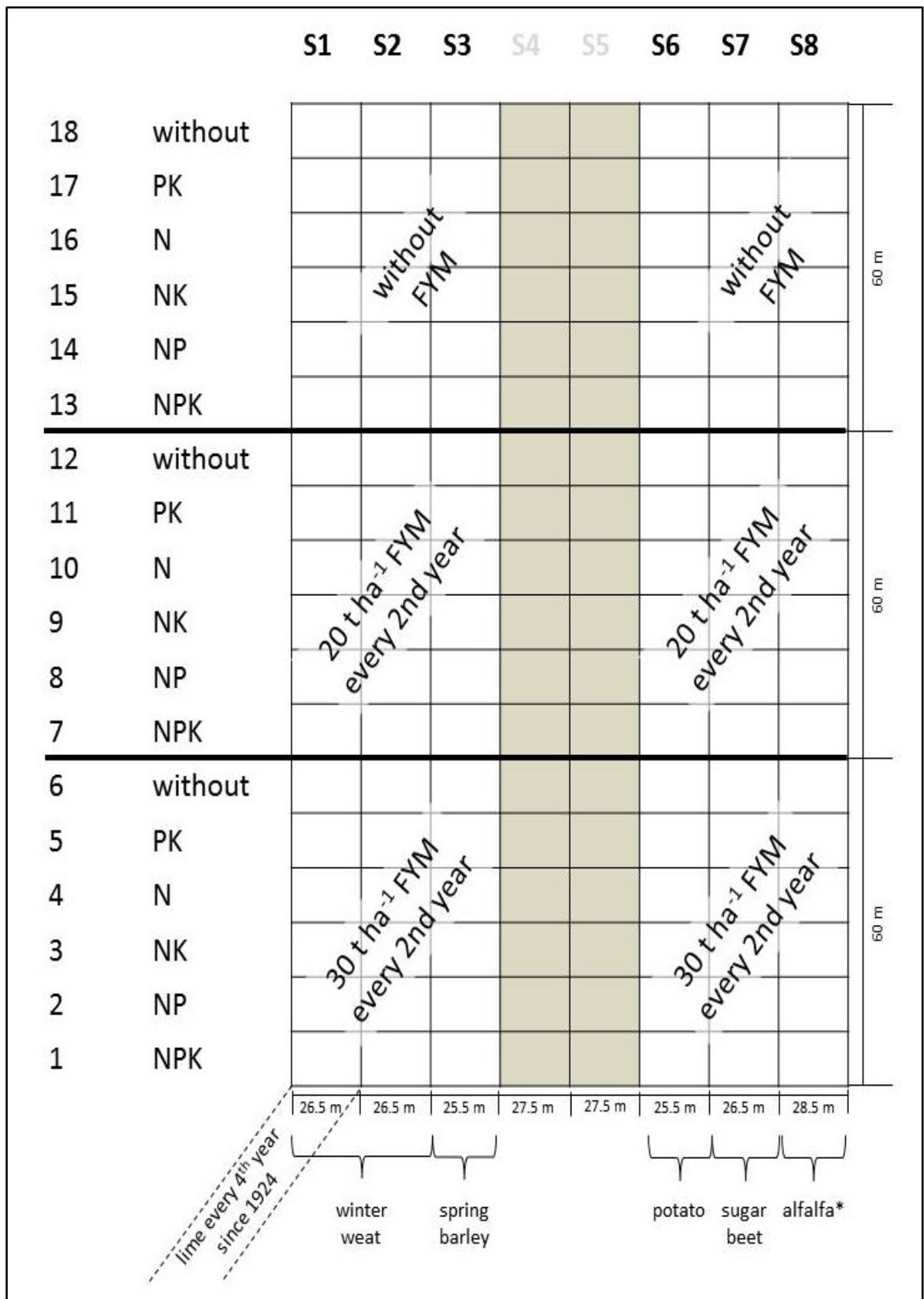
Figure 2: Experimental design of the Static Fertilization Experiment, Bad Lauchstädt, until 2015
* legumes since 1924; alfalfa since 1970

1.5. Objectives and thesis organization

Apart from the classical paradigm that SOM determines soil fertility and contributes to aggregation and thus, to the stabilization and sequestration of OC, it has been increasingly established that SOM is not inert but highly dynamic. Soil management, in turn, has been shown to strongly alter the processes standing in relation to the transformation and stabilization of SOM. This reactivity of SOM was in the focus of the priority program SPP1090 of the German Science Foundation (DFG) entitled "Soils as source and of sink of CO_2", in which more than 20 participant groups worked to characterize the different pools of SOM, their stability, and availability for transformation. The focus of the present thesis and the motivation behind was to provide deeper knowledge for the development of land use strategies leading to an accumulation and stabilization of SOC for reducing CO_2 emissions from soils.

In contrast to the majority of previous studies on SOC, which rather investigated the effects of management practices on SOC stocks in general, the present work specifically intended to elucidate the role of the microbial community on SOM quality and dynamics under the impact of long-term fertilization by considering both, the macro- and the fine-scale. The macro-scale at bulk soil level thereby relates to an overall consideration of physical and chemical soil conditions (e.g. bulk density, SOC, TN, pH) along with the analysis of content and composition of four different biomarkers (lipids, amino acids, amino sugars, neutral sugars), whereas the fine-scale represents the analysis of abiotic parameters and biomarkers in soil fractions presented in Figure 1. Linking biomarkers and abiotic soil properties along a fertilization gradient should provide information on the role of microorganisms on composition and stabilization of SOM and help to identify the main factors controlling turnover and/or stabilization of SOM in agricultural soils. In total, six of the SFEBL fertilization treatments were investigated (i.e. treatments 1, 6, 7,

12, 13, and 18 in Fig. 2). To elucidate specifically the role of plant-microbial relationships on SOM dynamics, samples were taken on two strips: Strip 2, cultivated with sugar beet at sampling and strip 8, where legumes are included in the crop rotation since 1924 (Fig. 2, *1.4*), cultivated with alfalfa at the time of sampling.
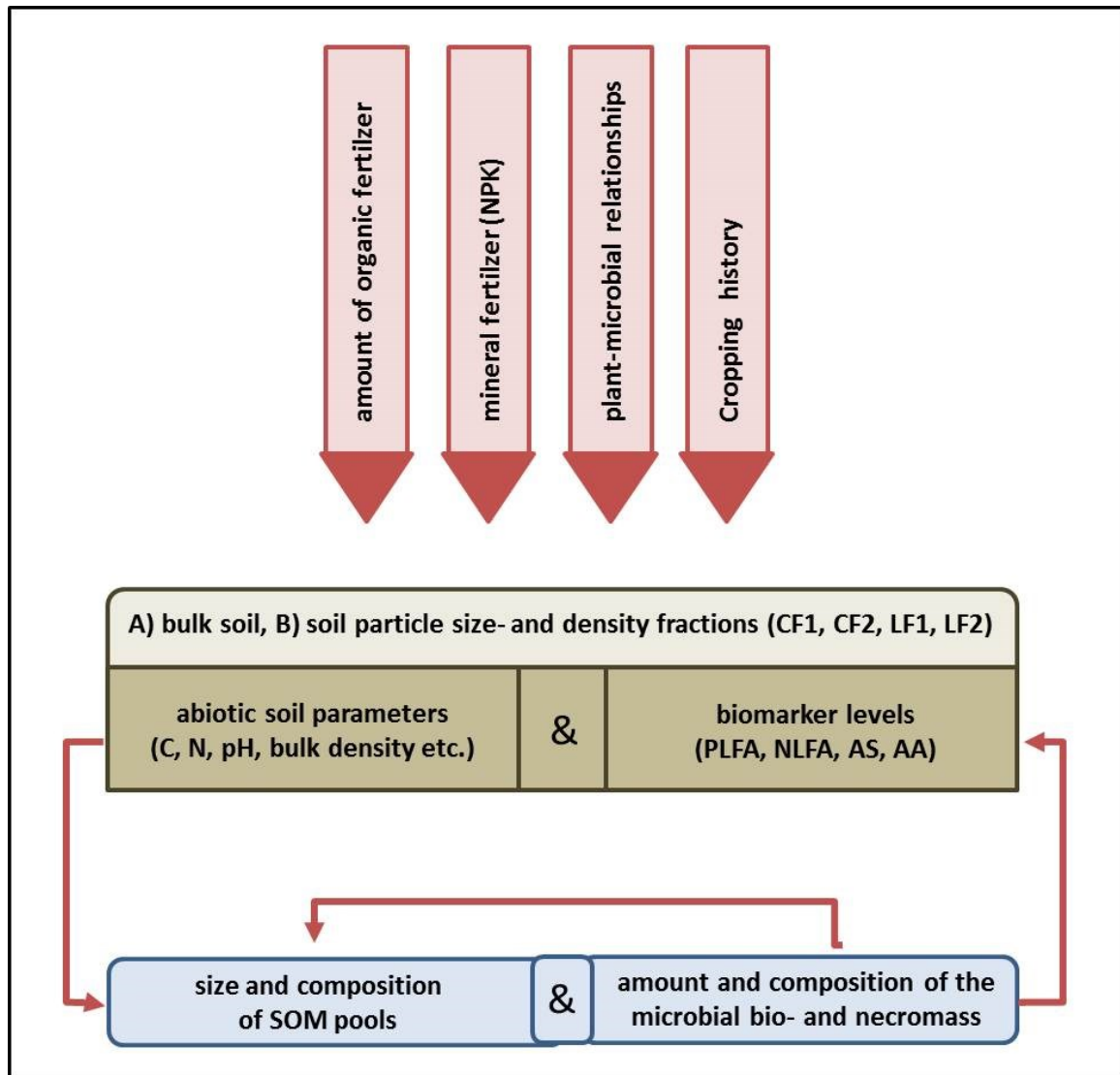
Figure 3: The primary objective this thesis was to investigate the impact of differentiated organic- and/or mineral long-term fertilization along with the presence and absence of symbiotic plant-fungal/bacterial relationships on SOM composition as well as on microbial bio- and necromass at **a)** bulk soil level and **b)** at the level of functional SOM pools represented by soil particle size- and density fractions (CF1, CF2, LF1, LF2) in order to draw conclusions on main factors affecting microbial mediated SOM dynamics.

According to the objectives and research scales the thesis is structured into three results chapters:

Chapter 2 primarily focuses on effects of long-term fertilization on SOC and total N dynamics under two different crop types (sugar beet, alfalfa) by examining the SOM composition with respect to the percentage contribution of each biomarker class to SOC and TN. The relationships between PLFA and the other biomarkers were analyzed to elucidate whether biomarker levels were associated with the living microbial biomass rather than stabilized SOM. Analysis of abiotic parameters, such as hot-water extractable carbon and nitrogen (HWC, HWN), NO_3^--N, NH_4^+-N, and pH should further provide information about changes in the availability of labile OC, mineral nutrients, and other soil conditions influenced by fertilization and/or crop type, thereby specifically taking into account the presence of plant-symbiotic relationships with rhizobia and arbuscular mycorrhizal fungi in association with alfalfa as crop.

Chapter 3 describes the composition of the living microbial biomass (PLFA) in presence of the legume (alfalfa) and a non-legume crop (sugar beet) to compare the effects of long-term fertilization vs. cropping history (legume included/not included in rotation) on the soil microbial necromass composition (amino sugar ratios).

While the investigations, described in the previous result chapters, were performed at bulk soil level, **Chapter 4** provides a more detailed look on the effects of long-term fertilization on the composition and microbial shape of SOM pools. In this context, biomarker levels were determined in two particle size, i.e. clay fractions (CF1: <1μm, CF2: 1-2μm) and two density fractions (LF1: <1.8 g cm^{-3}, LF2: 1.8-2 g cm^{-3}), which were generated from soil of the highest fertilization level (1) and the control (18) of the strip cultivated with alfalfa (strip 8).

2. Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon

Abstract

It is increasingly recognized that a detailed understanding of the impacts of land use on soil carbon pools and microbial mediated carbon dynamics is required in order to accurately describe terrestrial carbon budgets and improve soil carbon retention. Toward this understanding, we analyzed the levels of biomarkers such as phospholipid fatty acids, amino acids, monosaccharides, amino sugars, and several indicators of labile and stabilized carbon in soil samples from a long-term agricultural field experiment. Our results imply that the composition of soil organic carbon (SOC) depends strongly on both the applied fertilization regime and the cultivated crop. In addition, our approach allowed us to identify possible mechanisms of microbial growth and contributions to soil carbon storage under different long-term agricultural management regimes.

Amino acids and monosaccharides were quantitatively the most dominant biomarkers and their levels correlated strongly positively with microbial biomass. The relative contributions of the studied biomarkers to the total SOC varied only slightly among the treatments other than in cases of extreme fertilization and without any fertilizer. Under the case of extreme fertilization and with alfalfa as crop type, we found evidence for accumulation of microbially derived monosaccharides and amino acids within the labile

OC pool, probably resulting from soil C saturation. Interestingly, we also found an accumulation of microbially derived monosaccharides and amino acids in completely unfertilized plots, which we assumed to be caused by the lower pore space size and subsequent oxygen limitation for microbial growth. Mineral fertilization also had substantial effects on soil organic N when applied to plots containing alfalfa, a leguminous plant. Our results demonstrate that over-fertilization, fertilizer type, and the cultivated crop type can have major impacts on the turnover and composition of soil organic carbon, and should be considered when assessing management effects on soil C dynamics.

Keywords: SOC quality, microbial biomass, amino acids, amino sugars, monosaccharides, long-term fertilization

2.1   Introduction

Terrestrial ecosystems represent the largest global pool of organic carbon (Batjes 1998; Janzen 2005). There is an increasing need to understand the cycling and storage potential of organic carbon in terrestrial ecosystems to mitigate climate change and improve sustainability, especially in agriculturally managed systems since these represent around 40% of all land on earth (Smith et al. 2008). Efficient management of carbon and nitrogen flows will be required to reduce CO_2, CH_4, and N_2O emissions due to agriculture and to increase carbon stocks in agricultural ecosystems (IPCC 2007). However, it is difficult to evaluate the efficiency of a specific management strategy if one cannot account for all of the factors that may affect the dynamics of C and N in the soil and their interactions with one-another. One such factor is the use of organic and inorganic fertilizers, which directly or indirectly increase the input of C into the soil and change the availability of nutrients,

the soil structure, and the abiotic conditions in the soil environment (Campbell et al. 1991; Marschner et al. 2003). The crop type can also influence soil organic carbon (SOC) turnover due to the different physiological and morphological characteristics of different plant species. Important characteristics can include root architecture, nutrient demand, water use efficiency, and the quantity and composition of root exudate (Dakora and Phillips 2002; Jastrow et al. 2007; Marschner 2012). Due to the close interactions between plants and soil microorganisms, plant-microbe interactions and the growth, activity and composition of crop-specific microbial communities have profound effects on C and N dynamics. A well-known example of this is that legumes are often used in agriculture to improve the availability of N and other mineral nutrients based on their ability to form symbioses with N-fixing bacteria and arbuscular mycorrhizal fungi (Lal 2010; Paustian et al. 1997a; Wilson et al. 2009).

Investigations of soil properties using pyrolytic techniques (Py-FIMS, Py-GC/MS) have demonstrated that fertilization and crop rotation affect the SOC composition by altering C and N dynamics within or between several organic carbon pools (Leinweber et al. 2008; Schmidt et al. 2000). The organic carbon (OC) content of non-protected or labile carbon pools is affected directly by the supply of new nutrients and microbial growth, and can be rapidly degraded (Wander 2004). Management-induced effects on the availability of C, N and P or the oxygen content, pH or temperature of the soil can change the rates of microbial metabolism and decomposition, which can result in quantitative and qualitative changes in the labile OC pool (Curiel Yuste et al. 2007; Davidson and Janssens 2006; Hartman and Richardson 2013; Knorr et al. 2005; Rousk et al. 2009; Tiedje et al. 1984). Since rates of OC production and utilization may compensate each other, variations in contents of total labile OC may not be measurable. Even though the total labile OC content may not change, the decomposition and incorporation of plant derived labile OC

into microbial biomass may cause a shift in the proportions of OC from different sources, resulting in an altered composition of SOC.

Conversely, OC in protected carbon pools is more resistant to depletion and such pools exhibit low turnover rates. Factors that impede the decomposition of organic constituents include formation of stable complex chemical structures, physical protection by occlusion into micro-aggregates, and chemical protection by interaction with minerals and metal ions (Lützow et al. 2006; Sollins et al. 1996). Microorganisms can enhance the proportion of OC in the protected pool by producing complex organic compounds and promoting aggregate formation around fungal hyphae or bacterial exopolysaccharides. While estimates of total soil organic C are generally based on the size of the protected or stabilized OC pool, most C models predict that SOC levels increase linearly with C input, which has been confirmed for a large number of long-term agricultural field experiments (Huggins et al. 1998; Kong et al. 2005; Paustian et al. 1997b). However, the protective capacity of the soil may be limited, especially if it was originally rich in OC, resulting in C saturation, or in other words, a limit to observed linear responses despite further increase of C input (Hassink 1997; Hassink et al. 1997; Six et al. 2002; Wiesmeier et al. 2014). It is currently not entirely clear how saturation of the protected pool affects OC turnover within the non-protected OC pool. In addition, little is known about how microbial mediation of OC stabilization and cycling are influenced by the impact of fertilization on plant-microbe interactions. To address these questions, it is necessary to consider physical, chemical and microbiological factors simultaneously.

Biomarkers such as phospholipid fatty acids (PLFA) and amino sugars (AS) have been used extensively to study environmental impacts on living microbial communities and the accumulation of microbial residues, respectively (Amelung 2003; Amelung et al. 2001a;

Bossio et al. 1998; Guggenberger et al. 1999; Jangid et al. 2008). Although monosaccharides (MS) and amino acids (AA) are ubiquitous in the biosphere, they can also be used as biomarkers for microbially derived C and N to some extent. For instance, proportions of bacterial to plant derived monosaccharides can be reflected by the ratios of C6:C5 (mannose + galactose)/(xylose + arabinose) and deoxyC6:C5 (rhamnose + fucose)/(xylose + arabinose) (Murayama, 1984; Oades, 1984). For amino acids, meso-Diaminopimelic acid as well as D-enantiomers of alanine and glutamic acid build up the peptidoglycane layer of bacterial cell walls and have been used to indicate bacterial residues (Amelung 2001; Amelung and Zhang 2001; Grant and West 1986). However, it is important to recall that MS and AA account for a significant proportion of both microbial biomass C and SOC and TN (Friedel and Scheller 2002; Martens et al. 2004; Neidhardt 1990; Senwo and Tabatabai 1998). At present, little is known about the mechanisms that are responsible for the stabilization or destabilization of the biomarkers mentioned above, and the factors that govern these mechanisms are similarly challenging to understand.

Our aim in this study was to investigate the effects of long-term fertilization on C and N dynamics under different crop types, and the resulting impacts on SOC composition. This was done using an integrated approach in which chemical and physical parameters were monitored along with four separate biomarkers: PLFA, AS, MS, and AA. We hypothesized that high C inputs due to the long-term addition of organic and/or mineral fertilizer would enhance the stabilization of AA, MS and AS in the soil and increase the contribution of C from the selected biomarkers to the total SOC. We also expected the crop type to influence SOC composition by affecting OC decomposition rates, via plant-induced changes in the availability of labile OC and mineral nutrients, interactions with associated microbial communities, and changes in soil structure.

2.2. Materials and methods
2.2.1. Study site description and sampling

The experimental basis for the study was the Static Fertilization Experiment Bad Lauchstädt (SFEBL), Sachsen-Anhalt (Germany). This long-term experiment was initiated in 1902 in order to investigate the effects of organic and mineral fertilization on crop yield, crop quality, and soil fertility. The experiment's soil type is a Haplic Chernozem (FAO) (USDA: Mollisol) consisting of 21.0% clay, 67.8% silt and 11.2% sand. The mean annual temperature and precipitation at the site are 8.8°C and 480 mm, respectively (Körschens 2002). A comprehensive description of the experimental site and treatments has been given by Körschens (2002). The crop rotation is sugar beet, spring barley, potatoes, and winter wheat. On one of the six strips of the experiment, legumes have been included in the rotation since 1924, replacing sugar beet and spring barley every 7th and 8th year. Since 1970 alfalfa (*Medicago sativa*) has been the only legume species cultivated during this 7th and 8th year legume rotation. The level of mineral fertilization (NPK) depends on the crop that is being cultivated and the amount of farmyard manure (FYM) that is applied, and ranges from 60 to 170 kg ha^{-1} yr^{-1} N, 12 to 60 kg ha^{-1} yr^{-1} P, and 50 to 230 kg ha^{-1} yr^{-1} K. Farmyard manure is applied every second year during the cultivation of root crops (i.e. potatoes or sugar beet). When alfalfa is cultivated, it generally follows winter wheat and receives farmyard manure and only PK in the mineral fertilized treatments before seed drilling. The treatment effects on soil organic carbon and nitrogen as well as on crop yields are proved on the basis of repeated soil samplings (annually or biannually since the 70th of the last century) and annual measurements of crop yields and nutrient uptake by the crops and documented in a range

of research articles (Böhme and Böhme 2006; Kandeler et al. 1999; Merbach and Schulz 2012).

This work focused on five different fertilizer treatments and an unfertilized control treatment (NIL). The five fertilization regimes were: mineral fertilizer alone (NPK), 20 t FYM ha^{-1} 2yrs^{-1} (FYM1), 20 t FYM ha^{-1} 2yrs^{-1} plus NPK (FYM1+NPK), 30 t FYM ha^{-1} 2yrs^{-1} (FYM2), and 30 t FYM ha^{-1} 2yrs^{-1} plus NPK (FYM2+NPK). The influence of the crop type as a biotic factor was investigated by sampling soil from one strip that was cultivated with alfalfa (*Medicago sativa*) and another that was cultivated with sugar beet (*Beta vulgaris*) at the time of sampling, for each of the fertilization treatments listed above. Because the specific crops we sampled were from within the context of long-term management that was equal, with the exception of the 7th and 8th year alfalfa cultivation on the alfalfa strip, we were able to investigate both a long-term and immediate influence of including alfalfa in crop rotations. Further, while "sugar beet" is discussed, it must be noted that our sampling of sugar beet represents both immediate effects of this crop, and long-term effects of a crop rotation absent of alfalfa. Five representative subsamples per treatment were taken from the plough horizon (0-20 cm) at the end of cultivation (June 2010 for alfalfa, October 2011 for sugar beet). Soil samples were sieved to < 2 mm, and stones and visible plant residues were removed. Portions of the fresh and sieved soil were frozen at -20 °C immediately after the sampling for biomarker analysis (see below). Mineral N was analysed in fresh and frozen soil samples. Other chemical parameters were determined using air-dried soil samples.

2.2.2. Chemical and physical characterization of the soil

2.2.2.1. SOC, TN and labile OC and N

Total carbon (TC) and total nitrogen (TN) were determined via combustion in a C/H/N analyser (Vario El III, Elementar-Hanau). No inorganic C was detected, so the reported TC values represent the total soil organic carbon (SOC).

Hot water extraction was performed to quantify the labile OC pool, i.e. the potentially mineralizable and decomposable fraction of the total SOC (Weigel et al. 1998). This was done by boiling a soil/water suspension (1:5, w/v) for 1 h under reflux, according to the method of Schulz (2002). After cooling to room temperature, 0.1 ml of 1 M $MgSO_4$ was added to facilitate soil sedimentation, followed by centrifugation for 10 min at 6700 g to obtain clear extracts. All water extracts were filtered (0.45 μm Minisart single-use syringe membrane filters RC 25, PP-housing, Sartorius AG, Göttingen, Germany) prior to the determination of their hot water extractable C and N (HWC, HWN) concentrations (mg kg^{-1}), which was done using an elemental analyser for liquid samples (Micro N/C and Multi N/C, Analytik Jena, Germany).

2.2.2.2. Additional soil chemical parameters

Fresh soil samples from each treatment were used for the determination of the pH, ammonium-N (NH_4^+-N), and nitrate-N (NO_3-N). NH_4^+-N and NO_3-N were extracted with 1 M KCl (1:4 w/v, soil: KCl) from 10 g soil by shaking for 1.5 h. After sample filtration (Whatman Schleicher & Schuell 595 1/5 Ø 270 mm), the concentrations of NH_4^+-N and NO_3^--N were measured using a flow injection analyser (FIAstar 5000, Foss GmbH, Rellingen, Germany). The soil pH was measured from slurries prepared by mixing 25 ml of 0.01 M $CaCl_2$ and 10 g of air-dried soil. The soil bulk density was determined by measuring five soil cores of 5 cm depth and 5.6 cm diameter that were taken from each

plot after removing the uppermost 10 cm of soil. This was done to ensure that the measured bulk density was representative of the ploughing layer, which is located 10 to 15 cm below the soil surface.

2.2.3. Analysis of biomarkers

2.2.3.1. Phospholipid fatty acids

Phospholipid fatty acids were analysed using a modified variant of the method described by Bligh and Dyer (1959). In brief, soil samples (2 g each) were extracted three times using a chloroform–methanol–citrate buffer mixture (2:4:1.8 v/v/v). After phase separation, the chloroform layer dried under a flow of N_2 and redissolved in a smaller 300 µl volume of chloroform. Phospholipids were then separated from neutral- and glycolipids by solid phase extraction columns pre-conditioned with chloroform (SPE-SI; Bond 207 Elute, Varian, Palo Alto, USA). Phospholipids were converted into fatty acid methyl esters by mild alkaline methanolysis in a KOH solution prior identification and quantification.

Individual PLFA peaks were identified and quantified using coupled gas chromatography-mass spectrometry (GC/MS) with a 13:0 internal standard (Frostegård and Bååth 1996; Vestal and White 1989; Zelles et al. 1992). To ensure that PLFA-C levels could also be used to represent microbial biomass C, only phospholipid fatty acids with alkyl chains of fewer than 20 carbon atoms were considered.

2.2.3.2. Amino acids

For the determination of amino acids, 2 g of soil was hydrolysed with 6 M HCl for 22 h at 110°C (Macko et al. 1997). Trans-4-(aminomethyl)-cyclohexanecarboxylic acid was added to each sample prior to hydrolysis as an internal standard, at a concentration of 400 µg per sample. The hydrolysates were then filtered (GF6, Schleicher & Schuell, FRG), dried (Multivapor P-12, Büchi, Essen, Germany) and redissolved in 4 ml 0.1 M HCl. Prior to the first purification step a cation exchange resin DOWEX 50W-X8 (50-100 mesh) was filled in a glass column and prepared with 2 M NaOH, followed by 2 M HCl. Finally distilled water was added until the eluate was neutral (Boas 1953). After the samples had passed the column, the resin was washed with 0.1 M oxalic acid (pH 1.6-1.8), 0.01 M HCl, and distilled water. Amino acids were eluted from the column by an excess of 2.5 M NH_4OH (Amelung and Zhang 2001). The eluates were dried under a flow of N_2 after which the samples were redissolved in 0.1 M HCl and mineral particles were removed by centrifugation for 15 min at 4200 g. The carboxyl groups of the amino acids were esterified using acetyl chloride in isopropanol, and their amino groups were trifluoroacetylated using trifluoracetic anhydride in dichloromethane. The derivatized amino acids were then re-purified by the addition of phosphate buffer and chloroform, followed by centrifugation for 10 min at 11 000 g (Ueda et al. 1989). The lower, organic phase was dried again under an N_2 stream. The final extracts were redissolved in 500 µl of an 8:1 ethyl acetate: hexane mixture prior to GC/MS analysis. Individual amino acids were identified and quantified with reference to a standard mixture.

2.2.3.3. Monosaccharides

Monosaccharides were determined as described previously (Amelung et al. 1996). In brief, 10 ml of 4 M trifluoracetic acid was added to 0.5 g of soil and the resulting suspension was heated for 4 h at 105 °C. Myo-inositol (100 μg) was used as an internal standard and added to the mixture prior to hydrolysis. After filtration (GF6, Schleicher & Schuell, FRG), the hydrolysates were evaporated to dryness (Multivapor P-12, Büchi, Essen, Germany) and redissolved in 10 ml of water. To remove humic-like material, iron, and amino sugars, samples were passed through XAD-7 resin, eluting with 15 ml of water, followed by a further purification step using a cation exchange resin (Dowex 50W-X8). Saccharides were released from the resin by washing with 20 ml of water, after which the eluate was freeze dried. Sugar oxime derivatives were prepared by mixing the dried samples with 200 μl of the derivatisation reagent (a 20 mg ml^{-1} solution of O-methylhydroxylamine hydrochloride in pyridine) and heating the resulting mixture at 75 °C for 30 minutes. BSTFA (400 μl) was then added, and the mixture was maintained at 75 °C for another five minutes. Analyte recovery was determined using 3-O methylglucose as a second standard (200 μl per sample at a concentration of 5 mg in 25 ml N-methyl-pyrrolidone), which was added to the samples just before derivatization. A standard mixture of D-(+)-xylose, L-(+)-arabinose, L-(+)-rhamnose, L-(-)-fucose, D-(+)-mannose, D-(+)-galactose and D-(+)-glucose was used to identify and quantify individual monosaccharides.

The proportion of microbial sugars in the samples was determined from the ratios of C6:C5 sugars, i.e. (mannose + galactose)/(xylose + arabinose), and deoxyC6:C5 sugars, i.e. (rhamnose + fucose)/(xylose + arabinose), as described by Oades (1984) and Murayama (1984).

2.2.3.4. Amino sugars and muramic acid

Amino sugars and muramic acid were extracted as described by Zhang and Amelung (1996). Myo-inositol (100 µg) was added to a 1 g soil sample as an internal standard and the resulting mixture was hydrolysed by heating in 10 ml of 6 M HCl at 105 °C for 8 h. The sample was then filtered (GF6, Schleicher & Schuell, FRG) and evaporated to dryness (Multivapor P-12, Büchi, Essen, Germany). To remove salts, the dried samples were neutralized with 0.4 M KOH and centrifuged at 1750 g for 10 min. The supernatant was then freeze-dried, redissolved in 3 ml methanol and centrifuged again for 10 min at 1750 g. N-methylglucamine (100 µg) was added to the samples immediately prior to derivatisation and used as a standard to determine the percent recovery. Aldononitrile derivatives of the amino sugars were prepared according to Guerrant and Moss (1984) using 300 µl of the derivatisation reagent (32 mg ml^{-1} of hydroxylamine hydrochloride and 40 mg ml^{-1} of 4-dimethylaminopyridine in 4:1 pyridine-methanol). After heating for 30 min at 75 °C, 1 ml of acetic anhydride was added and the samples were heated for an additional 20 min. Excess derivatisation reagent was removed by adding 1.5 ml of dichloromethane to the cooled samples and washing the organic phase four times with 1ml of 1M HCl and 1ml of water each. The organic phase was then evaporated to dryness and redissolved in 300 µl ethyl acetate-hexane (1:1) prior to GC/MS analysis.

2.2.3.5. Gas chromatographic analysis of biomarkers and calculation of biomarker C and N contents

All of the biomarker derivatives discussed above were determined by gas chromatography–mass spectrometry (GC/MS) using a HP 6890 gas chromatograph coupled to a HP 5973 mass-selective detector (Hewlett Packard, Wilmington, USA). The

GC/MS was equipped with a DB-5ms column (60 m x 0.25 mm x 0.25 µm film thickness). The temperature programs were adapted individually for each compound class of interest.

Because the biomarkers considered in this work are groups of individual compounds, the terms PLFA-C, AA-C, AS-C and MS-C refer to the summed carbon of individual compounds within the relevant group (µg carbon per mol compound), expressed in mg kg^{-1}. AA-N and AS-N were calculated in the same way. Finally, the carbon and nitrogen contents across biomarkers were summed; these sums are denoted $\Sigma C_{biomarker}$ and $\Sigma N_{biomarker}$, respectively.

2.2.4. Statistical analysis

When the Static Fertilization Experiment Bad Lauchstädt was established in 1902, no replicate plots were included in the experimental design. It is therefore important to point out that our results are based on pseudoreplicates and that our ANOVA analyses may suffer from type I and type II errors. All statistical analyses were conducted using R version 2.15.1 (RCoreTeam 2012). Data were analysed for normality and, if necessary, transformed. Pseudoreplicates were tested for homogeneity of variances within the plots. For regression analyses, treatments were separated into a) plots without mineral fertilizer (NIL, FYM1, FYM2) and b) plots with added mineral fertilizer (NPK, FYM1+NPK, FYM2+NPK). Multiple comparisons of means were based on the conservative Tukey´s honest significant difference test (P<0.05) to reduce the risk of type I errors.

2.3. Results

2.3.1. Influence of crop type and fertilization on SOC, labile OC, and other abiotic parameters

SOC and TN levels were similar for both crops. Increasing the amount of fertilizers beyond that applied in the FYM1+NPK treatment did not cause appreciable increases in SOC values (Tab. 6). The SOC levels achieved under the FYM1+NPK, FYM2, and FYM2+NPK treatments were clearly separated from those observed in the control plots and in those treated with NPK alone or FYM alone. In general, the SOC and TN only responded significantly to treatment with greater quantities of FYM in cases without NPK fertilization: the SOC values for plots treated with both mineral and organic fertilizers were similar irrespective of the amount of applied FYM (Tab. 6). On average, the labile OC (HWC) content of the soil in plots planted with sugar beet was 12% lower than in plots planted with alfalfa (Tab. 6). In addition, it was only in the alfalfa strips that the labile OC increased with the amount of added FYM. The greatest differences between the crop types were found for NH_4^+-N and NO_3^--N: the levels of these forms of nitrogen in the soil were 38% and 52% lower, respectively, in the strip planted with sugar beet. Although NH_4^+-N and NO_3^--N both responded generally positively to fertilizer addition, the differences between fertilized treatments and the control were larger with alfalfa. In contrast, the soil pH in the sugar beet strip was generally higher than in the alfalfa strip and decreased with increasing fertilization; the opposite occurred in the alfalfa strip. The bulk density of the soil did not differ significantly between any of the investigated treatments under either crop, but did tend to decline as the intensity of fertilization increased (Tab. 6).

Table 6: Abiotic and physical soil properties under the studied treatments

Treatment	SOC	TN	HWC	HWN	NO_3^--N	NH_4^+-N	pH	bulk density
	%		mg kg^{-1}					g cm^{-1}
Alfalfa								
FYM2+NPK	2.60[a]	0.21[b]	751.44[a]	63.28[a]	6.94[a]	2.28[a]	6.38[bc]	1.46[ab]
FYM2	2.54[ab]	0.22[b]	677.81[ab]	58.40[ab]	6.43[a]	2.36[a]	6.04[cde]	1.47[b]
FYM1+NPK	2.49[ab]	0.21[bc]	647.11[b]	60.44[a]	5.29[ab]	2.00[ab]	5.62[ef]	1.46[b]
FYM1	2.15[c]	0.18[de]	540.84[cd]	47.82[cd]	4.46[abc]	1.77[abc]	5.92[de]	1.48[ab]
NPK	1.93[cd]	0.16[ef]	504.42[d]	40.18[de]	1.73[de]	1.38[bcd]	5.08[g]	1.53[ab]
NIL	1.61[d]	0.13[f]	399.64[e]	30.05[f]	0.44[e]	1.19[cd]	5.26[fg]	1.55[ab]
Sugar beet								
FYM2+NPK	2.48[ab]	0.24[a]	642.68[b]	63.88[a]	3.21[bcd]	1.24[cd]	6.54[b]	1.44[ab]
FYM2	2.31[b]	0.23[ab]	614.56[bcd]	59.51[ab]	2.44[cde]	1.01[d]	6.70[b]	1.53[ab]
FYM1+NPK	2.30[b]	0.23[ab]	591.13[bc]	55.49[abc]	2.23[cde]	1.60[bcd]	6.15[cd]	1.49[ab]
FYM1	1.96[c]	0.19[cd]	503.92[d]	51.51[bcd]	1.71[de]	0.89[d]	6.85[b]	1.58[ab]
NPK	1.85[cd]	0.17[de]	399.20[e]	37.35[ef]	1.55[de]	1.18[cd]	6.44[bc]	1.55[ab]
NIL	1.68[d]	0.14[f]	346.37[e]	43.31[cde]	0.93[de]	0.92[d]	7.33[a]	1.63[a]

SOC = soil organic carbon; TN = total nitrogen; HWC = hot water extractable carbon; HWN = hot water extractable nitrogen. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Values labeled with different superscripted letters differ significantly according to Tukey´s honestly significant difference tests (P<0.05). Multiple comparisons of means were conducted including the treatments on both strips.

2.3.2. Biomarker C and N contents and their contributions to SOC and TN

On average, $\Sigma C_{biomarker}$ and $\Sigma N_{biomarker}$ were only slightly higher under alfalfa than under sugar beet (Tab. 7). The greatest differences between the values of these sums in strips planted with sugar beet and those planted with alfalfa occurred under the FYM2+NPK, NPK, FYM1, and control treatments. While the soil's content of PLFA-C in strips planted with sugar beet was generally lower than in those planted with alfalfa, this biomarker exhibited similar responses to fertilization for both crops (Tab. 7). In a similar vein to SOC and TN, the PLFA-C values observed under the three most intense fertilization

regimes did not differ significantly for either crop. In contrast, MS-C in the alfalfa strip under the FYM2+NPK fertilization regime was substantially greater than that observed under less intense regimes. However, in all other cases the levels of this biomarker did not statistically vary based on fertilization regime or planted crop (Tab. 7). Like MS-C, the AA-C level in the most intensely fertilized alfalfa plot was much higher than in the other alfalfa treatments. In addition, the AA-C values for the alfalfa strips were around 37% higher than the corresponding values for the sugar beet strips. The AA-N levels planted with alfalfa were more than 100% greater than those planted with sugar beet. While the levels of AS-C planted with alfalfa were approximately 1.3 times lower than in those planted with sugar beet, the levels of AS-N were generally highest under the lightly fertilized (FYM1, NPK) or unfertilized treatments (NIL) for both crops (Tab. 7).

Table 7: C and N contents of individual biomarkers (mg kg^{-1} soil) under the studied treatments for both crop types

Treatment	PLFA-C	AA-C	MS-C	AS-C	ΣC$_{biomarker}$	AA-N	AS-N	ΣN$_{biomarker}$
	mg kg^{-1}							
Alfalfa								
FYM2+NPK	23.11^a	1386.66^a	2870.68^a	126.97^d	4423.00^a	474.59^a	23.14^c	497.72^a
FYM2	19.93^{ab}	800.18^{bc}	894.35^{bc}	146.05^c	1875.28^{bcde}	261.93^{bd}	25.89^c	287.82^{bc}
FYM1+NPK	19.03^{abc}	919.82^b	1093.29^b	117.52^d	2162.72^{bc}	322.78^{bc}	20.93^c	343.71^b
FYM1	15.90^{bc}	536.98^{cde}	966.82^{bc}	275.63^{bc}	1811.99^{cde}	159.83^e	49.26^{bc}	209.09^{cde}
NPK	14.65^{cd}	446.01^{def}	882.03^{bc}	532.79^a	1886.67^{bcde}	138.68^{cef}	100.01^b	238.69^{bcd}
NIL	10.29^{de}	605.45^{cd}	916.93^{bc}	131.51^d	1674.93^{def}	220.43^{de}	23.50^c	243.93^{bcd}
Sugar beet								
FYM2+NPK	15.27^{bc}	726.11^{bc}	1162.37^b	402.20^b	2313.65^b	233.12^{fg}	76.00^a	309.12^{bc}
FYM2	16.83^{bc}	609.02^{cd}	1139.65^b	384.42^b	2165.17^{bcd}	186.47^{fg}	72.37^a	258.85^{bcd}
FYM1+NPK	14.70^{cd}	662.97^{bcd}	1099.31^{bc}	253.91^b	2041.91^{bcde}	202.94^g	47.48^a	250.42^{bcd}
FYM1	6.18^e	403.14^{def}	967.91^{bc}	265.37^b	1651.02^{ef}	111.41^g	53.18^b	164.59^{de}
NPK	6.80^e	301.78^{ef}	935.36^{bc}	359.19^b	1610.82^{ef}	91.79^{fg}	68.44^b	160.23^{de}
NIL	6.16^e	207.00^f	737.02^c	343.72^b	1300.64^{cf}	58.43^{fg}	65.68^{bc}	124.11^e

PLFA-C = phospholipid fatty acid carbon (PLFA<20-C-atoms); AA-C = amino acid carbon; MS-C = monosaccharide carbon; AS-C = amino sugar carbon; ΣC$_{biomarker}$ = summed carbon contents of the studied biomarkers; AA-N = amino acid nitrogen; AS-N = amino sugar nitrogen; ΣN$_{biomarker}$ = summed nitrogen contents of amino acids and amino sugars. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Values labeled with different superscripted letters differ significantly according to Tukey´s honestly significant difference tests (P<0.05). Multiple comparisons of means were conducted including the treatments on both strips.

The total carbon content of the different biomarkers accounted for 7.4-17.2% of the SOC, and their total nitrogen content represented 8.9-23.9% of the TN (Fig. 4). Of the biomarkers, MS-C and AA-C made the greatest contribution to the SOC (5.7-16.5%), followed by AS-C (0.5-2.8%) and PLFA-C (<1%). The values of ΣC$_{biomarker}$ (Fig. 4A) and ΣN$_{biomarker}$ (Fig. 4C) were particularly high in the most intensely fertilized plot (FYM2+NPK) planted with alfalfa, due to the very high levels of C and N from monosaccharides and amino acids in its soil. With the exception of this plot, the

contribution of $\Sigma C_{biomarker}$ to the SOC decreased with increasing fertilizer addition in the alfalfa strip whereas the opposite was observed in the sugar beet strip (Fig. 4B). The contribution of $\Sigma N_{biomarker}$ to the TN did not differ significantly between fertilization regimes in the sugar beet strip (Fig. 4D). However, the contributions of amino sugars to the SOC and TN were generally highest under the control treatment and the treatments with FYM alone (FYM1) or NPK alone (NPK; Fig. 4).
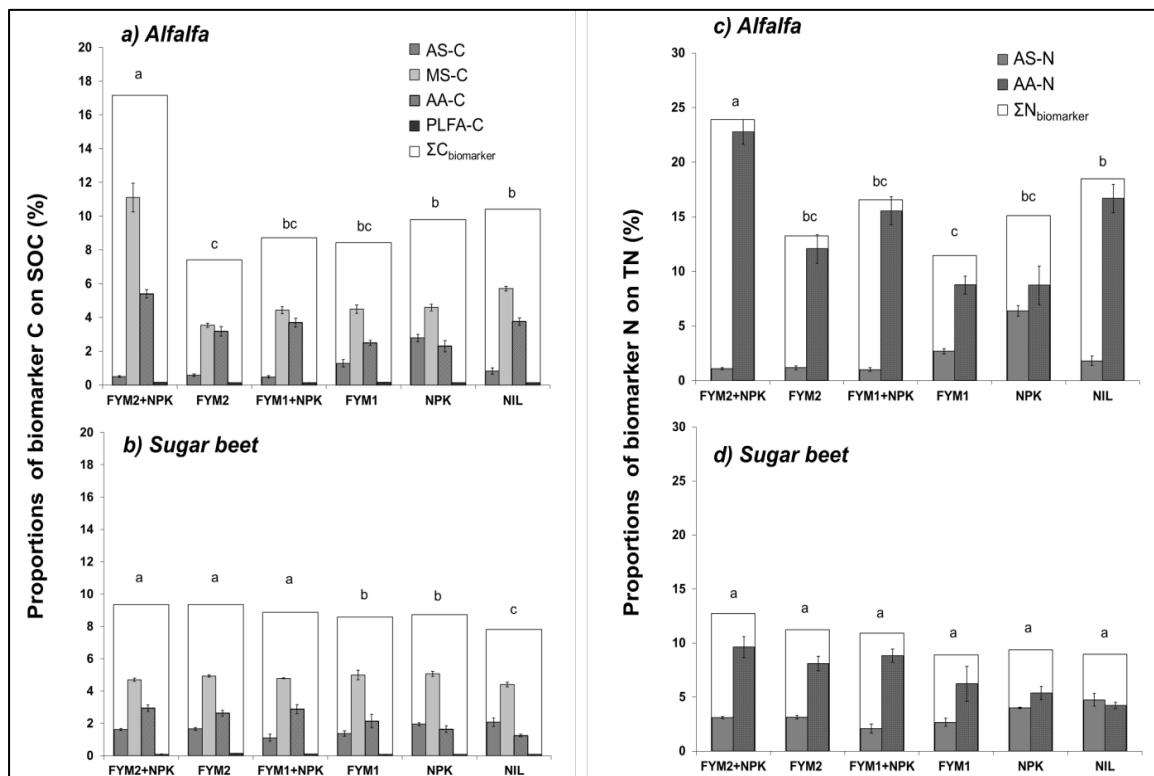
Figure 4: Contributions of individual biomarkers and combined biomarker groups ($\Sigma C_{biomarker}$ and $\Sigma N_{biomarker}$) to SOC and TN for each fertilization regime and crop type. a+c) alfalfa strip; b+d) sugar beet strip. Bars labelled with different letters correspond to values of $\Sigma C_{biomarker}$ and $\Sigma N_{biomarker}$ that differ significantly according to Tukey´s honestly significant difference test (P<0.05). Multiple comparisons of means were conducted separately for each strip.

2.3.3. Relationships among total and relative abundances of microbial biomarkers

PLFA-C is widely used as an index of microbial biomass C. The relationships between PLFA-C and the biomarkers AA-C, MS-C, and AS-C differed between crop types and fertilization regimes. AA-C exhibited the strongest positive correlation with PLFA-C in general ($p<0.001$). In plots planted with sugar beet, MS-C was always positively correlated with PLFA-C. However, in plots planted with alfalfa, this positive correlation was only observed under mineral fertilizer treatments. There was a negative correlation between AS-C and PLFA-C, but only in plots treated with mineral fertilizer (Tab. 8).

Table 8: Relationships between amino acid-, monosaccharide-, and amino sugar carbon and carbon from microbe-derived phospholipid fatty acids among treatments involving exclu-sively organic fertilization (NIL, FYM1, FYM2) and mineral fertilized treatments (NPK, FYM1+NPK, FYM2+NPK) for both crop types.

Biomarker-C	farmyard manure only			farmyard manure on mineral fertilized soil		
	PLFA-C			PLFA-C		
	t	P	R^2	t	P	R^2
Alfalfa						
AA-C	2.625	**<0.01**	0.35	4.615	**<0.001**	0.62
MS-C	0.012	NS	0.00	3.916	**<0.01**	0.54
AS-C	0.400	NS	0.01	-3.828	**<0.01**	0.53
Sugar beet						
AA-C	3.765	**<0.01**	0.52	5.930	**<0.001**	0.73
MS-C	3.777	**<0.01**	0.52	5.106	**<0.001**	0.67
AS-C	2.093	NS	0.25	-0.358	NS	0.00

PLFA-C = phospholipid fatty acid carbon (PLFA<20 C-atoms); AA-C = amino acid carbon; MS-C = monosaccharide carbon; AS-C = amino sugar carbon. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. R^2, multiple R-squared; t- and P-statistics base on regressions by a linear model values shown in bold text are statistically significant at the $\alpha = 0.05$ level.

The ratios of AA-C, MS-C, and AS-C to PLFA-C revealed differences in the abundance of different biomarkers relative to microbial biomass C among the investigated treatments and between both crop types (Figs. 5 & 6). Under sugar beet, the ratio of AA-C to PLFA-C was more or less independent of the applied treatment (Fig. 5b). Conversely, in the alfalfa strips, AA-C was enriched relative to PLFA-C under the control treatment and the most intense fertilization regime (FYM2+NPK; Fig. 5a). There were also differences between the two crops with respect to the monosaccharides. In the sugar beet strip, the highest MS-C to PLFA-C ratios were found under the control treatment and the less intense fertilization regimes NPK and FYM1 (Fig. 5d). Conversely, for alfalfa, the ratios of MS-C to PLFA-C were highest in the most intensely fertilized plot (FYM2+NPK) and the control plot (Fig. 5c). For both crop types, the lowest AS-C to PLFA-C ratios were observed under treatments with both mineral and organic fertilization (FYM1+NPK and FYM2+NPK) as well as the most intense FYM-only treatment (FYM2; Fig.6). Under the control treatment, the relative degree of AS-C enrichment was approximately 4 times greater for sugar beet versus alfalfa.
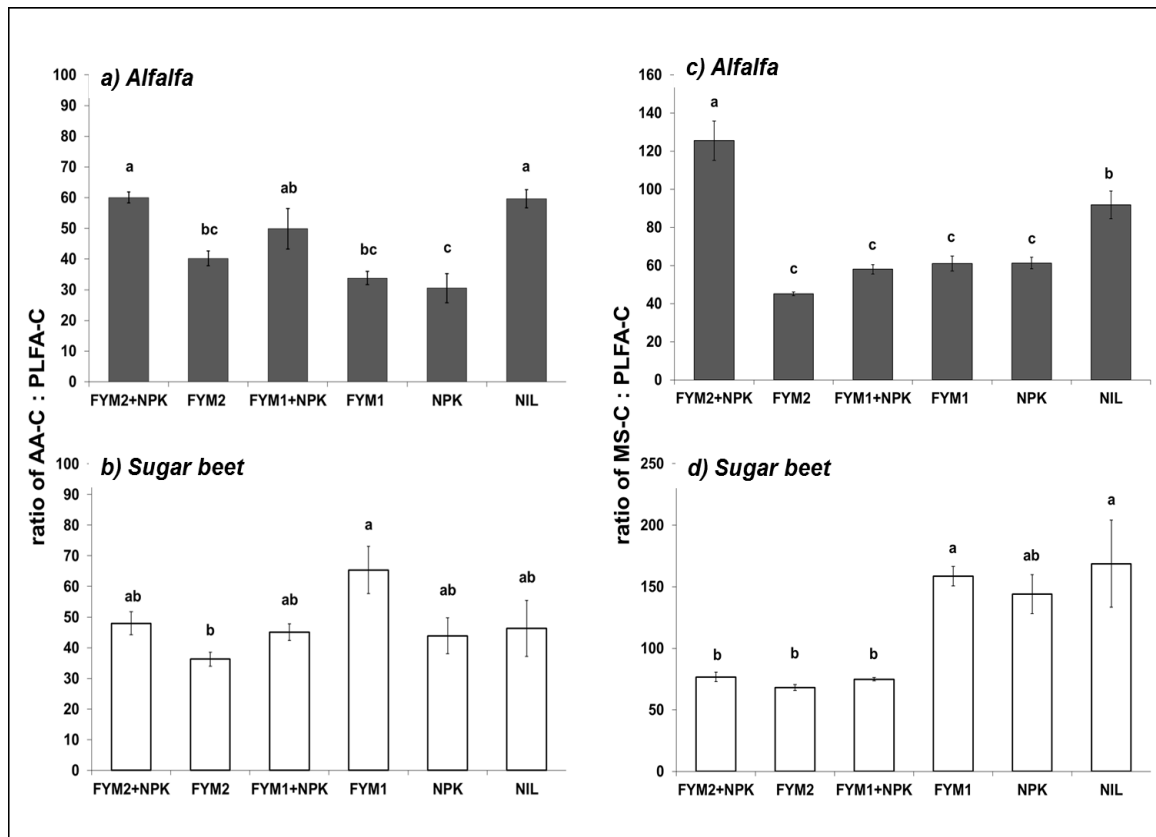
Figure 5: Ratios of amino acid (a+b) and monosaccharide carbon (c+d), respectively, to phospholipid fatty acid carbon for each studied treatments in both strips. a+c) alfalfa strip; b+d) sugar beet strip. Bars labelled with different letters correspond to values that differ significantly according to Tukey′s honestly significant difference test (P<0.05). Multiple comparisons of means were conducted separately for each strip.

The ratios of C6:C5 (mannose + galactose)/(xylose + arabinose) and deoxyC6:C5 (rhamnose + fucose)/(xylose + arabinose) monosaccharides revealed that microbial carbohydrates made a greater contribution to the total soil carbohydrate content under alfalfa compared to sugar beet, with the highest values occurring under the most intense fertilization regime (FYM2+NPK) and the control treatment (Tab. 9). In keeping with this, the total and relative abundances of meso-Diaminopimelic acid (DAP) (percentage of DAP in the AA fraction) were highest under the most intense fertilization regime (FYM2+NPK) and in the control plot on the alfalfa strip (Fig. 7).

42
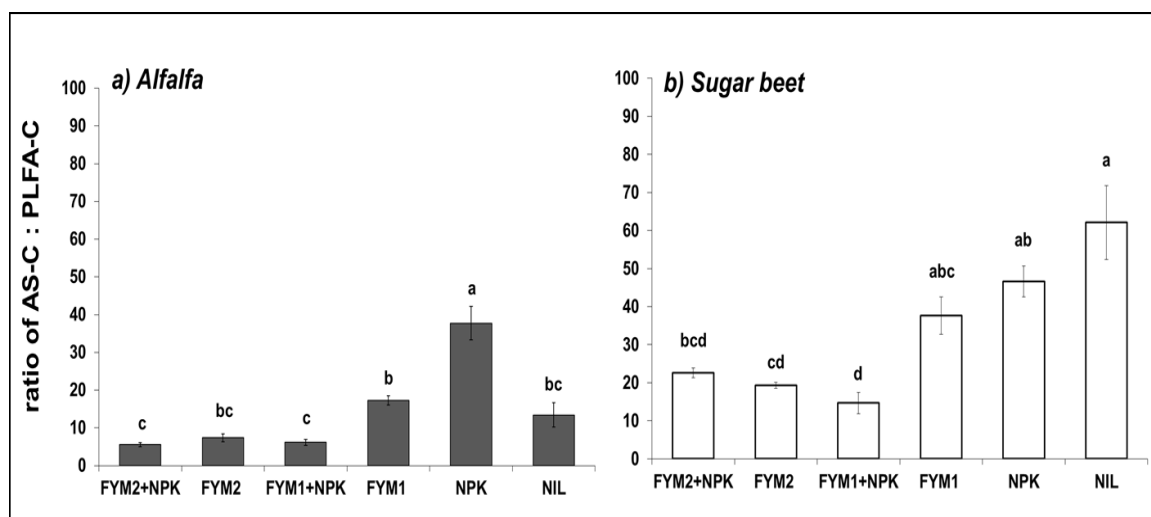
Figure 6: Ratios of amino sugar carbon to microbial carbon for each studied treatment in both strips. a) alfalfa strip; b) sugar beet strip. Bars labelled with different letters correspond to values that differ significantly according to Tukey´s honestly significant difference test (P<0.05). Multiple comparisons of means were conducted separately for each strip.

Table 9: Monosaccharide C6:C5 (mannose + galactose) / (xylose + arabinose) and deoxyC6:C5 (rhamnose + fucose)/(xylose + arabinose) ratios for the studied treatments.

Alfalfa	C6:C5	deoxyC6:C5
FYM2+NPK	1.26[abc]	0.38[a]
FYM2	1.35[ab]	0.39[a]
FYM1+NPK	1.16[bcde]	0.34[bc]
FYM1	1.19[bcd]	0.34[bc]
NPK	1.20[abcd]	0.31[c]
NIL	1.37[a]	0.36[ab]
Sugar beet	C6:C5	deoxyC6:C5
FYM2+NPK	1.03[d]	0.16[d]
FYM2	1.14[bcde]	0.17[d]
FYM1+NPK	1.13[cde]	0.17[d]
FYM1	1.02[e]	0.17[d]
NPK	1.11[cde]	0.17[d]
NIL	1.00[e]	0.17[d]

Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Values labeled with different superscripted letters differ significantly according to Tukey´s honestly significant difference tests (P<0.05). Multiple comparisons of means were conducted including the treatments on both strips.
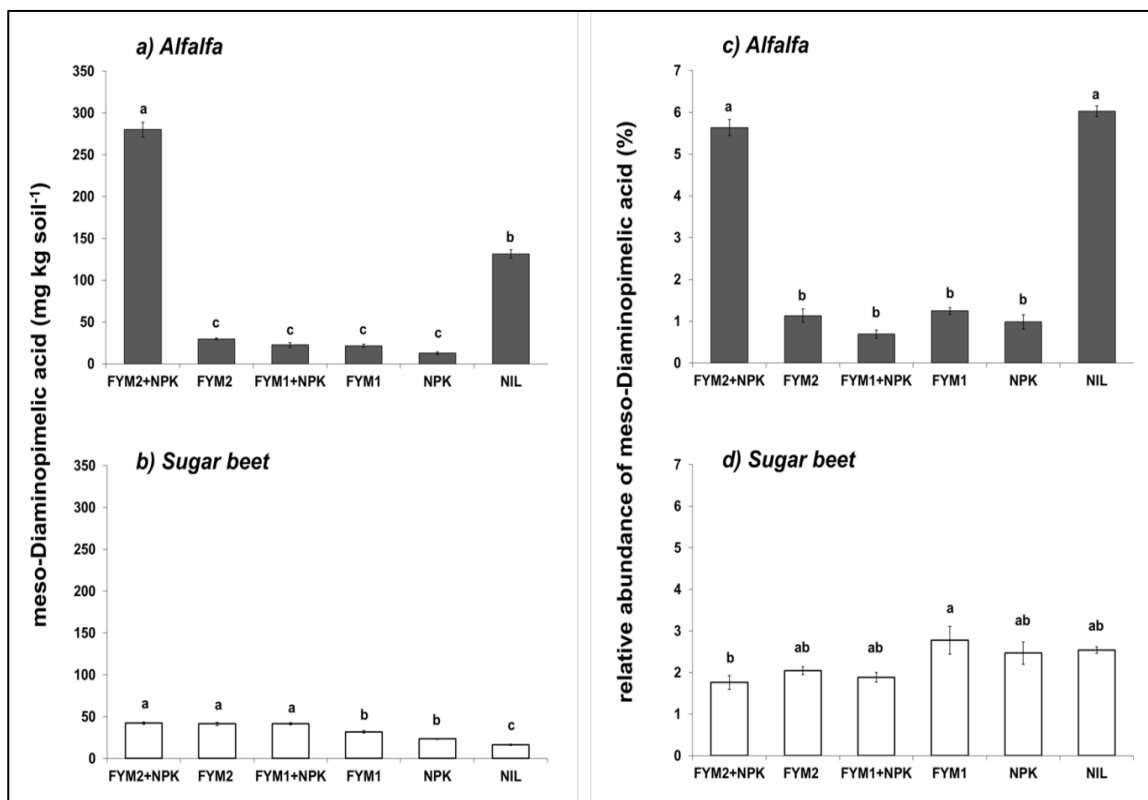
Figure 7: Total (mg kg soil^{-1}) and relative abundances of meso-Diaminopimelic acid (% of the sum of all determined amino acids) for each studied treatment in both strips. a+c) alfalfa strip; b+d) sugar beet strip. Bars labelled with different letters correspond to values that differ significantly according to Tukey´s honestly significant difference test (P<0.05). Multiple comparisons of means were conducted separately for each strip.

2.3.4. Relationship of biomarkers with SOC and labile OC

Labile OC was positively correlated with $\Sigma C_{biomarker}$ in all cases, but there was no general correlation between $\Sigma C_{biomarker}$ and total SOC (Tab. 10). Overall, PLFA-C exhibited a strong positive relationship with SOC and labile OC. Changes in the labile OC content of the soil explained between 46% and 95% of the observed variation in PLFA-C. It also appeared that the strength of these relationships was somewhat dependent on the crop type and applied fertilization regime. For example, in the sugar beet strip, the linearity of the relationship between PLFA-C and labile OC was strengthened by treatments with mineral fertilizer. However, no such trend was observed in the alfalfa strip (Tab. 10).

For both crops, there was a very strong correlation between AA-C and SOC based on regression analyses using data for plots treated with mineral fertilizer. This correlation disappeared for alfalfa under treatments using only farmyard manure. Conversely, for sugar beet, mineral fertilization strengthened the relationship between AA-C and SOC. The clearest differences between the two crop types were found for carbohydrates. For sugar beet, MS-C always exhibited strong positive correlations with both SOC and labile OC. However, for alfalfa, MS-C only correlated with labile OC and SOC in mineral-fertilized plots. In general, AS-C did not correlate with SOC or labile OC, with the exception of a negative relationship under the mineral fertilized treatments in the alfalfa strip (Tab. 10).

Table 10: Relationships of biomarker C with the SOC and labile OC (HWC) under treatments involving exclusively organic fertilization (NIL, FYM1, FYM2) and mineral fertilization (NPK, FYM1+NPK, FYM2+NPK) for both crop types.

	SOC			HWC		
Alfalfa	t	P	R^2	T	P	R^2
farmyard manure only						
PLFA-C	8.706	**<0.001**	0.86	6.538	**<0.001**	0.76
AA-C	2.035	NS	0.24	2.740	**<0.05**	0.37
MS-C	-0.320	NS	0.01	-0.308	NS.	0.01
AS-C	0.390	NS	0.01	0.499	NS.	0.02
ΣCbiomarker	1.848	NS	0.21	2.482	**<0.05**	0.32
farmyard manure on mineral fertilized soil						
PLFA-C	4.008	**<0.01**	0.55	5.801	**<0.001**	0.72
AA-C	6.472	**<0.001**	0.76	6.535	**<0.001**	0.77
MS-C	2.621	**<0.05**	0.34	3.531	**<0.01**	0.49
AS-C	-7.054	**<0.001**	0.79	-5.043	**<0.001**	0.66
ΣCbiomarker	2.878	**<0.05**	0.39	3.813	**<0.01**	0.53

	SOC			HWC		
Sugar beet	t	P	R^2	T	P	R^2
farmyard manure only						
PLFA-C	3.113	**<0.01**	0.43	3.328	**<0.01**	0.46
AA-C	3.095	**<0.01**	0.42	5.862	**<0.001**	0.73
MS-C	5.905	**<0.001**	0.73	13.054	**<0.001**	0.93
AS-C	0.452	NS	0.02	0.225	NS.	0.00
ΣCbiomarker	4.086	**<0.01**	0.56	6.994	**<0.001**	0.79
farmyard manure on mineral fertilized soil						
PLFA-C	9.681	**<0.001**	0.88	15.885	**<0.001**	0.95
AA-C	5.705	**<0.001**	0.71	5.428	**<0.001**	0.69
MS-C	7.142	**<0.001**	0.80	5.664	**<0.001**	0.71
AS-C	-0.011	NS	0.00	-0.157	NS	0.00
ΣCbiomarker	6.718	**<0.01**	0.78	5.725	**<0.001**	0.72

SOC = soil organic carbon; HWC = hot water extractable carbon; PLFA-C = phospholipid fatty acid carbon (<20 C-atoms); AA-C = amino acid carbon; MS-C = monosaccharide carbon; AS-C = amino sugar carbon; $\Sigma C_{biomarker}$ = summed carbon contents of the investigated biomarkers. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. R^2 = multiple R-squared; t- and P-statistics were estimated by regression using a linear model. Values shown in bold text are statistically significant at the α = 0.05 level.

2.4. Discussion

2.4.1. Responses of SOC and labile OC to fertilization

No differences in SOC for either crop were observed between the three most intense fertilization regimes (FYM1+NPK, FYM2, FYM2+NPK). It has been hypothesized that no or only little increase of SOC despite of increased C inputs, indicates C saturation of the given soil system (Hassink et al. 1997; Six et al. 2002). Indeed, there is already complementary evidence that C saturation may be occurring at the SFEBL in the most intensively fertilized plots (Eden et al., 2012). Similar phenomena have also been observed for SOC in various other long-term experiments (Campbell et al. 1991; Gulde et al. 2008; Solberg 1998).

In accordance with this logic, the labile OC pool at the sugar beet strip also appears C saturated, showing no further increase in HWC contents in the three most intensely fertilized treatments (FYM1+NPK, FYM2, FYM2+NPK). However with alfalfa, all increases in the intensity of fertilization led to corresponding increases of HWC. This is consistent with the findings of Stewart et al. (2008) and Gulde et al. (2008), which provided evidence that labile OC can continue to accumulate even when mineral-associated C fractions appear to be C-saturated in some cases. In the following sections we will discuss underlying mechanisms, considering possible influencing factors in relation to the crop type, such as differences in root exudation, root architecture, and increased nitrogen fertility based on the symbiotic relationship of alfalfa to N-fixing bacteria and mycorrhizal fungi.

2.4.2. Influence of fertilization and crop type on SOC and TN composition

The different contributions of biomarker C and N to SOC and TN across our study plots support our hypothesis that both fertilization level and crop type affect SOC composition.

Direct or indirect increases in C input based on higher plant productivity due to organic fertilization may have increased the contribution of $\sum C_{biomarker}$ to the SOC. Our results imply that mineral fertilization alone also had a noticeable effect on SOC in the alfalfa strip, together with changes in the SOC composition that presumably stemmed from changes in the nitrogen budget. Notably, the application of NPK alongside organic fertilizer seems to have caused a pronounced increase in the contribution of amino acids to TN. There is increasing evidence that mycorrhizal fungi assimilate and transfer soil organic N to their host plant to considerable degree (Leigh et al. 2009; Talbot and Treseder 2010; Whiteside et al. 2012a; Whiteside et al. 2012b; Whiteside et al. 2009). We have complementary evidence to believe that AMF dynamics play a role in our treatment responses, based on colonization rates of mycorrhiza (unpublished data). Thus, it is likely that treatment with mineral fertilizer would have reduced either the fungal acquisition of N or the degree of mycorrhization, in either case reducing uptake from the AA pool (Gryndler et al. 2006; Olsson et al. 1997; Whiteside et al. 2012b). Our results also imply though that the general microbial community may counterbalance the consumption of amino acids when organic N acquisition by AM-fungi is reduced, except in cases of extremely high fertilization, since the differences in $\sum N_{biomarker}$ and amino acid N percentages between the mineral and organic fertilized treatments were only significant under the most intense fertilization regime (FYM2+NPK).

2.4.3. The effects of fertilization and crop type on microbial biomass and OC turnover

The generally strong relationship between PLFA-C and SOC is consistent with past observations from long-term agricultural experiments and reflects a strong adaptation of the living microbial community to C input due to long-term fertilization in the SFEBL

(Smith et al. 1990; Witter et al. 1993). PLFA-C also correlated closely with labile OC, indicating that the size of the labile OC pool is related to a high degree by the growth and associated metabolism of soil microorganisms and their responses to changes in management and site conditions (Ghani et al. 2003; Hoffmann et al. 2006; Kalbitz et al. 2000; McDowell 2003). This result also confirms that microbial biomass represents a considerable part of the labile OC pool (Ghani et al. 2003; Hoffmann et al. 2006; Sparling et al. 1998).

The availability of mineral nutrients to microorganisms in agricultural soils depends on the type and amount of fertilizer amendment and the specific needs of the cultivated crop (Fageria et al. 2011; Marschner 2012). Because the strength of the relationship between PLFA-C and labile OC differed between crop types and between mineral and non-mineral fertilized soils, microbial biomass was likely affected by both crop- and fertilization-related variations in the availability of mineral nutrients. In this work, PLFA-C levels indicated that treatment with NPK caused an increase in microbial biomass. However, the only significant difference was found on the sugar beet strip, when mineral fertilizer was added to 20 t FYM ha^{-1} 2yrs^{-1}. Thus, in keeping with previous studies, there was no clear and consistent evidence that the addition of mineral N or full mineral fertilizer increased the microbial biomass (Allison et al. 2008; Bardgett et al. 1999; Moore et al. 2000; Smolander et al. 1994; Treseder 2008).

However, the standing microbial biomass does not necessarily reflect growth rates. Growth rates may increase even with no apparent change in total microbial biomass if death rates remain similar to growth rates (Stapleton et al. 2005). Analyses of the other biomarkers examined in this work did indeed suggest that the addition of NPK enhanced microbial growth in the presence of high C inputs due to fertilization. With alfalfa as the planted crop and within the crop rotation, treatment with 30 t FYM ha^{-1} 2yrs^{-1} + NPK

(FYM2+NPK) caused strong increases in the C6:C5 and deoxyC6:C5 ratios and in DAP. This suggests a strong increase in the contribution of bacteria-derived monosaccharides and amino acids to the SOC (Grant and West 1986; Murayama 1984; Oades 1984), implying microbial synthesis of these compounds through growth.

In addition to evidence of increased microbial growth under the most intensive fertilization regime with alfalfa, we observed increases in the levels of soil MS-C and AA-C contents not only in total, but also relative to PLFA-C. This implies that MS-C and AA-C were increasing beyond what can be explained by microbial growth alone. Taking together all individual indications we hypothesized that under intense fertilization there was initially a rapid increase in microbial growth and respiration that was then suppressed when soil pore space became enriched in carbon dioxide and oxygen became limiting (Dixon and Kell 1989a; Gök and Ottow 1988).

In the absence of any fertilization, we found evidence that nitrogen promotes the degradation and incorporation of labile carbon into microbial biomass. The AA-N levels under the control treatment with alfalfa were approximately four times higher than those with sugar beet, suggesting that the legume improved the availability of organic N due to symbiotic N-fixation. This enhanced N availability in the alfalfa strip might have promoted the transformation of plant into microbial organic carbon to a greater degree than would have been the case without alfalfa. This would explain the results of monosaccharide C6:C5 and deoxyC6:C5 ratios, and DAP contents, which were clearly higher in the alfalfa control plot than in the sugar beet strip under equivalent conditions. The microbial contributions to MS and AA were also higher under the control treatment of alfalfa than under any fertilization regime other than the most intense (i.e. FYM2+NPK). Despite the low productivity of control plots (Körschens and Pfefferkorn 1998), alfalfa has a root architecture that promotes labile carbon and microbial activity in

the rhizosphere. Therefore, the predominance of microbial OC compounds under the control treatment may be due to a greater proportion of microbe- versus plant-derived carbon, and/or to preferential consumption of plant-derived carbon by microorganisms.

As discussed previously, the ratios of MS-C and AA-C to PLFA-C were enriched in control plots relative to most treated plots, although PLFA-C, and thus microbial biomass, was lowest in controls. However, there was a non-significant tendency for control plots to have higher soil bulk densities than the fertilized plots. Eden et al. (2012) also found that the control plots of the SFEBL had smaller pore volumes, lower air-filled porosities and lower levels of relative diffusivity at field capacity than their fertilized counterparts, which were attributed to the low levels of organic matter input. The control plots were thus more likely to have experienced critical pO_2 or pCO_2 levels, which inhibit aerobic catabolism (Bronick and Lal 2005; Haynes and Naidu 1998).

2.4.4. The relationships of AA, MS, and AS with microbial biomass, SOC and labile OC

Because microbial amino acid biosynthesis is tightly regulated (Wendisch 2007), soil amino acid levels are tightly coupled to microbial growth and activity. This was reflected in the general and significant correlation between AA-C and PLFA-C observed in this work and in the studies of Friedel and Scheller (2002), who reported a close relationship between amino acid contents from bulk soil and microbial amino acid-N levels from chloroform fumigation extracts across soil types. As discussed above, deviations from this normally strong correlation are indicative of changes in microbial physiology and catabolism under no or extreme fertilization. It was also striking, that the correlations between AA-C and PLFA-C and between AA-C and SOC were stronger on plots treated with mineral fertilizer than on those without, regardless of crop type. There are two

possible explanations for this trend. The first is that mineral fertilization may affect the interactions between AA and clay minerals. Clays can retain significant amounts of ammonium (Dontsova et al. 2005; Shen et al. 1997). It is therefore possible that mineral fertilization causes the occupation of clay binding sites by inorganic cations, which could increase the concentration of organic molecules in solution and better reflect microbial growth. Thus, we would see a stronger correlation between PLFA-C (always extracted with a high-ionic strength buffer for the same result), and AA-C under mineral fertilization. Alternatively, decreased enzyme production under mineral fertilization, with less AA-C present as degradation products, may explain this result. The suppression of soil enzyme production and activity following N and P addition has been observed in several studies (Bandick and Dick 1999; Dick et al. 1988; Marschner et al. 2003; Olander and Vitousek 2000).

Reducing the scope for interaction with the clay surface would also affect the stabilization of poly- and monosaccharides. In keeping with this observation, the correlations of MS-C with PLFA-C and SOC were also stronger in soils treated with mineral fertilizer. The correlations of MS-C with PLFA-C and SOC on the alfalfa strip were generally weaker than in the sugar beet strip, and became non-significant in the absence of mineral fertilizer. This may have been due to a greater contribution of non-biomass related MS-C: monosaccharides account for ten times less of the dry mass of bacteria than amino acids (Neidhardt 1990). This decrease in the relationship between MS-C and PLFA-C would be further strengthened by any increase in the mineralization of carbohydrates or any reduction in their utilization as C-sources with increasing fertilizer concentrations (Fischer et al. 2010).

Of the tested biomarkers, amino sugars had the least significant relationship to PLFA-C. This supports the assumption that AS mainly represent accumulated microbial necromass

(Amelung et al. 2001a; Glaser et al. 2004; Zhang et al. 1999; Zhang et al. 1998). With the exception of the negative correlation between AS-C and PLFA-C in the alfalfa strip under mineral fertilization, AS-C appeared to be independent of both SOC and labile OC. This stands in contrast to the findings of Liang et al. (2008); the apparent contradiction between their results and ours may be because there were different amounts of clay in the soils at the two sites. In addition, AS-C has been reported to account for only 0.1-7% of microbial biomass C (Glaser et al. 2004), which is approximately one-tenth of the amount contributed by MS. From this we conclude that the amino sugars extracted in this work primarily reflect the stabilized microbial contribution to the OC, and that the acid hydrolysis step during the extraction procedures caused the release of some mineral-associated (i.e. stabilized) AS in the same way as for AA and MS. This implies that non-protected AS that are not associated with biomass are rapidly degraded in the soil. The proposed rapid degradation of non-protected AS is consistent with the findings of Liang et al. (2007b), who reported a decrease in amino sugar contents after an initial increase during a 12 week microcosm experiment involving maize stalks and soybean leafs. However, it is perplexing that a fertilization regime that causes soil C-saturation (i.e. FYM2+NPK) yielded a strong increase in the levels of MS-C and AA-C but had no effect on AS-C in the alfalfa strip. This result becomes even more confusing when one considers the high abundance of DAP, which is combined with N-acetylglucosamine and N-acetylmuramic acid to form the peptidoglycan layer of bacterial cell walls. Since it seems unlikely that non-protected amino sugars would have appreciably shorter half-lives in the soil than either amino acids or neutral sugars, this difference may indicate that amino sugars are rapidly transformed, possibly by enzymatic deamination. However, it is not currently clear how or to what extent this mechanism contributes to the degradation of amino sugars in soils.

2.5. Conclusions

We attempted to understand the result of both microbial metabolic and growth dynamics under long-term management by linking data from simultaneous measurements of four biomarkers with a large set of soil chemical and physical parameters. Under consideration of all findings we concluded that including alfalfa into crop rotations can have a great impact on carbon storage and microbial metabolism, despite underlying long-term fertilization regimes. Especially in the case of fertilization beyond plant nutrient needs (indicated from long-term assessments of SFEBL crop yields, nutrient contents, and leaching), e.g. C saturation, the special characteristics of legumes might enable a greater retention of soil labile C. Due to the lacking statistical power further investigation is needed to substantiate our assumptions and, on the other hand to answer the questions which emerged from this study.

3. Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem

Abstract

We examined the impact of arbuscular mycorrhizal fungi and rhizobia on the living microbial community and microbial necromass under different long-term fertilization treatments at the long-term Static Fertilization Experiment Bad Lauchstädt (Germany). Phospholipid fatty acids (PLFA) and amino sugars plus muramic acid, were used as biomarkers for soil microbial bio- and necromass, respectively, and analysed from six treatments imposed on two crop rotations, varying only in the inclusion/non-inclusion of a legume. Treatments included: two levels of only farmyard manure (FYM), only mineral fertilizer (NPK), the combined application of both fertilizer types and a non-fertilized control. PLFA profiles differed clearly between the investigated crop rotations and were significantly related to labile C, mineral N, and soil pH. This emphasizes the role of carbon, and of mycorrhizal and rhizobial symbioses, as driver for changes in the microbial community composition due to effects on the living conditions in soil. We found some evidence that legume associated symbiosis with arbuscular mycorrhizal fungi and rhizobia act as a buffer, reducing the impact of varying inputs of mineral nutrients on the decomposer community. While our results support former findings that living microbial populations vary within short-term periods and are reflective of a given crop grown in a given year, soil necromass composition indicates longer term changes across

the two crop rotation types, mainly shaped by fertilizer related effects on the community composition and C turnover. However, there was some evidence that specifically the presence of a legume, affects the soil necromass composition not only over the whole crop rotation but even in the short-term.

3.1. Introduction

Proper management of agricultural soils can contribute to the mitigation of atmospheric CO_2 increases both by reducing respiratory C losses from soil and by sequestering photosynthetically fixed CO_2 (Smith et al. 2014). These processes are mainly driven by soil microorganisms, which play a central role for the functioning of terrestrial ecosystems, encompassing much more than simply decomposition of organic matter or cycling of mineral nutrients (Nannipieri and Badalucco 2003). Almost all organic material which enters the soil passes through the pool of living microbes, where it gets degraded, transformed, or incorporated into fungal or bacterial cells (Paterson et al. 2009). It is estimated that up to 80% of soil organic carbon (SOC) may be derived from microbial cellular components, highlighting the significance of the soil microbial community as source of soil organic matter (SOM) genesis and long-term C sequestration (Liang et al. 2011). It is not fully understood, however, how specific management practices affect the sequestration, or in turn, the mineralization of microbial residues in soils. Some of these open questions might be answered by a direct comparison between the living microbial biomass and long-term sequestered necromass.

Since most soil microorganisms are not cultivable, culture independent methods relying on biochemical indicators, such as lipids or amino sugars, are frequently used to analyse

microbial communities (Frostegård and Bååth 1996; Liang and Balser 2012). Phospholipid derived fatty acids (PLFA) are structural components of all cellular membranes, constantly synthesized during microbial growth, and experiencing a rapid turnover in the soil (White et al. 1979; Zelles 1999). Thus, PLFA's can be applied as an indicator for the living microbial biomass and for the current structure of the microbial community. If neutral lipid fatty acids (NLFA) are also considered, it is possible to have additional information about the physiological state of fungi, since NLFA mainly derive from triacylglycerols, which are storage products of eukaryotic cells (Bååth 2003).

In contrast to PLFAs, amino sugars (AS) and muramic acid (MurA) are largely stabilized in the soil and are assumed to represent mainly microbial necromass (Glaser et al. 2004). While MurA occurs exclusively in the peptidoglycane layer of bacterial cell walls, glucosamine (GluN) is the basic component of fungal chitin and is present to a lesser extent in bacterial cell walls. Only negligible quantities of GluN in soil have been assigned to sources not related to fungal or bacterial biomass, such as skeletons of arthropods, earthworm gut lining, nematode egg shells, mollusk polysaccharides, or snail gelatine (Amelung 2001; Chantigny et al. 1997). Galactosamine (GalN) is a frequent component of bacterial capsular- or exopolysaccharides but also found in significant amounts in fungi (Glaser et al. 2004). Little is known about the biological function of mannosamine (ManN), which also seems to be derived mainly from bacteria (Kenne and Lindburg 1983; Rüde and Goebel 1962). In the past, the ratios of GluN:MurA or GluN:GalN have been used to estimate the contribution of fungal and bacterial residues into the SOM pool (Said-Pullicino et al. 2007; Solomon et al. 2001).

Previous studies examining the role of microorganisms in SOM dynamics have either focused only on structural and functional changes of the living microbial community or on shifts in the bacterial and fungal contributions to SOM. Based on the need of

understanding SOM dynamics for sustainable food production, a large proportion of this research has been performed in agricultural soils. The type and application rate of fertilizers as well as the plant species and plant-microbe interactions have all been found to affect the biomass, activity and structure of the microbial community (Fließbach et al. 2007; Lambers et al. 2009; Ngosong et al. 2010).

Compared to the great number of PLFA-based studies, the accumulation and turnover of microbial residues in agricultural soils has been researched less intensively. Long-term cropping has been found to reduce total AS contents, which was explained by microbial substrate limitation due to cultivation (Zhang et al. 1998). In this context, a preferential degradation of bacterial derived AS was assumed, resulting in a higher fungal contribution (Zhang et al. 1999). Amelung et al. (2001a) even demonstrated that the amount of C and N sequestered in the microbial necromass can be manipulated by changing the input of labile C and N into soil. Finally, evidence on crop- and crop rotation related effects on amounts of total and individual amino sugars in soil was reported recently by Zhang et al. (2014).

To our knowledge, there are only a handful of studies in which the living (PLFA) and non-living (AS) biomass has been measured simultaneously in relation to each other (Appuhn and Joergensen 2006; Liang et al. 2008; Liang et al. 2015; Zhang et al. 2013), while the impact of fertilization, crop, or symbiotic relationships of crops with rhizobia or AMF in agricultural soils has not been considered. Such work, however, would improve our knowledge about the production and stabilization of amino sugars and thus could have the potential to increase our understanding of the microbial mediation of long-term dynamics of soil organic C and N.

From the background of very long-term, likely steady state conditions at the Static Fertilization Experiment Bad Lauchstädt (SFEBL) we compared the abundance and

composition of the living microbial community with the amount of microbial residues by determining both PLFA and AS. In addition to fertilization treatments we considered plant-microbe interactions as influencing factors by sampling two crop rotations that vary only in the inclusion or exclusion of alfalfa (*Medicago sativa)* every 7th and 8th year having a dual symbiosis (AMF and rhizobia) which has a huge impact on the rhizosphere in terms of N and C exudates. We examined changes in crops and cropping history from two perspectives using these different tools: first we examined the effects of long-term fertilization treatments on the living, active microbial community associated with the currently grown crop (alfalfa or sugar beet) using lipid analysis, AMF colonization, and rhizobia nodulation rates. Second, we examined the effects of fertilization and cropping history using the amino sugar microbial necromass pool, assumed to change more slowly over time and reflect longer term patters associated with agricultural management.

3.2. Materials and methods

3.2.1. Study site description and sampling

This study took place at the long-term Static Fertilization Experiment Bad Lauchstädt (SFEBL), Sachsen-Anhalt (Germany), which was initiated in 1902 (Körschens and Pfefferkorn 1998). The soil is a Haplic Chernozem (FAO) (USDA: Mollisol) consisting of 21% clay, 67.8% silt and 11.2% sand. The mean annual temperature and precipitation are 8.8°C and 480 mm, respectively (Körschens 2002). The Static Fertilization Experiment Bad Lauchstädt was laid out in a split plot design with the main-plot factor FYM (three levels: no FYM, 20t FYM ha^{-1} 2yrs^{-1}, 30t FYM ha^{-1} 2yrs^{-1}) and mineral fertilization as a sub-plot factor (6 levels: no, PK, N, NK, NP, NPK) which are all realised in each main plot. The experiment is further stratified in 5 different cropping strips. Four

of the cropping strips represent different phases (a different crop on each strip, each year) of a 4 crop rotation (sugar beet, spring barley, potatoes, and winter wheat). In addition, legumes have been included in the crop rotation exclusively on the 5th strip of the experiment since 1924, replacing sugar beet and spring barley every 7th and 8th year.

Since 1970 alfalfa (*Medicago sativa*) has been the only legume species cultivated on this strip. Mineral fertilization (NPK) varies annually in adaption to the nutrient demand of each crop (60 to 170 kg ha^{-1} yr^{-1} N, 12 to 60 kg ha^{-1} yr^{-1} P, 50 to 230 kg ha^{-1} yr^{-1} K). Application of farmyard manure takes place every second year with the cultivation of root crops (potatoes, sugar beet). Alfalfa follows winter wheat in the rotation, receives farmyard manure before seed drilling, and only receives PK in the mineral fertilized treatments.

Soil sampling was timed to correspond with plant maturity, which occurred in June under alfalfa on the legume strip (sampled in 2010) and in October under sugar beet (*Beta vulgaris*) on the non-legume crop rotation (sampled in 2011). Samples were taken from 6 plots of each rotation type, comprising five fertilization treatments with increasing fertilizer addition as well as an unfertilized control (NIL). In detail the fertilized treatments were: mineral fertilizer (NPK), 20t farmyard manure ha^{-1} 2yrs^{-1} (FYM1), 20t farmyard manure ha^{-1} 2yrs^{-1} plus mineral fertilizer (FYM1+NPK), 30t farmyard manure ha^{-1} 2yrs^{-1} (FYM2) and 30t farmyard manure ha^{-1} 2yrs^{-1} plus mineral fertilizer (FYM2+NPK). Since the main-plot factor and the main-plot/sub-plot combinations were not replicated, soil was taken directly next to five replicate plants in the plough horizon (0-20 cm) in each plot and treated as replicates in further analysis. In the crop rotation including legumes, roots from the same replicate alfalfa plants were removed gently from the soil, washed and used for the determination of rhizobial nodule numbers and arbuscular mycorrhizal colonization rates. Soil samples were sieved to < 2mm and stones

60

and visible plant residues were removed. Portions of the fresh and sieved soil were frozen at -20°C immediately after sampling.

3.2.2. Soil parameters

3.2.2.1. SOC, TN and labile OC and N

Total carbon (TC) and total nitrogen (TN) were determined via combustion in a C/H/N combustion analyzer (Vario El III, Elementar-Hanau). No inorganic C was detected; so TC represents the total soil organic carbon (SOC).

Cold-water extraction was performed to assess the pool of labile and highly available organic carbon, representing the nutritional conditions at the time of sampling (Zsolnay 1996). A volume of 30 ml of a 0.01 M $CaCl_2$ - solution was added to 15 g air dried soil, and samples were shaken for 10 min and centrifuged at 4000 rpm for 15 min. Water extracts were filtered (0.45 μm Minisart single-use syringe membrane filters RC 25, PP-housing, Sartorius AG, Göttingen, Germany) prior to the determination of labile C and N concentrations (mg kg^{-1}) (CWC, CWN), using an elemental analyzer for liquid samples (Micro N/C and Multi N/C, Analytik Jena, Germany).

Fresh soil was used for determination of pH, ammonium-N (NH_4^+-N) and nitrate-N (NO_3-N). NH_4^+-N and NO_3-N were extracted with 1 M KCl (1:4 w/v, soil: KCl) from 10 g soil by shaking for 1.5 h. After sample filtration (Whatman Schleicher & Schuell 595 1/5 Ø 270 mm) the concentrations of NH_4^+-N and NO_3^--N were measured using a flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany).

Soil pH was measured from a slurry of 25 ml of 0.01 M $CaCl_2$ and 10 g air-dried soil.

3.2.3. Analysis of microbial biochemical indicators

A description of the extraction and quantification of the phospho- and neutral-lipid fatty acids (PLFA and NLFA) and of amino sugars and muramic acid is given in Schmidt et al. (2015). External standards were included in the GC/MS analysis to allow identification of the individual fatty acids and amino sugars plus muramic acid, respectively, based on their retention times.

From the obtained PLFA data we created three different data sets, to elucidate effects of crop and fertilization on the composition of the microbial community as well as the size and composition of the microbial biomass, including physiological aspects:

1.) Total PLFA profile used for NMDS: A data frame consisting of the relative abundances (mol %) of all detected PLFA′s up to a chain length of 20 C-atoms, representing the composition of the total microbial biomass. Beside the key-indicator PLFA′s (see below) this data frame contains also non-specific PLFA′s, which are often excluded from microbial community structure analysis, either because they are widely distributed or, in contrast, rarely found. The total PLFA profile was assumed to include information about both community changes and variations in the PLFA composition triggered by microbial physiological responses to environmental factors (Russell and Fukunaga 1990; Wixon and Balser 2013).

2.) Key-indicator PLFA profile used in SIMPER analysis: Contains the relative abundances (mol%) of only those PLFA′s indicating specific microbial groups, as follows: iso and anteiso-branched (e.g. i13:0, a13:0) = Gram-positive bacteria; methyl branched (e.g. 16:0 10me, 16:0 11me, 17:0me, 18:1me, 18:0 12me) = actinomycetes; hydroxy (e.g. 10:0 2OH, 14:0 3OH, 16:0 2OH, 18:1 2OH) and monounsaturated (14:1, 16:1ω9c, 16:1ω7c, 16:1ω7t, 17:1 11c or 9c, 17:1 7c or 8c, 18:1ω12c, 18:1ω9t, 18:1ω7c, 18:1ω5c, 19:1) = Gram-negative bacteria; 16:1ω5c = arbuscular mycorrhizal fungi

(AMF); 18:1ω9c, 18:2ω6,9c = general fungi (Balser 2005; Federle 1986; Frostegård et al. 1993; Frostegård et al. 1991; Stahl and Klug 1996; Vestal and White 1989; Wilkinson 1988; Zelles et al. 1992).

3.) The biomass (nmol lipid g dry soil^{-1}) of broad groups within the microbial community, used for ANOVA analysis was indicated by considering the content of one representative member of a respective group. This decision is based on the circumstance that for AMF there is in general only one key-indicator PLFA available. The selected key PLFA´s representing the biomass of a specific microbial group were: 16:1ω5c for AMF (Balser 2005); 18:2ω6,9c for general fungi (GF) excluding AMF (Balser 2005; Frostegård et al. 2011); 16:1ω7c for Gram-negative bacteria (Wilkinson et al. 2002); and i15:0 for Gram-positive bacteria (Wilkinson et al. 2002).

Additionally, the neutral lipid fatty acids 16:1ω5c (AMF) and 18:2ω6,9c (GF) were used as an indicator of storage lipids (nmol lipid g dry soil^{-1}) from arbuscular mycorrhizal and general fungi, respectively. The physiological state of fungal communities was then determined using the ratios of NLFA/PLFA (Bååth 2003). The fungal to bacterial ratio was calculated by dividing the concentrations (nmol lipid g dry soil^{-1}) of phospholipids indicative of general and AM fungi by the concentrations of phospholipid indicators for actinomycetes, Gram-positive and Gram-negative bacteria (Frostegård and Bååth 1996). Similar to this, the ratio of Gram-negative bacteria to Gram-positive (Gm$^-$/Gm$^+$) was calculated based on the biomass of both groups (see data set 3).

The relative contribution of fungal to bacterial residues was estimated by ratios of GluN/MurA, (Zhang and Amelung 1996), GalN/MurA, and ManN/MurA (Glaser et al. 2004).

3.2.4. Root nodules and estimation of arbuscular mycorrhizal colonization rates

Root samples of alfalfa obtained as described above were used to estimate mycorrhizal and rhizobial colonization rates. All samples were processed separately, with five resultant replicates (one each from five samples plants) per plot. The numbers of root nodules were counted under a stereomicroscope. Numbers were counted for two root size classes (class 1: < 1 mm; class 2: >1 mm), and for the purpose of our analysis both size classes have been added together. For estimation of arbuscular mycorrhizal root colonization percentage, roots were stained according to (Vierheilig et al. 1998) using a stain from Sheaffer (Sheaffer, Ft. Madison, IA, USA). Approximate colonization percentage was determined under a stereomicroscope.

3.2.5. Statistical analysis

We are aware that the sampling design of the Static Fertilization Experiment Bad Lauchstädt (1902) as described before (see study site description in chapter 2.1) can be interpreted as pseudoreplication in the sense of Hurlbert (1984). It should be noted that historical field experiments almost never fulfil the assumptions of statistical models. The same is the case for studies on disturbance events, landscape studies and large-scale manipulations. Following Davies and Gray (2015), adopting "a militant stance to pseudoreplication" necessarily leads to lost opportunities to learn from such studies. In the case of long-term experiments like ours, considerable treatment differences as a result of long-term trends over decades represent the inherent value of the studies. We therefore argue for a pragmatic solution of this problem and an adequate discussion of the results. Conservative statistics using both univariate and multivariate methods were performed to partially

compensate for the pseudoreplicated nature of our experiment. All statistical analysis was carried out in R version 2.15.1 (RCoreTeam 2012). Farmyard manure, mineral fertilizer, and their interactions were included as fixed effects in two-way analysis of variance (ANOVA) of our microbial response variables, i.e. the lipid biomass of specific microbial groups (nmol lipid g dry soil^{-1}), and the ratio of fungal to bacterial and Gram-negative to Gram-positive biomass. For regression analyses of the bacterial PLFA biomass and the contents of muramic acid, treatments were separated into a) plots without mineral fertilizer (NIL, FYM1, FYM2) and b) plots with added mineral fertilizer (NPK, FYM1+NPK, FYM2 +NPK). Multiple comparison of means was based on the conservative Tukey´s honest significant difference test ($p < 0.05$) to reduce the risk of type I errors.

Nonmetric Multidimensional Scaling (NMDS) was used to determine the variation in total PLFA profiles among the treatments and crop rotation type (McCune et al. 2002). Soil physical and chemical parameters were included in the model as environmental variables. Distance matrices for analysis were constructed based on binomial distance as evaluated by the function rankindex (vegan package, (Oksanen 2011). Finally, NMDS was performed using metaMDS (vegan package). Thirty independent runs were executed on raw data to ensure that global solutions, and not local minima, were obtained. Environmental factors were fitted onto the ordination with the function envfit, based on 1000 random permutations (vegan package). To complement NMDS, the Permanova permutational analysis of variance was performed on total PLFA profiles using the adonis function. Finally, the similarity percentages procedure (SIMPER) was applied on the key-indicator PLFA profile in order to identify those individual PLFA's which contributed most to the average dissimilarity between the groups (Clarke 1993).

3.3. Results

3.3.1. Composition of the microbial biomass and community structure

Nonmetric multidimensional scaling demonstrated that total PLFA profiles were clearly clustered according to the current crop at sampling time (Fig. 8). In addition, while total PLFA profiles between fertilization treatments were fairly similar under alfalfa except the NIL treatment, under sugar beet there were distinct separations between plots with high (FYM2+NPK, FYM2, FYM1+NPK) and low or no fertilization (FYM1, NPK, NIL) (Fig. 8).

Figure 8: NMDS ordination of total phospholipid profiles based on relative abundances of all determined PLFAs from the investigated treatments on both rotation types. Fitted environmental variables: pH; CWC = cold water extractable carbon (mg kg^{-1}); CWN = cold water extractable nitrogen (mg kg^{-1}); NH$_4^+$ = ammonia (mg kg^{-1}); NO$_3^-$ = nitrate (mg kg^{-1}). Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Stress (according to Clarke, 1993): <0.05 = excellent, <0.10 = good, <0.20 = usable, >0.20 = not acceptable

PERMANOVA analysis confirmed the clustering we observed with NMDS analysis. The current crop seemed to be the main factor influencing the composition of soil PLFAs ($p<0.001$, $r^2=0.33$), while significant effects were also implied for farmyard manure ($p<0.001$, $r^2= 0.14$) and mineral fertilizer ($p<0.001$, $r^2= 0.05$). With respect to the crop specific effects of fertilization regime, farmyard manure appeared to be of greater importance than mineral fertilizer, as shown by the interactions between farmyard manure and crop ($p<0.001$, $r^2= 0.21$) and between mineral fertilizer and crop ($p<0.05$, $r^2= 0.02$). The combined application of both fertilizer types resulted in no significant changes in PLFA multivariate analysis.

All investigated abiotic parameters showed significant correlations with the first two dimensions of the NMDS analysis. The strongest relationships between environmental variables and multivariate clustering were found for changes in pH, labile C and total N ($r^2 > 0.3$). The relationship between PLFA profiles and nitrate and ammonia were weaker though, with r^2 values of 0.18 and 0.21, respectively (Tab. 11).

Table 11: Output of the function *envfit* showing the relationship between the investigated abiotic parameters and the first and second axes of phospholipid nonmetric multidimensional scaling (NMDS) scores

Factor	NMDS1	NMDS2	r^2	Pr(>r)
CWC	-1.00	0.04	0.37	0.000999 ***
CWN	-0.89	-0.45	0.39	0.000999 ***
NH_4^+	-0.99	-0.14	0.21	0.000999 ***
NO_3^-	-0.61	-0.80	0.18	0.003996 **
pH	0.65	-0.76	0.68	0.000999 ***

The r^2 values represent the proportion of variance explained by the ordination. P-values represent the significance of correlations based on a post hoc permutation test (n = 999). The NMDS 1 and NMDS 2 columns give the directional cosines of the vectors associated with the first and second axes of NMDS, respectively. Factor labels: CWC = cold water extractable carbon (mg kg soil^{-1}); CWN = cold water extractable nitrogen (mg kg soil^{-1}); NH_4^+ = ammonium-N and NO_3^- = nitrate-N (mg kg soil^{-1})

NMDS and SIMPER analysis of the total and key PLFA profiles also provide indications that fertilization effects were most pronounced under sugar beet. Table 12 lists the fatty acids that were identified by SIMPER as being mainly responsible for group separation. Only eight of the 34 individual fatty acids were found under the five most important species listed by SIMPER. The high frequency of *cis*-oleic acid (18:1ω9c) and linoleic acid (18:2ω 6,9c) shows that fungi were, in general, of great relevance, causing dissimilarities between the investigated crops and fertilizer treatments. Gram-positive bacteria, indicated by branched pentadecanoic acids (i15:0, a15:0), were of specific importance in presence of sugar beet, where they represented the most greatly affected microbial group (Tab. 12). In contrast, the fatty acids 16:0 10me, 16:1ω5c and 17:1ω7c or 8c were associated with changes in the community structure exclusively under alfalfa, indicating relevance of actinomycetes, AMF, and Gram-negative bacteria in the presence of the legume.

Table 12: Similarity percentage analysis (SIMPER) of microbial related fatty acid contribution to the dissimilarity between the investigated crop rotation types (n=2) and treatments (n=6).

	overall between-group dissimilarity	Fatty acids	Contribution of FA´s to group dissimilarity	Average FA abundance	
Alfalfa & Sugar beet	12.75%			*Alfalfa*	*Sugar beet*
		i15:0	1.86%	7.13%	5.29%
		18:1ω9t	1.81%	6.31%	8.51%
		18:1ω9c	1.43%	5.85%	7.56%
		a15:0	1.26%	4.90%	3.61%
		18:2ω6,9c	1.03%	2.06%	3.25%
Legume rotation (Alfalfa)					
FYM_Y & FYM_N	7.45%			**FYM_Y**	**FYM_N**
		18:1ω9t	0.85%	6.60%	5.72%
		18:1ω9c	0.64%	6.09%	5.37%
		16:0 10me	0.59%	5.03%	4.43%
		17:1ω7c or 8c	0.55%	3.10%	3.76%
		16:1ω5c	0.53%	2.95%	2.33%
Min_Y & Min_N	6.28%			**Min_Y**	**Min_N**
		18:1ω9t	0.74%	5.91%	6.78%
		16:0 10me	0.56%	4.61%	5.06%
		18:1ω9c	0.49%	5.71%	6.00%
		16:1ω5c	0.45%	2.53%	2.96%
		18:2ω6,9c	0.43%	2.17%	1.95%
Non-legume rotation (Sugar beet)					
FYM_Y & FYM_N	15.88%			**FYM_Y**	**FYM_N**
		i15:0	2.74%	6.37%	3.13%
		18:1ω9t	2.24%	7.59%	10.33%
		a15:0	2.00%	4.47%	1.90%
		18:1ω9c	1.73%	6.97%	8.75%
		18:2ω6,9c	1.30%	2.81%	4.12%

	overall between-group dissimilarity	Fatty acids	Contribution of FA´s to group dissimilarity	Average FA abundance	
Min_Y & Min_N	13.24%			**Min_Y**	**Min_N**
		i15:0	2.23%	6.20%	4.38%
		18:1ω9t	1.77%	7.87%	9.15%
		18:1ω9c	1.71%	6.60%	8.53%
		a15:0	1.50%	4.13%	3.09%
		18:2ω6,9c	1.01%	3.08%	5.63%
Comb_Y & Comb_N	14.30%			**Comb_Y**	**Comb_N**
		i15:0	2.52%	7.31%	4.28%
		18:1ω9t	2.08%	6.77%	9.37%
		18:1ω9c	1.82%	5.97%	8.36%
		a15:0	1.74%	5.05%	2.89%
		18:2ω6,9c	1.06%	2.49%	3.63%

Grouping is based on results of NMDS. FA = fatty acid, FYM_Y = with farmyard manure, FYM_N = without farmyard manure, Min_Y = with mineral fertilizer, Min_N = without mineral fertilizer, Comb_N = mineral or organic fertilizer, Comb_Y = combined application of mineral and organic fertilizer

3.3.2. Fungal and bacterial biomass

Fungal and bacterial biomass responded differently to fertilization, again depending on crop type (Tabs. 13 + 14). Fungi revealed the most obvious differences in response to fertilization between the two crops, with no responses to either NPK or farmyard manure under sugar beet, but with significant and positive responses to both fertilizer types under alfalfa. In general, all bacteria and actinomycetes were strongly influenced by farmyard manure. In the presence of alfalfa the effect of mineral fertilizer on bacteria was similar to that of farmyard manure (Tab. 13). Under sugar beet, however, the biomass of Gram-positive bacteria was significantly increased by mineral fertilizer irrespective of farmyard

manure addition, where the biomass of Gram-negative bacteria and actinomycetes responded to mineral fertilizer only when combined with moderate farmyard manure amendments (Tab. 14).

Table 13: P-values of two-way analysis of variance (ANOVA) determining the effects of farmyard manure, mineral fertilizer as well as their interaction on the lipid biomass of certain taxonomic groups (nmol lipid g dry soil^{-1}), on the ratio of fungal to bacterial and Gram-negative to Gram-positive biomass

Taxon	*Legume-rotation (Alfalfa)*			*Non-legume-rotation (Sugar beet)*		
	Mineral	**FYM**	**Mineral X FYM**	**Mineral**	**FYM**	**Mineral X FYM**
GF	<0.05	<0.01	0.32	0.95	0.28	0.76
Gm+	<0.001	<0.001	0.40	<0.01	<0.001	0.25
Gm-	<0.001	<0.001	0.37	<0.05	<0.001	0.36
Actino	<0.001	<0.001	0.29	<0.05	<0.001	0.44
fung/bact	0.17	<0.01	1.00	<0.001	<0.001	0.49
Gm-/Gm+	<0.05	0.28	0.38	<0.001	<0.001	0.29

Column headings: Mineral = NPK mineral fertilizer; FYM = farmyard manure. Row labels: GF = general fungi (*18:2ω6,9c*); Gm$^+$ = Gram-positive bacteria (*i15:0*); Gm$^-$ = Gram-negative bacteria (*16:1ω7c*); actino = actinomycetes (*18:0 10me*); fung/bac = fungal to bacterial ratio; Gm-/Gm+ = ratio of Gram-negative to Gram-positive bacteria.

Differences between the two crops were also found for the ratios of fungi to bacteria and Gram-negative to Gram-positive bacteria. Under alfalfa, the fung/bact ratio varied only slightly among the treatments (Tab. 14). Although the fungal proportion was significantly increased by farmyard manure (p<0.01), neither the addition of mineral fertilizer only nor the combined application of both fertilizer types seem to have had an effect. In turn, the ratio of Gm$^-$/Gm$^+$ showed a weak but also significant positive response to mineral

fertilizer ($p<0.05$). In presence of sugar beet, both ratios were strongly negatively affected by the application of mineral as well as organic fertilizer ($p<0.001$) (Tabs. 13 + 14).

Table 14: PLFA biomass of different microbial groups, ratios of Gram-negative to Gram-positive bacteria, and fungal to bacterial biomass in comparison to amino sugar ratios indicating the fungal and bacterial contribution to SOM

Treatment	GF	Gm$^+$	Gm$^-$	actino	Gm$^-$/ Gm$^+$	fung/ bac	GluN/ MurA	GalN/ MurA	ManN/ MurA
	nmol lipid g soil^{-1}								
Legume-rotation (Alfalfa)									
FYM2 + NPK	7.37^a	8.43^{ab}	0.68^a	2.72^c	0.08^a	0.25^{ab}	17.16^{ab}	7.29^a	1.35^a
FYM2	6.55^{ab}	7.25^{ab}	0.55^a	2.42^c	0.08^a	0.27^a	8.32^b	3.49^b	0.73^{bc}
FYM1 + NPK	5.56^{bc}	6.49^b	0.55^a	2.49^b	0.08^a	0.25^{ab}	9.60^b	3.49^b	1.02^a
FYM1	4.98^{cd}	5.82^{ab}	0.50^a	1.94^{abc}	0.09^a	0.26^{ab}	7.90^b	2.71^b	0.61^c
NPK	3.95^{de}	5.61^a	0.48^a	1.91^a	0.08^a	0.23^b	21.03^a	9.76^a	1.21^a
NIL	2.85^e	3.84^a	0.32^a	1.33^a	0.08^a	0.23^{ab}	11.97^b	4.63^b	0.94^{ab}
Non-legume-rotation (Sugar beet)									
FYM2 + NPK	4.63^{ab}	6.36^a	0.56^{ab}	1.79^b	0.09^d	0.26^c	10.78^{bc}	4.70^{bc}	0.51^b
FYM2	5.98^a	6.87^a	0.61^{ab}	1.92^b	0.09^{cd}	0.32^{bc}	9.38^c	4.26^c	0.48^b
FYM1 + NPK	4.80^{ab}	5.19^b	0.54^b	1.91^b	0.10^c	0.29^{cb}	7.44^c	3.43^c	0.42^b
FYM1	3.07^b	1.32^c	0.22^b	1.00^a	0.17^{ab}	0.38^{ab}	8.75^c	4.33^c	0.52^b
NPK	2.89^b	1.50^c	0.29^a	1.13^a	0.20^b	0.37^{ab}	14.24^{ab}	6.47^{ab}	0.73^a
NIL	2.25^b	0.56^d	0.16^{ab}	0.68^a	0.32^a	0.46^a	16.58^a	7.02^a	0.74^a

Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Column headings: GF = general fungi; Gm$^+$ = Gram-positive; Gm$^-$ = Gram-negative; actino = actinomycetes; GluN = Glucosamine, MurA = Muramic acid; GalN = Galactosamine; ManN = Mannosamine. Different letters in each column represent significant differences according to Tukey´s honestly significant difference tests ($P<0.05$). Multiple comparisons of means were performed separately for each strip.

3.3.3. Legume associated symbiotic interactions with AMF and rhizobia

Mineral fertilization had a clear negative impact on the colonization of alfalfa roots by arbuscular mycorrhizal fungi (Tab. 15). Correspondingly, NLFA/PLFA ratios of the AMF key fatty acid (16:1ω5c) strongly declined under alfalfa in the presence of NPK and reached values comparable to those found among the fertilized treatments under sugar beet. The only significantly increased NLFA/PLFA ratio under sugar beet was found on the control, which was, in turn, comparable to the NLFA/PLFA ratio determined for the alfalfa control plot.
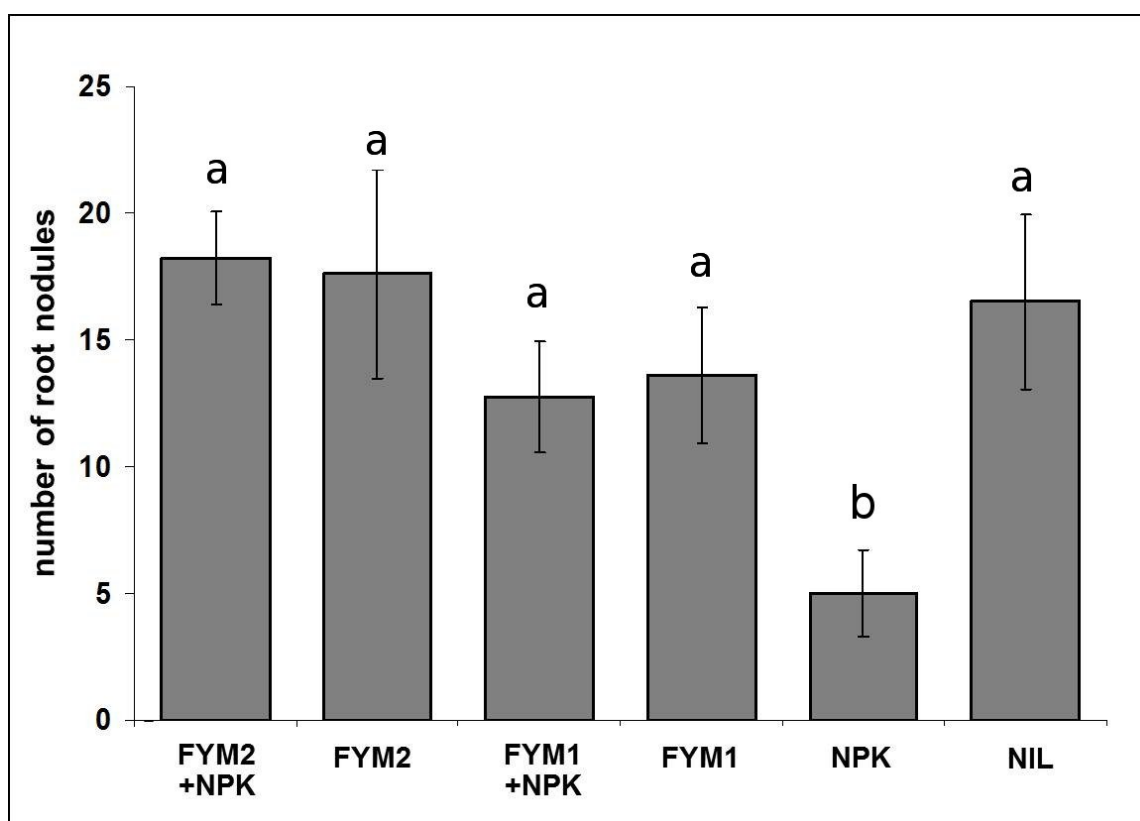
Figure 9: Effect of fertilization on nodule formation (number of nodules) on roots of *Alfalfa*, determined at the end of the cultivation period. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Error bars represent standard errors. Different letters in each column represent significant differences according to Tukey´s honestly significant difference tests (P<0.05).

Table 15: Ratios of neutrallipid- to phospholipid- (NLFA/PLFA) key indicator fatty acids 16:1ω5c (AMF = arbusculuar mycorrhizal fungi) and 18:2ω6,9c (general fungi) and AMF root colonization (%).

Treatment	NLFA/PLFA AMF	NLFA/PLFA general fungi	AMF root colonization %
Legume-rotation (Alfalfa)			
FYM2 + NPK	0.55[b]	1.91[a]	15.0[cd]
FYM2	1.52[a]	1.83[a]	30.0[b]
FYM1 + NPK	0.56[b]	2.58[a]	23.8[bc]
FYM1	3.09[a]	3.96[a]	28.0[b]
NPK	0.79[b]	3.23[a]	11.0[cd]
NIL	2.53[a]	3.76[a]	61.3[a]
Non-legume-rotation (Sugar beet)			
FYM2 + NPK	0.63[b]	1.43[b]	n.d.
FYM2	0.83[b]	1.89[b]	n.d.
FYM1 + NPK	0.83[b]	1.81[b]	n.d.
FYM1	1.31[b]	3.94[a]	n.d.
NPK	1.09[b]	1.86[b]	n.d.
NIL	2.48[a]	2.54[ab]	n.d.

Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs[-1] plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs[-1]; FYM1+NPK = 20t manure ha^{-1} 2yrs[-1] plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs[-1]; NPK = mineral fertilizer; NIL = unfertilized control. Different letters in each column represent significant differences according to Tukey´s honestly significant difference tests (P<0.05). Multiple comparisons of means were performed separately for each rotation.

Symbiosis between alfalfa and rhizobial bacteria appeared mainly unaffected by fertilizer application with one exception (Fig. 9). When mineral fertilizer was given exclusively, nodule formation was decreased by more than 50% as compared to all other plots including the unfertilized control.

3.3.4. Fungal and bacterial necromass

Because of the slow turnover time, amino sugar data are described in terms of the longer crop rotation type instead of the specific crop grown at the time of sampling, given the longer term perspective of this microbial biomarker. On average, total amino sugars were approximately 35% higher within the crop rotation without legumes as compared to the rotation including legumes, specifically with MurA exhibiting the largest differences between the two crop rotations. Within each rotation ManN, GluN, and GalN contents were mostly similar among the investigated fertilization treatments (Fig. 10). Where cropping did not include legumes (sugar beet grown at the time of sampling), these amino sugars were only slightly decreased on plots that had received 20t manure ha^{-1} 2yrs^{-1}, irrespective of the addition of mineral fertilizer (Figs. 10, b + d). On the exclusively mineral fertilized treatment of the rotation including legumes, however, ManN, GluN, and GalN were approximately 1.7, 2.5, and 3 times higher, respectively, compared to the average content of all other treatments (Figs. 10, a + c). The response of MurA to fertilization was different than that of ManN, GluN, and GalN and varied between the two crop rotations. Under sugar beet at the non-legume rotation, MurA increased consistently with each increasing fertilization level and was, independently from the addition of NPK, significantly positively correlated to the bacterial lipid biomass among the gradient of FYM addition ($p<0.001$). At the crop rotation with legumes, however, there was a significant negative relationship between MurA and bacterial lipid biomass in response to the increase of farmyard manure among the mineral fertilized treatments ($p<0.01$).
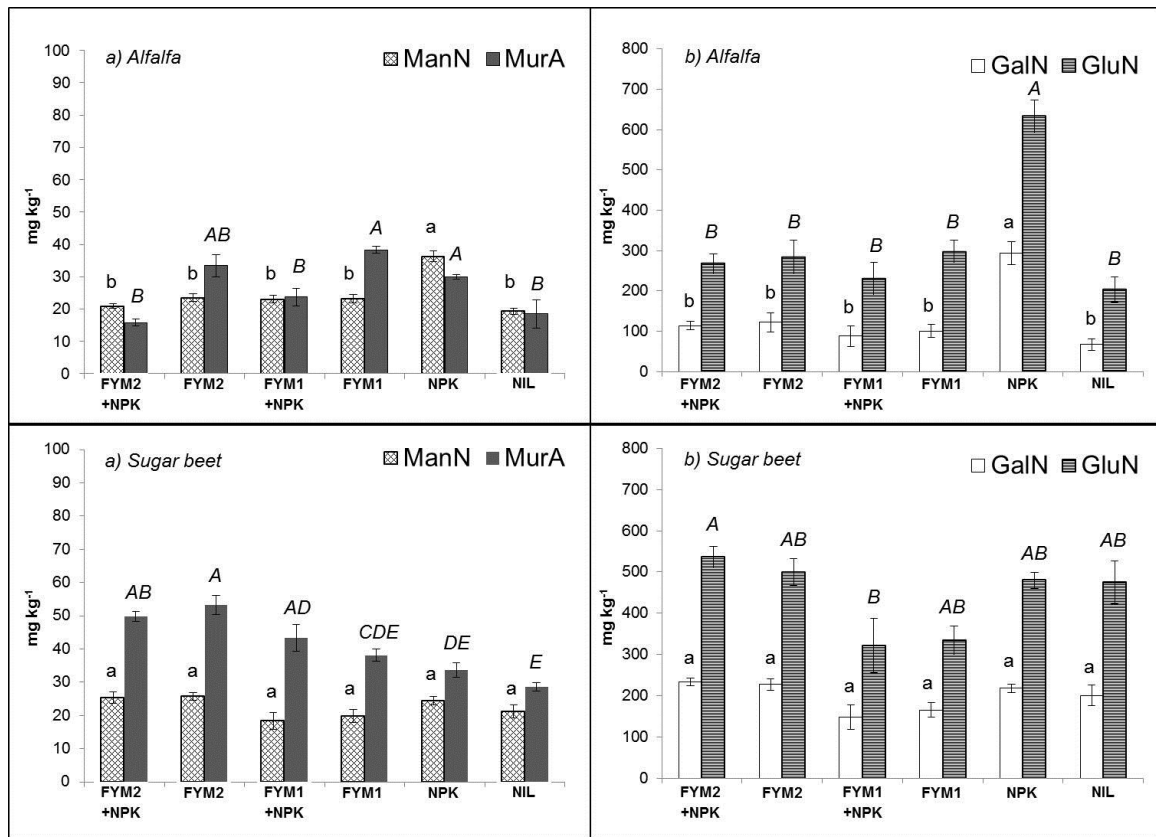
Figure 10: Total amounts of GluN (glucosamine), GalN (galactosamine), ManN (mannosamine) and MurA (muramic acid) (mg kg^{-1}) in the investigated treatments and rotation types. a) ManN and MurA in the rotation type cultivated with *Alfalfa*; b) ManN and MurA in the rotation type cultivated with *Sugar beet*; c) GluN and GalN in the rotation type cultivated with *Alfalfa*; d) GluN and GalN in the rotation type cultivated with *Sugar beet*. Treatment labels: FYM2+NPK = 30t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM2 = 30t manure ha^{-1} 2yrs^{-1}; FYM1+NPK = 20t manure ha^{-1} 2yrs^{-1} plus mineral fertilizer; FYM1 = 20t manure ha^{-1} 2yrs^{-1}; NPK = mineral fertilizer; NIL = unfertilized control. Error bars represent standard errors. Different letters in each column represent significant differences according to Tukey´s honestly significant difference tests (P<0.05).

On both crop rotations the GluN/MurA and GalN/MurA ratios indicated that the fungal contribution to SOM was highest on the most highly fertilized treatment, the treatment with mineral fertilizer only, and the control (FYM2+NPK, NPK, NIL) (Tab. 14). In contrast, only on the crop rotation including legumes the ManN/MurA ratio was increased on the mineral fertilized plots (Tab. 14).

77

3.4. Discussion

The composition of the living microbial biomass (PLFA pattern) differed clearly between the two crops (Fig. 8). This implies a plant species specific microbial community, however, it should be taken into account that our crop effects inherently include effects of seasonality or annual variation, because each crop type was sampled on different dates in our study, and seasonality is known to have a large impact on the soil microbial community (Bossio et al. 1998). The development of plant species specific rhizosphere communities is tightly associated with impacts by the plant on environmental and nutritional conditions in the soil (Costa et al. 2006; O'Donnell et al. 2001), which are assumed to be mediated primarily by root exudates (Berg and Smalla 2009a). This idea matches well with the results of our study, showing a significant correlation between multivariate PLFA profiles and soil pH, as well as labile C and N (Fig. 8, Tab. 11). These factors have been widely demonstrated to influence soil microbial community structure (de Graaff et al. 2010; Frostegård et al. 1993; Rousk et al. 2010).

Additionally, the clear separation between lower and higher levels of fertilization under sugar beet, based on both permanova and NMDS (Fig. 8) implies that fertilization had a strong impact on the composition of the microbial community, which was not the case in the presence of alfalfa. This seems logical assuming that rhizosphere microorganisms must compete for nutrients both with other soil organisms and with the sugar beet crop, in contrast to alfalfa, which meets its nutrient demand with help of the symbiotic partners and with less competition for nutrients between the plant and its symbionts. In line with this is a plant dependent response of soil microorganisms to mineral fertilizer as implied by our results, while microbial responses to farmyard manure were large in magnitude regardless of the crop grown (Tab. 13). This varied response to different fertilizer sources, i.e. levels of carbon and nutrient availabilities, has been previously documented

(Esperschütz et al. 2007; Widmer et al. 2006). Altogether under alfalfa, the similarity of PLFA profiles, the higher total microbial biomass, the weak responses of Gm-/Gm+, and fung/bac ratios to any form of fertilizer application imply that fertilization affected soil nutrient availability only slightly in presence of the legume, which likely already had a higher soil nutrient status based on the symbiotic N-fixation (Bais et al. 2006; Dakora and Phillips 2002). Of much higher significance than mineral fertilizer, however, was the microbial response to both labile C and organic fertilization in both crop rotations, supporting that C availability, irrespective of its source, was the main abiotic factor driving the observed changes in the size and composition of the microbial community (Demoling et al. 2007).

A more detailed look at specific microbial fatty acid indicators provided a deeper insight about how the microbial community was shaped by crop type (Tab. 12). In the legume strip, the PLFA 16:1ω5c contributed greatly to group separation using SIMPER analysis. This PLFA is often used as a key indicator of AM fungi, but because it also may be found in bacteria, it should be used carefully as tool to interpret AM-fungal biomass (Frostegård et al. 2011). This is especially of relevance in bacterial dominated sites, such as in agricultural systems. In our study, however, there is evidence that in the presence of alfalfa this key PLFA does indeed indicate AM fungal biomass. Thus, the declining relative abundance of 16:1ω5c, but not of other Gram-negative indicators, with NPK addition is consistent with the observed reduction in AMF root colonization (Tabs. 14 + 15), and the often reported negative impact of mineral fertilizer on AM-formation (Gryndler et al. 2006; Olsson et al. 1997). A consequence of this negative impact is that less carbon gets allocated to fungal storage structures, which is revealed by the low NLFA/PLFA ratios of 16:1ω5c in the NPK treated plots of alfalfa. The PLFA 16:1ω5c was detected also in the presence of sugar beet as non-host plant in the non-legume

rotation. In contrast to the legume strip, however, neither the PLFA nor the NLFA/PLFA ratios of this FA varied among the fertilization treatments, supporting bacterial origin in that case. Taking into account that neutral lipids are degradation products of phospholipids (Bååth 2003) the similar ratios imply comparable rates of lipid degradation in all treatments. In turn, the increased ratio of the control could result from a slowed OM turnover. A possible explanation would be the higher soil compaction at this plot (Eden et al. 2012; Schmidt et al. 2015), caused by the poorer soil aggregation in absence of any fertilizers, and the less intensive root penetration by sugar beet.

Another PLFA, the methyl-branched 16:0 (10Me), indicating actinomycetes, was only responsible for group separation under alfalfa. This finding supports the idea that there is a strong relationship between actinomycetes and legumes associated with high abundances of these bacteria in the plant rhizosphere (Sharma et al. 2005; Trujillo et al. 2010).

Within the non-legume strip grown with sugar beet during our sampling, the dissimilarities between fertilization regimes were mainly based on Gram-positive bacteria (iso and anteiso 15:0), Gram-negative bacteria (18:1w9t) and general fungi (18:1w9c, 18:2w6,9c). Gram-positive bacteria responded positively to all fertilization regimes, in contrast to the behaviour of the Gram-negative bacteria. This is in line with previous studies, in which it has been reported that the abundance of Gram-positive bacteria increases relative to Gram-negative bacteria following the addition of manure or mineral fertilizer (Giacometti et al. 2013).

In contrast with the variation in living fungal to bacterial biomass ratios in response to the fertilization treatments and crop rotation type, the ratios of fungal to bacterial necromass residues tended to respond in a similar way to the fertilization gradient on both rotation

types (Tab. 14). This could support the assumption that amino sugar ratios reflect the community structure integrated over a longer time period (Glaser et al. 2004) rather than lipids, which act more as a snap shot of the current biomass. These results also imply that over long time periods, fertilization management may alter microbial communities more than changes in crop rotation or inclusion of legumes. Both, GluN/MurA and GalN/MurA ratios indicate an increased proportion of fungal residues on the exclusively mineral fertilized plots and the extremes, e.g. the highest fertilization level (FYM2+NPK) and the unfertilized control (NIL). This must not necessarily be caused by a higher production of fungal in relation to bacterial biomass but can also simply result from a preferential degradation of bacterial residues (Zhang et al. 1999) and/or a reduced bacterial activity and biomass production in absence of manure. Thus, manure addition was reported to increase the proportion of bacterial deposits over long-term due to positive effects on microbial metabolism, such as the increased C input and an improved soil structure (Bronick and Lal 2005). In a similar respect, soil compaction may reduce the production and stabilization of muramic acid (Amelung et al. 2001a).

However, this does not explain the increased GluN/MurA and GalN/MurA ratios at the fully-fertilized treatments which are in contrast to the more general patterns observed along the fertilization gradient described above. More clarification on this issue was given from observing the responses of MurA specifically, being different to that of GluN, GalN, and ManN. Similar observations have been also made in a range of other studies (Bai et al. 2013; Ding et al. 2013), suggesting faster degradation of MurA in contrast to a higher recalcitrance of GluN and GalN.

Both the turnover and accumulation of amino sugars depend on the availability of labile C and mineral nutrients. In agricultural soils labile C increases following the addition of FYM, shifting the microbial community from dormancy into a state of higher metabolic

activity, where bacteria assimilate easily decomposable substrates faster than fungi (Engelking et al. 2007; Meidute et al. 2008). Several studies have shown that MurA contents rapidly increase during the first weeks after addition of readily mineralizable substrates, such as glucose, followed by a constant decrease back to the initial value (He et al. 2011; Liang et al. 2007a). This implies that MurA accumulates in soil only until labile C sources are exhausted, after which it is used as a microbial source of C. Interestingly, we found a significant positive relationship between MurA and PLFA biomass under sugar beet in the crop rotation without legumes (p<0.001), supporting MurA accumulation with the build-up of bacterial biomass. The positive relationship between MurA and PLFA changed however in the crop rotation with a legume included, where we found no (treatments without NPK) or even a negative relationship between MurA and PLFA biomass (p<0.01, treatments with NPK) with increasing FYM application rates (NIL, FYM1, FYM2). This could result from higher decomposition of bacterial cell-wall residues in the presence of the legume, which might be additionally enhanced by mineral N. In fact, there is a series of implications that C turnover was accelerated in presence of alfalfa (Schmidt et al. 2015). From this one could conclude that microbial residue decomposition rates are strongly influenced by the availability of N, which is in line with other suggestions that N addition accelerates C cycling (Chen et al. 2014). Taking together the positive effect of labile C on microbial biomass production with the positive effect of N on the decomposition of MurA, the increased GluN/MurA and GalN/MurA ratios at the highest fertilization level (FYM+NPK) could again result from faster decomposition of MurA due to the additional application of NPK. To validate our hypothesis, however, further investigation is needed.

Higher amounts of fungal residues in the absence of manure on the one hand, and faster C cycling with manure and NPK on the other, would also explain that net accumulation of

GluN, GalN, and ManN was comparable among the treatments for each strip. All of these considerations, however, cannot explain the remarkably increased contents of these AS on the plot with NPK only in the crop rotation with legumes. An explanation to this may be provided in the evidence that we observed dramatically lower root nodule numbers in the NPK treatment, taking into account that metabolites secreted as chemical signals from plant roots and microorganisms within soil microbe interactions significantly contribute to the pool of labile SOM in rhizospheres (Monreal 2015; Monreal and Schnitzer 2015). Indeed, amino sugars play an important role in the establishment of the symbiosis between rhizobia and their host plant. NOD-factors, which are signalling compounds secreted by rhizobia, contain a backbone of 3-5 glucosamine molecules (Mylona et al. 1995). Attachment of NOD-factors onto the root hair surface elicits various plant responses, including the process of nodule formation. GalN, in turn, occurs in capsular- and exopolysaccharides, which are involved in the recognition and attachment of rhizobial cells to the root surface of the host plant, and thus, are necessary for efficient establishment of the legume–rhizobia symbiosis (Rinaudi et al. 2006; Smol´kina et al. 2012). In addition, ManN has been found to be a glycosyl component of lipopolysaccharides in some rhizobia strains (Wang and Hollingsworth 1994).

The establishment of the symbiosis is mainly controlled by the plant, driven by the goal of optimizing the cost–benefit ratio of nitrogen acquisition. Thus, it is conceivable that the addition of mineral N could change the amount of those AS in the rhizosphere that are involved in the multi-step process of nodulation. Effects on GluN, for instance, could be related to the release of NOD-gene inducing flavonoids (Pan and Smith 2000), NOD-factor perception (Broghammer et al. 2012; D´Haeze and Holsters 2002), and NOD-factor degradation or abortion of infection threads (Staehelin et al. 1995; Vasse et al. 1993). The accumulation of GluN could occur when N-feedback mechanisms within the symbiotic

relationship are primarily based on degradation but not expression of NOD-factors. However, up to now the endogenous auto-regulation of the plant is not well understood and there is no study available describing possible effects of mineral N on NOD-factor concentrations *in situ*. Therefore it remains hypothetical whether a relationship exists between the high contents of GluN, GalN and ManN and the plant-rhizobial interaction.

3.5. Conclusions

In this study, carbon, either from plants or organic fertilizers, was the main driver of microbial community growth and structure in the soil. The general importance of organic fertilization highlights the substantial effect of long-term C addition on the quantity and quality of microbial populations in the rhizosphere. Short term (annual) effects are primarily related to C-input via plant specific allocation of photosynthetically assimilated carbon, to the availability of mineral nutrients, and to soil environmental conditions. Multivariate analysis of PLFA data revealed that rhizobia and AMF are two further factors besides of carbon driving dissimilarities for the microbial community in the rhizosphere of the two study crop rotations: (i) the growth of the symbiotic partners is enhanced by or even depends on the presence and nutritional state of a host, and (ii) because of their impact on the amount of labile C and N in the soil, inducing direct and/or plant mediated effects on decomposing microorganisms. In comparison to the living community, there was evidence that mainly fertilization, and somewhat the composition of crop rotations also shape the soil microbial necromass based on the amount of fungal and bacterial biomass synthesized, and by controlling microbial decomposition rates in association with the supply of nitrogen and labile carbon. From our results it may be hypothesized that rhizobia serve as a significant source of amino sugars, but this idea is still in need of further examination.

4. Biomarker levels of soil organic matter fractions indicate long-term fertilization effects on C stabilization and turnover

Chapter source: Schmidt et al. 2018. Biomarker levels of soil organic matter fractions indicate long-term fertilization effects on C stabilization and turnover (submitted in Biology and Fertility of Soils)

Abstract

Identifying factors influencing the processes of microbial-mediated carbon storage in soil is important for understanding how soil C sequestration in agricultural lands can be improved. Previously, we found that biomarker C responded in varying degrees to long-term fertilization regimes within bulk soil samples from the Static Fertilization Experiment, Bad Lauchstädt. Here, for a more detailed analysis, we investigated whether fertilization also affects the enrichment and abundance of biomarkers in specific soil organic matter (SOM) pools. We extracted amino acids (AA), amino sugars (AS), and monosaccharides (MS) from the following fractions: $<1\mu m$ clay sized (CF1), 1-$2\mu m$ clay sized (CF2), a <1.8 g cm^{-3} density fraction (LF1) and a 1.8-2 g cm^{-3} density fraction (LF2). These fractions individually represent pools of stabilized, passive, active and intermediate SOM, respectively. Phospholipid fatty acids (PLFA) were only extracted from clay fractions. All soil fractions were subjected to a hot-water-extraction in order to determine the potentially labile and easily degradable part of OC (HWC) within each fraction. Enrichment factors were calculated to indicate accumulation or depletion of OC, labile C, or biomarker-C in the investigated fractions as compared to the bulk soil. Biomarkers contributed between 5% and 19% to SOC and up to 44% to total N of the investigated fractions. Buildup and stabilization of microbial-derived OM in response to

fertilization was indicated by an enrichment of PLFA-C in the CF2 fraction and a shift to higher proportions of AA-C from the total biomarker-C of both clay fractions. In general, stabilized SOM was characterized by a higher share of microbial-derived OM than non-stabilized SOM, as reflected by higher C6:C5 ratios in clay as compared to light fractions. Among microbial groups, Gram-positive bacteria seem to benefit most from fertilization, suggesting that these bacteria play an important role in the process of C-stabilization.

Keywords: long-term fertilization, SOM-fractions, PLFA, amino acids, carbohydrates

4.1. Introduction

Depending on management practices and soil type, carbon can be re-sequestered in agricultural soils, partially compensating for the immense historical losses of soil organic carbon (SOC) following the conversion of natural land to cultivation (Lal et al. 2007). However, just a direct or indirect increase in C-input, for example by addition of fertilizers, does not necessarily lead to higher SOC stocks. In contrast to organic fertilization, which may increase SOC to levels even exceeding natural ones (Six et al. 2002), inorganic fertilization, especially if applied intensively, may exacerbate C-loss by accelerating the decomposition of labile SOM despite ongoing C stabilization (Neff et al. 2002).

Whether a management strategy will successfully increase SOC stocks, directly depends on how it affects microbial activity and soil C turnover. Each management induced change in soil aeration, water balance or the availability of readily degradable soil organic matter (SOM) and essential nutrients will alter the metabolism and growth of the soil microbial community (Amelung et al. 2001a; Joergensen et al. 2010; Lehmann et al.

2011; O'Donnell et al. 2001). Thus, even labile and easily decomposable SOM will accumulate in soil if microbial activity is reduced due to unfavorable conditions, such as oxygen depletion (Dixon and Kell 1989b; Schmidt et al. 2015; Veen and Kuikman 1990). This kind of C storage is sensitive to management though, and any disturbance, for instance tillage, may revive microbial activity and lead to rapid consumption of the accumulated SOM. In contrast, stable retention of soil C ultimately depends on protection of SOM from microbial decomposition either by association with the soil mineral matrix, i.e. clay, or by inclusion in soil aggregates (Lützow et al. 2006; Schmidt et al. 2011).

Soil fractionation methods have been used to assess management effects on certain hypothetical pools of rapidly and easily decomposable vs. stabilized SOM (Hoffmann et al. 2006; Schulz 2004; von Lützow et al. 2007). Most of these investigations have evaluated effects of management practices at a quantitative level, i.e. C- and N-contents, while only a handful of studies have focused on qualitative changes using different techniques, such as Fourier transform spectroscopy (FTIR and DRIFTS) (Demyan et al. 2012; Poirier et al. 2005), nuclear magnetic resonance spectroscopy (NMR) (Guggenberger et al. 1995), or the analysis of individual organic compounds (lignin, amino acids, monosaccharides) (Guggenberger et al. 1994; Poirier et al. 2005). Although these studies revealed remarkable differences in the SOM composition between light- and organo-mineral fractions, land use was found to affect SOM composition of the individual soil fractions only less and seemed to influence mainly the part of non-clay associated SOM. In this context, Guggenberger et al. (1995) succeeded to recognize differences by modifying the calculation of enrichment factors following Christensen (2001) for individual C species, i.e. by relating the content of the C species in a fraction to its content in bulk soil. Independently from this and from the fractionation method used,

many studies implied that SOM of clay fractions is supposed to be mainly of microbial origin. Still limited, however, is our knowledge regarding the role of individual microbial groups for SOM stabilization and C composition of the different SOM pools.

In order to contribute to opening this black box, we extracted amino acids (AA), amino sugars (AS), and monosaccharides (MS) from two particle size, i.e. clay fractions (CF1: <1 μm, CF2: 1-2 μm) and two density fractions (LF1: <1.8 g cm^{-3}, LF2: 1.8-2 g cm^{-3}) of a long-term fertilized soil that has received 30t farmyard manure ha^{-1} 2yrs^{-1} and NPK, as well as a non-fertilized control at the Static Fertilization Experiment (SFEBL), Bad Lauchstädt, Germany. In addition to the organic compounds mentioned above, phospho-lipid fatty acids (PLFA) were also analyzed in the two clay fractions. The clay fractions were considered to represent the "passive" pool of stabilized SOM, which is tightly associated with the clay surface and therefore unavailable for degradation. Whereas the CF2 still contains OM occluded in very small microaggregates, the CF1 represents a fraction of OM bound on primary and secondary clay particles (Lützow et al. 2006; Totsche et al. 2018). In contrast, the density fractions were considered to represent that part of SOM which is not (LF1) or only loosely (LF2) associated with the soil mineral matrix, thus representing "active" and "intermediate" SOM pools with more rapid C-turnover rates (Böhm et al. 2010; Breulmann 2011; Christensen 2001; Lützow et al. 2006; Schulz et al. 2011).

Even though the extracted organic compound classes (AA, AS, MS, PLFA) are ubiquitous, they are also the most important ones within the total microbial biomass regarding abundance and function. Thus, amino acids as basic components of proteins account to around 50% (Miltner et al. 2011), while phospholipid fatty acids are essential constituents of microbial biomembranes (Zelles 1999). Amino sugars, contained in fungal- and bacterial cell walls, can make up, in turn, up to 7% of the total microbial

biomass C (Glaser et al. 2004). Beside of this, previous studies revealed, that they significantly contribute to SOC and TN (Friedel and Scheller 2002; Martens et al. 2004; Zhang et al. 2007) and can be used to characterize the structure of the microbial community and/or the microbial contribution to SOC (Murayama 1984; Oades et al. 1970; Schmidt et al. 2015; Zelles 1999).

Based on the long-term, unchanged fertilization and cultivation practices, almost steady-state conditions can be assumed at the SFEBL, supported by there having been no significant increase in SOC contents for several decades (Merbach and Schulz 2012).

Since biomarker levels have been found to be strongly affected by fertilization at the bulk soil level (Schmidt et al. 2015), we hypothesized that SOM composition of the different soil fractions was significantly altered as well. Following the approach of Guggenberger et al. (1995), enrichment factors were calculated for amino acids, amino sugars, monosaccharides and fatty acids to indicate potential changes. In line with former findings and indications, we also expected significant differences regarding SOM pool sizes of the fertilized and non-fertilized soils (Schulz 2004).

4.2. Materials and Methods

4.2.1. Study site description and sampling

The study site is located at the long-term Static Fertilization Experiment Bad Lauchstädt (SFEBL), Sachsen-Anhalt (Germany), which was initiated in 1902 (Körschens and Pfefferkorn 1998). The soil is a Haplic Chernozem (FAO) (USDA: Mollisol) consisting of 21% clay, 67.8% silt and 11.2% sand. The mean annual temperature and precipitation are 8.8 °C and 480 mm, respectively (Körschens, 2002). The Static Fertilization Experiment Bad Lauchstädt was laid out in a split plot design with the main-plot factor FYM (three levels: no FYM, 20 t FYM ha^{-1} 2 years^{-1}, 30 t FYM ha^{-1} 2 years^{-1}) and

mineral fertilization as a sub-plot factor (6 levels: no, PK, N, NK, NP, NPK) which are all realized in each main plot. Each sub-plot has a size of 28.5 m x 10 m. In a crop rotation, sugar beet, spring barley, potatoes, and winter wheat are cultivated, while legumes have been included in the rotation on one sampling strip since 1924, replacing sugar beet and spring barley every 7th and 8th year, respectively. At the time of sampling, the soil was cultivated with alfalfa (*Medicago sativa*). Amendments of mineral fertilizers (NPK) are adapted to the nutrient demand of each specific crop in its year of cultivation (60 to 170 kg ha^{-1} yr^{-1} N, 12 to 60 kg ha^{-1} yr^{-1} P, 50 to 230 kg ha^{-1} yr^{-1} K). Alfalfa receives only PK in the mineral fertilizer treatments. Every second year, farmyard manure is applied together with the cultivation of root crops (potatoes, sugar beet), or before seed drilling in the first year of cultivation with alfalfa.

Soil samples were taken from the most intensely fertilized treatment, receiving 30 t farmyard manure ha^{-1} 2yrs^{-1} plus mineral fertilizer (FM), and the unfertilized control (NIL). The fertilized treatment chosen was originally implemented to represent an extreme case of fertilizer application.

The sampling date was chosen to correspond with plant maturity of alfalfa and took place in June 2010. Since the main-plot factor and the main-plot/sub-plot combinations were not replicated, soil was taken directly next to four replicate plants in the plough horizon (0–20 cm) in each plot and treated as replicates in further analysis. The soil was sieved to < 2 mm and frozen at -20 °C immediately after sampling.

4.2.2. Soil-fractionation

The applied size-density fractionation method based on Shaymukhametov et al. (1985) with modifications after Schulz (2004). It was used to isolate two fractions of clay-associated SOM (CF1 <1 µm, CF2 = 1-2 µm) and two fractions of SOM with specific densities (LF1 < 1.8 g cm^{-3}, LF2 = 1.8-2.0 g cm^{-3}) from the soil. The remaining fractionation residue (FR) was almost free of SOM (OC <0.01%). In contrast to the original method, fresh soil was used, defrosted shortly before fractionation. Fractionation was performed using 20 g soil per subsample, which was initially dispersed in 60 ml dest. H$_2$O, and centrifuged for 10 min at 2000 rpm. The supernatant, containing undecomposed plant and root residues was then decanted to remove POM (<1 g cm^{-3}).

The subsequent **particle-size fractionation** was performed in three steps: (1.) The soil was again suspended in 60 ml of dest. H$_2$O. Low energy ultrasonication (30.2 J sec^{-1}) was applied for 1 min to liberate the SOM associated with an easily dispersible clay fraction. (2.) Following the ultrasonic treatment, the suspension was centrifuged at 1000 rpm for 3 min. The supernatant, now containing the clay fraction of < 2 µm particle size, was then transferred into a second tube. (3.) By centrifugation for 3 min at 2000 rpm, this suspension was again divided into the CF1 (supernatant) and the CF2 (pellet). The supernatant was finally centrifuged at 4000 rpm for 10 min after addition of 500 µl 2N MgSO$_4$ solution to remove the water from CF1. Subsequently, each of the clay fractions was transferred with ethanol into a pre-weighed glass dish and dried at 60°C in a water bath. Steps 1-3 were repeated 15 times in total. The remaining solid phase after clay separation was dissolved and transferred with ethanol into a Falcon tube. The suspension was topped up with 50 ml ethanol, shaken, and centrifuged at 3000 rpm for 10 min. The washing step was repeated three times.

Thereafter, **density fractionation** was performed in two steps. (1.) A mixture of bromoform (tribrommethane)/ethanol with a density of 2.0 g cm^{-3} was added to the washed residue. The sample was shaken for 10 min and centrifuged at 3000 rpm for 15 min. The supernatant was transferred into an Erlenmeyer flask, already filled with 50 ml dest. H_2O. The procedure was repeated until no sizable amounts of the fraction were visible in the supernatant after centrifugation. The fractionation residue was again washed with ethanol and dried in the water bath. (2.) After phase separation overnight, the lower, OM-free phase was drained and the interphase, containing the OM, was collected in a Falcon tube. Bromoform was removed from this suspension by repeated washing with ethanol, shaking and centrifugation at 3000 rpm for 5 min. Separation of the LF1 from LF2 was achieved by performing the same procedure as described above, this time using a bromoform/ethanol mixture with a density of 1.8 g cm^{-3}. The LF2 remaining in the Falcon tube was repeatedly washed with ethanol and subsequently dried in the water bath. The same procedure was applied to the LF1, which was collected the next day from the Erlenmeyer flask.

Weight was determined for all fractions and the fractionation residue after drying. Before further analysis, all fractions were milled.

4.2.3. SOC, TN and labile OC and N

Total carbon (TC), total nitrogen (TN), labile C and labile N were determined for bulk soil as well as for each fraction. TC and TN were measured via combustion (Vario El III, Elementar-Hanau). No inorganic C was detected and thus TC represents TOC. Labile C and N (HWC, HWN), i.e. the potentially mineralizable and decomposable part of the organic carbon (Schulz et al. 2011; Weigel et al. 1998), were extracted with hot water for one hour under reflux according to Schulz (2002). An elemental analyzer for liquid

samples (Micro N/C and Multi N/C, Analytik Jena, Germany) was then used to measure C and N-contents of the hot-water extracts.

4.2.4. Biomarker analysis

Detailed descriptions of the procedures used for the extraction of amino acids, amino sugars, monosaccharides as well as for phospholipid fatty acids are given in (Schmidt et al. 2015). The weight of samples taken was adapted in light of the C- and N-content of a fraction ($\geq$ 5 mg C and $\geq$0.2 mg N). Thus, 600 mg (CF1 and CF2), 100 mg (LF1), and 200 mg (LF2) were used for determination of amino acids and amino sugars. For monosaccharides, 300 mg of the CF1 and CF2 fractions and 55 mg and 200 mg of the LF1 and LF2 fractions, respectively, were weighed into the extraction vessels. Phospho lipid fatty acids were determined only in the clay fractions, since light fractions were almost free of lipids due to the use of bromoform in the liquid-mixture used for density separation.

Biomarker derivatives were analyzed by gas chromatography-mass spectrometry (GC/MS) using a HP 6890 gas chromatograph coupled to a HP 5973 mass-selective detector (Hewlett Packard, Wilmington, USA). Individual compounds were separated by passing through the stationary phase of a DB-5ms column (60 m x 0.25 mm x 0.25 mm film thickness). Temperature programs were adapted individually for each compound class of interest. Identification and quantification of the biomarkers were based on external standards, containing a set of the substances of interest for each substance class. Carbon and nitrogen contents of the different biomarker substance classes (AA, MS, AS, PLFA) were determined based on µg carbon/nitrogen per mol individual compound.

The relative contribution of fungal and bacterial amino sugars was reflected in the ratios of GluN/MurA (Zhang and Amelung 1996) and GalN/MurA (Glaser et al. 2004). The

share of microbial to plant derived neutral sugars in the SOM pools was determined according to Oades (1984) and Murayama (1984) by calculating the ratios of C6:C5 sugars, i.e. (mannose + galactose)/(xylose + arabinose), and deoxy-C6:C5 sugars, i.e. (rhamnose + fucose)/(xylose + arabinose).

The biomass (nmol lipid g dry soil^{-1}) of each specific microbial group was indicated by the content of one specific representative PLFA biomarker: 16:1ω5c for AMF (Balser 2005); 18:2ω6,9c for general fungi (GF) excluding AMF (Balser 2005; Frostegård et al. 2011); 16:1ω7c for Gram-negative bacteria (Wilkinson et al. 2002); and i15:0 for Gram-positive bacteria (Wilkinson et al. 2002). This decision is based on the circumstance that for AMF there is in general only one key-indicator PLFA available. Regarding the validity of the PLFA biomarker 16:1ω5c for AMF for the soil used in our study see Schmidt et al. (2015). The relative abundance of a microbial group represents its percentage on the total microbial biomass (sum of extracted PLFA up to a chain length of 20 C-atoms).

4.2.5. Calculation of carbon enrichment factors ($E_{SOC-fraction}$, E_{HWC}, E_{AA-C}, E_{AS-C}, E_{MS-C}, E_{PLFA-C})

Carbon enrichment factors of functional SOM pools and biomarkers were calculated according to (Christensen 1985). With respect to the SOM pools, the SOC content of each fraction was divided by the SOC content of the bulk soil:

$$SOC_{fraction} [g] / SOC_{bulk} [g]$$

Following the example of Guggenberger et al. (1994) the approach of Christensen was then adopted in order to investigate the enrichment of C from AA, MS, AS, and PLFA in soil fractions as compared to bulk soil SOC. The enrichment factor of a biomarker was

thereby determined as being the sum of C of all individual compounds within the respective substance class related to the SOC of bulk soil:

$$C_{biomarker} \ [g] \ * SOC_{Fraction} \ [kg] \ / \ C_{biomarker} \ [g] \ * SOC_{bulk \ soil} \ [kg]$$

In the same way as for C, enrichment factors were calculated for N as well.

4.2.6. Statistical analysis

For all analyzed parameters, multiple comparisons of means within and between the plots were conducted by ANOVA and using the conservative Tukey´s honest significant difference test ($p < 0.05$) in order to reduce the risk of type I and type II errors. All statistical analyses were performed in R version 2.15.1 (RCoreTeam 2012). The similarity percentages procedure (SIMPER) was applied to evaluate which microbial taxon mostly contributed to changes in the composition of the microbial biomass in response to fertilization. Like most historical field experiments, the Static Fertilization Experiment Bad Lauchstädt does not include replicate plots in its experimental design. Therefore, our results are based on pseudoreplicates (n=4) in the sense of Hurlbert (1984), leading to the circumstance that our ANOVA analyses may suffer from type I and type II errors. Being aware of this, we argue for a pragmatic solution rather than "a militant stance to pseudo-replication" (Davies and Gray 2015), taking into account the value of historic long term treatments such as the SFEBL, we address this problem and take the pseudoreplication into consideration in the discussion of our results.

4.3.   Results

4.3.1. SOC, TN and labile OC and N

Dry weight based carbon and soil mass balances of the isolated fractions resulted in recovery rates of $\geq$ 99%, while TN recovery was >80% on average. Significant effects of

fertilizer addition were found for the bulk soil, with an increase in SOC, TN, labile hot water extractable C and N (HWC; HWN), and the HWC/HWN ratio (Tab. 16). Regarding the fractions, only OC and TN of the CF2 and HWN of the LF1 were significantly higher under long-term FM compared to NIL. No fertilization effect was found on the size of the stable or stabilized pool, indicated by the mass (%) of the two clay fractions, making up together ~ 26% of bulk soil on both non-fertilized and fertilized plots. In contrast, fertilization increased the proportion of LF1 by around 150%, whereas LF1 and LF2 together accounted for only 1.4-2.9% of the bulk soil.

On average one tenth of the carbon associated with clay fractions and light fractions, respectively, could be assigned to the sum of biomarkers (CF1+CF2: FM ~ 12%, NIL ~ 14%; LF1+LF2: FM ~10%, NIL ~7%). Among the light fractions, biomarker C made up between 5-11% (FM) and 5-8% C (NIL) of the total measured organic carbon, respectively (Fig. 11A). With respect to nitrogen, around 40% of N in the light fractions derived from AA and AS, whereas 7-28% of the total N associated with clay was biomarker N (Fig. 11B).
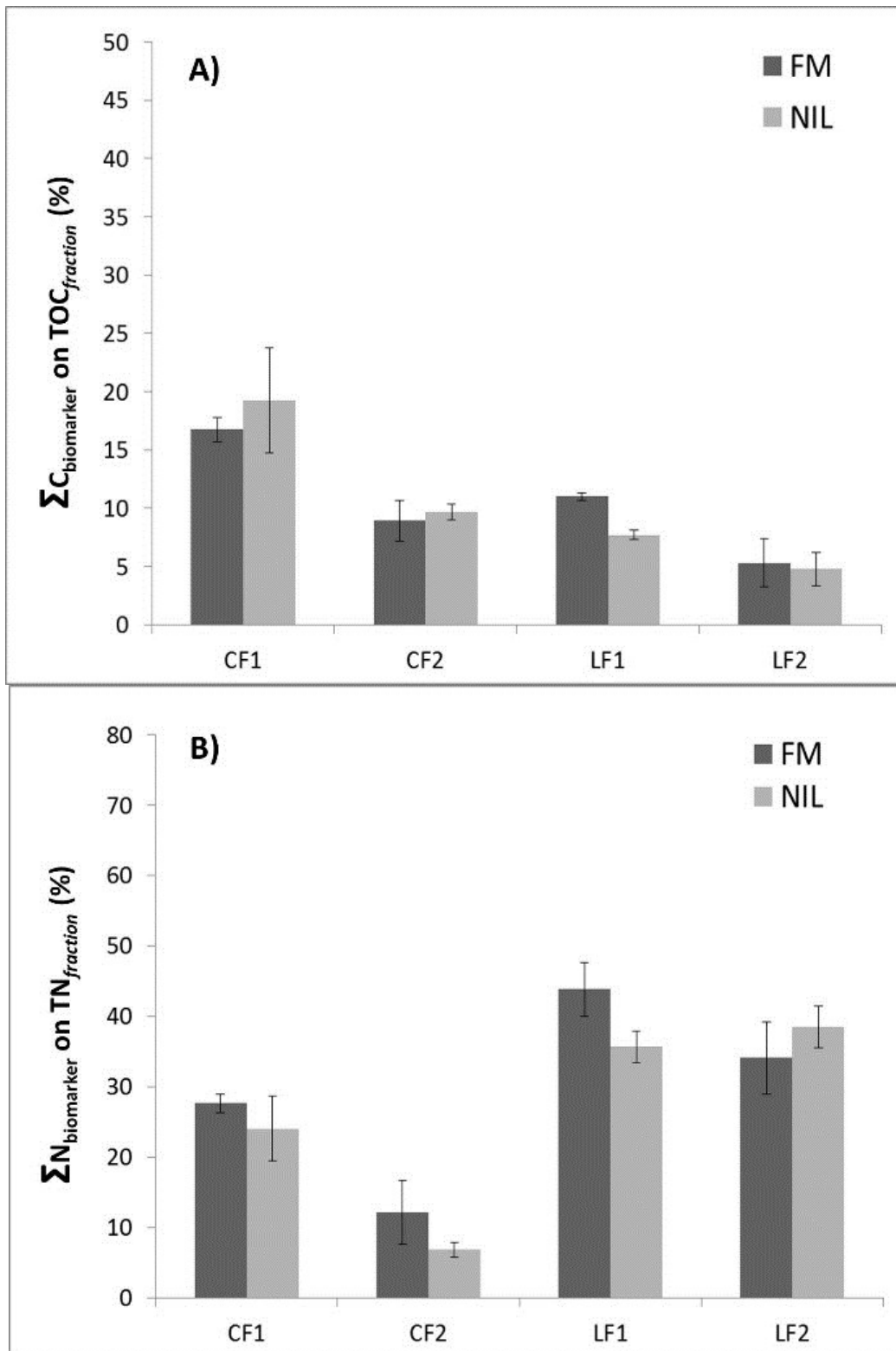
Figure 11: Percentage of the A) total OC and B) total N of each fraction explained by the C and N of the extracted biomarkers (AA, AS, MS, PLFA).

Table 16: Contents of total and labile C and N of bulk soil and soil fractions for the fertilized and non-fertilized soil, including the proportion of each fraction on bulk soil regarding mass and C content

Treatment/Fraction	C [%]	N [%]	HWC	HWN	HWC /HWN	Proportion on bulk soil	
			[mg/kg fraction]			weight [%]	SOC [%]
FM-bulk	2.6 [A]	0.2 [A]	751.4 [A]	63.3 [A]	6.0 [A]	100.0	100.0
FM-CF1	5.0 [c]	0.5 [cd]	1469.3 [bd]	190.2 [b]	7.7 [ab]	15.9	33.0
FM-CF2	7.8 [b]	0.6 [c]	1959.9 [b]	259.0 [b]	7.6 [ab]	9.9	32.2
FM-LF1	41.9 [a]	2.5 [a]	5236.5 [a]	572.1 [a]	9.2 [ab]	1.8	32.2
FM-LF2	5.0 [c]	0.1 [f]	1638.1 [bd]	212.3 [bd]	7.7 [ab]	1.1	2.3
FM-FR	<0.01	<0.01	ND	ND	ND	71.8	0.3
NIL-bulk	1.6 [B]	0.1 [B]	399.6 [B]	30.1 [B]	10.5 [B]	100.0	100.0
NIL-CF1	3.5 [ce]	0.3 [df]	1193.4 [bd]	106.1 [cde]	11.3 [ab]	15.9	38.0
NIL-CF2	4.9 [c]	0.4 [de]	1167.0 [bd]	96.2 [de]	12.1 [ab]	10.7	39.2
NIL-LF1	41.1 [a]	1.9 [b]	5112.5 [a]	342.9 [b]	14.9 [a]	0.7	20.3
NIL-LF2	3.8 [cd]	0.1 [f]	1871.8 [bc]	149.0 [cde]	12.6 [ab]	0.7	2.0
NIL-FR	<0.01	<0.01	ND	ND	ND	71.8	0.5

HWC = hot-water extractable carbon, HWN = hot-water extractable nitrogen, CF1 = clay fraction <1µm, CF2 = clay fraction 1-2µm, LF1 = light fraction <1.8 g cm^{-3}, LF2 = 1.8 -2 g cm^{-3}; and FR = fractionation residue. FM is the fertilization treatment and NIL is the unfertilized control. With the exception of C%, N%, HWC and HWN for bulk soil (n=5) all results are based on four (pseudo)replicates.

Table 17: Enrichment factors for total and labile C and N as well as for biomarker C and N of each fraction in response to fertilization

Treatment/ Fraction	E_{SOC}	E_{SON}	E_{HWC}	E_{HWN}	E_{AA-C}	E_{AA-N}	E_{AS-C}	E_{AS-N}	E_{MS-C}	E_{PLFA-C}
FM-CF1	2.0 [c]	2.3 [cd]	5.8 [c]	5.5 [c]	3.2 [b]	0.03 [c]	2.2 [c]	0.02 [c]	2.0 [c]	1.9 [b]
FM-CF2	3.1 [c]	3.0 [c]	12.1 [c]	9.8 [c]	3.4 [b]	0.02 [c]	3.6 [c]	0.02 [c]	3.0 [c]	5.7 [a]
FM-LF1	16.5 [b]	12.1 [b]	173.9 [b]	87.2 [b]	223.0 [a]	1.15 [a]	183.1 [a]	0.92 [a]	60.8 [b]	ND
FM-LF2	2.0 [c]	0.5 [e]	6.4 [c]	1.2 [c]	1.4 [b]	<0.01 [c]	1.9 [c]	<0.01 [c]	0.4 [c]	ND
NIL-CF1	2.2 [c]	2.2 [cd]	10.4 [c]	10.0 [c]	1.5 [b]	0.01 [c]	1.4 [c]	0.01 [c]	2.9 [c]	1.4 [b]
NIL-CF2	3.1 [c]	2.8 [c]	14.4 [c]	11.5 [c]	0.9 [b]	0.00 [c]	2.2 [c]	0.01 [c]	3.0 [c]	1.9 [b]
NIL-LF1	25.5 [a]	14.1 [a]	526.0 [a]	211.5 [a]	192.6 [a]	0.74 [b]	116.1 [b]	0.46 [b]	93.6 [a]	ND
NIL-LF2	2.3 [c]	0.9 [de]	17.1 [c]	5.7 [c]	1.2 [b]	<0.01 [c]	0.8 [c]	<0.01 [c]	0.5 [c]	ND

HWC = hot-water extractable carbon, HWN = hot-water extractable nitrogen, AA-C = amino acid carbon, AS-C = amino sugar carbon, MS-C = monosaccharide carbon, PLFA-C = phospholipid fatty acid carbon, CF1 = clay fraction <1µm, CF2 = clay fraction 1-2µm, LF1 = light fraction <1.8 g cm^{-3}, LF2 = 1.8 -2 g cm^{-3}; with exception of C%, N%, HWC and HWN for bulk soil (n=5) all results are based on four (pseudo)replicates.

4.3.2. $E_{SOC-fraction}$, E_{HWC}, and $E_{biomarker-C}$

Enrichment factors relate the SOC, HWC or biomarker-C in a fraction to the respective values in the bulk soil. Enrichment factors were mostly >1, indicating enrichment of OC or the specific biomarker-C within a fraction representing a functional SOM pool. Considering the fractions of the fertilized and non-fertilized plots on their own, all fractions were similarly enriched in $E_{SOC-fraction}$, E_{HWC}, E_{AA-C}, E_{AS-C}, and E_{MS-C}, except the LF1, where these enrichment factors were significantly higher than in the other fractions (Tab. 17).

The strongest response of $E_{biomarker-C}$ to fertilization was found for PLFA, followed by monosaccharides, amino acids, and then amino sugars (Tab. 17). In the CF2 fraction, fertilization increased the enrichment of PLFA-C. In addition, amino acids and amino sugars became further enriched in response to fertilization within the LF1. At the same time, however, monosaccharides became depleted in the LF1 fraction, probably explaining the overarching decrease in the $E_{SOC-fraction}$ and E_{HWC} of the LF1. With respect to the LF2, it is noteworthy that there was high variance in all biomarker analyses across replicates, which is in contrast to the low standard deviations observed for the other fractions. For this reason, clear and reliable statements about the influence of fertilization on the LF2 are not possible. However, considering average values, the LF2 seemed to be more enriched in amino sugars in response to fertilization, but appeared to be generally depleted in monosaccharides.

4.3.3. Biomarker levels and biomass composition of SOM fractions

In the following, we analyzed whether fertilizer addition altered the biomass composition of SOM-pools, starting with a broad look at the distribution of biomarker classes (Fig. 12), followed by a detailed consideration of relative abundances of individual compounds

within the most abundant biomarker compound classes (AA, MS), and finally, by evaluating the composition of the microbial necro- and biomass (here clay fractions only), respectively, within the fractions.
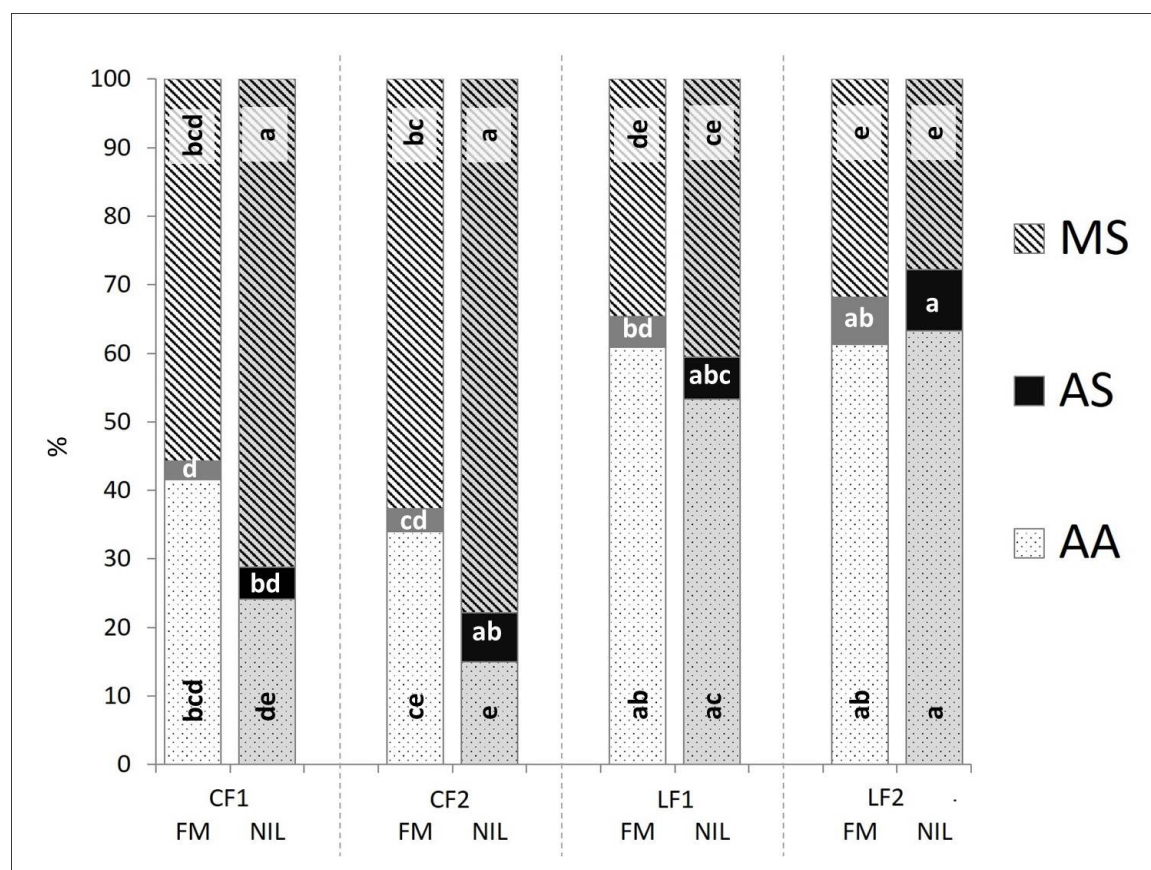
Figure 12: Effect of fertilization on the distribution of biomarker-C within a fraction; % of biomarker compound class on total biomarker-C (=sum of AA-C, AS-C, MS-C). PLFA-C not included since it was not determined for the light fractions and contributes <1% to the clay fractions (FM-CF1 = 0.43%, FM-CF2 = 0.97%, NIL-CF1 = 0.38%, NIL-CF2 = 0.55%). Bars labelled with different letters correspond to values of biomarker-C that differ significantly according to Tukey´s honestly significant difference test (P<0.05).

Because PLFA data were only available for the two clay fractions, only AA-C, AS-C, and MS-C were considered in the first step. Clay fractions were dominated by monosaccharide-C, while light fractions had higher proportions of amino acid-C (Fig. 12). Only in the clay fractions did fertilization lead to significant changes in the

distribution of the investigated compound classes. In this context, fertilizer addition increased the proportion of AA-C at the cost of MS-C in both, the CF1 and CF2 fraction. However, only in the CF2 the proportion of AS-C on biomarker-C was significantly lower in the FM treatment than in the control.

The composition of the most abundant compound classes, amino acids and monosaccharides, was significantly altered by fertilization, almost exclusively in the clay fractions, whereas significant differences were found only for some of the detected 15 individual amino acids. In both CF1 and CF2, alanine increased by a factor of 1.6 ($p < 0.05$) with fertilization. Additionally, glycine was 2.6 times higher in the CF2 of the FM samples than in the samples from the unfertilized control ($p < 0.01$). Proline, leucine and phenylalanine, however, were significantly reduced by fertilization within this fraction ($p < 0.05$).

Relative abundances of the 11 determined individual monosaccharides, including glucuronic- and galacturonic acids, were even less affected by fertilization than those of amino acids. Only rhamnose was significantly increased within the CF1 ($p < 0.001$), CF2 ($p < 0.1$), and LF1 ($p < 0.001$) fractions from the fertilized soil. Further significant fertilization effects were found in the relative decreases in glucoronic acid ($p < 0.01$) and fucose ($p < 0.05$) associated with the CF1 and LF2 fraction, respectively, as well as in the increase of xylose within LF2 ($p < 0.1$). Uronic acids (glucuronic acid and galacturonic acid) showed at least a tendency to rise with fertilization within the LF1 and at least galacturonic acid within the CF2 fraction as well.

The C6:C5 ratio, i.e. the ratio of (mannose + galactose) / (xylose + arabinose), did not indicate any influence of fertilization on the contribution of microbial-produced monosaccarides relative to plant derived carbohydrates. Comparing the individual soil fractions, however, the complexed SOM in clay fractions showed a higher proportion of

microbial carbohydrates than the uncomplexed SOM in light fractions, whereas the CF2 and LF1 contained more bacterial-derived MS than the CF1 and LF2, respectively (Fig. 13).
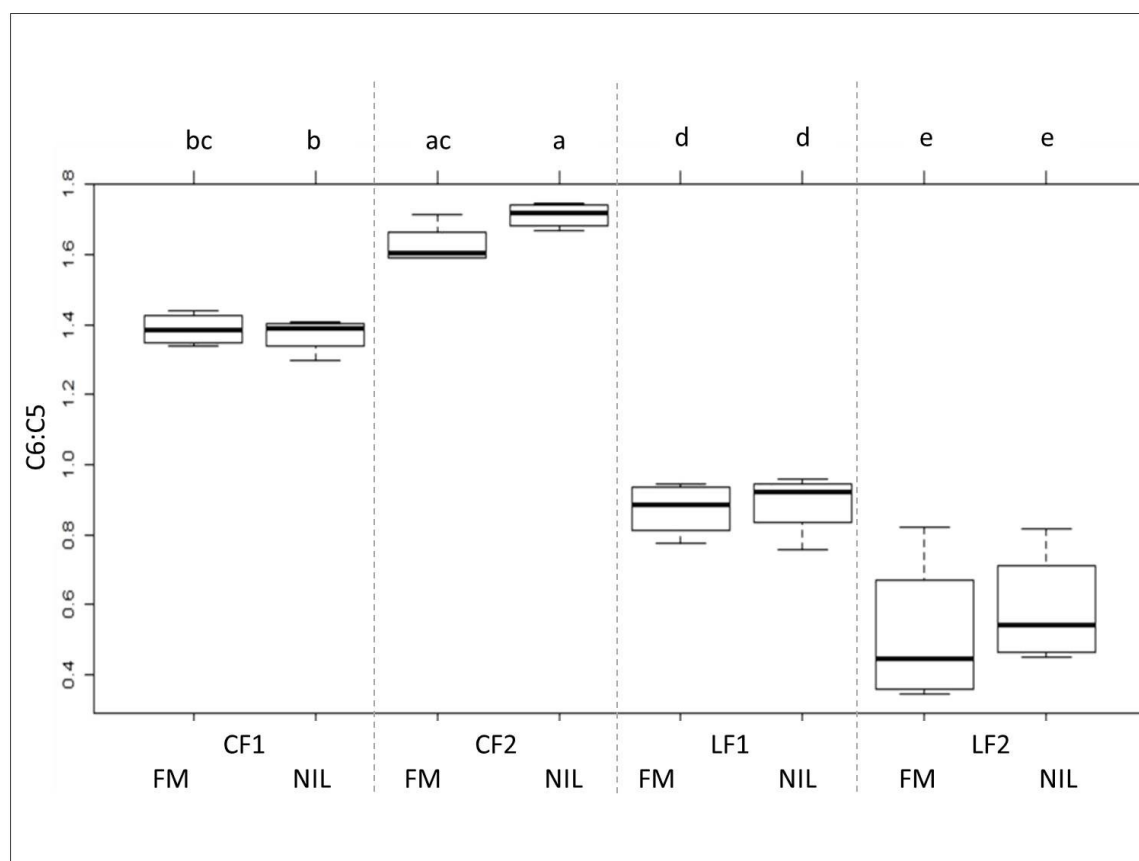
Figure 13: Effect of fertilization on the ratio of of individual SOM pools. Bars labelled with different letters correspond to C6:C5 ratios that differ significantly according to Tukey´s honestly significant difference test (P<0.05).

The GalN/MurA ratio was significantly lower in the clay fractions of the fertilized plot than in the control, while no fertilization effect was observed among the light fractions, which showed lower average values than the clay fractions. The GluN/MurA ratio responded in a similar way to fertilization, although the highest ratio was found in the LF1 of the control plot (Fig. 14A & B).
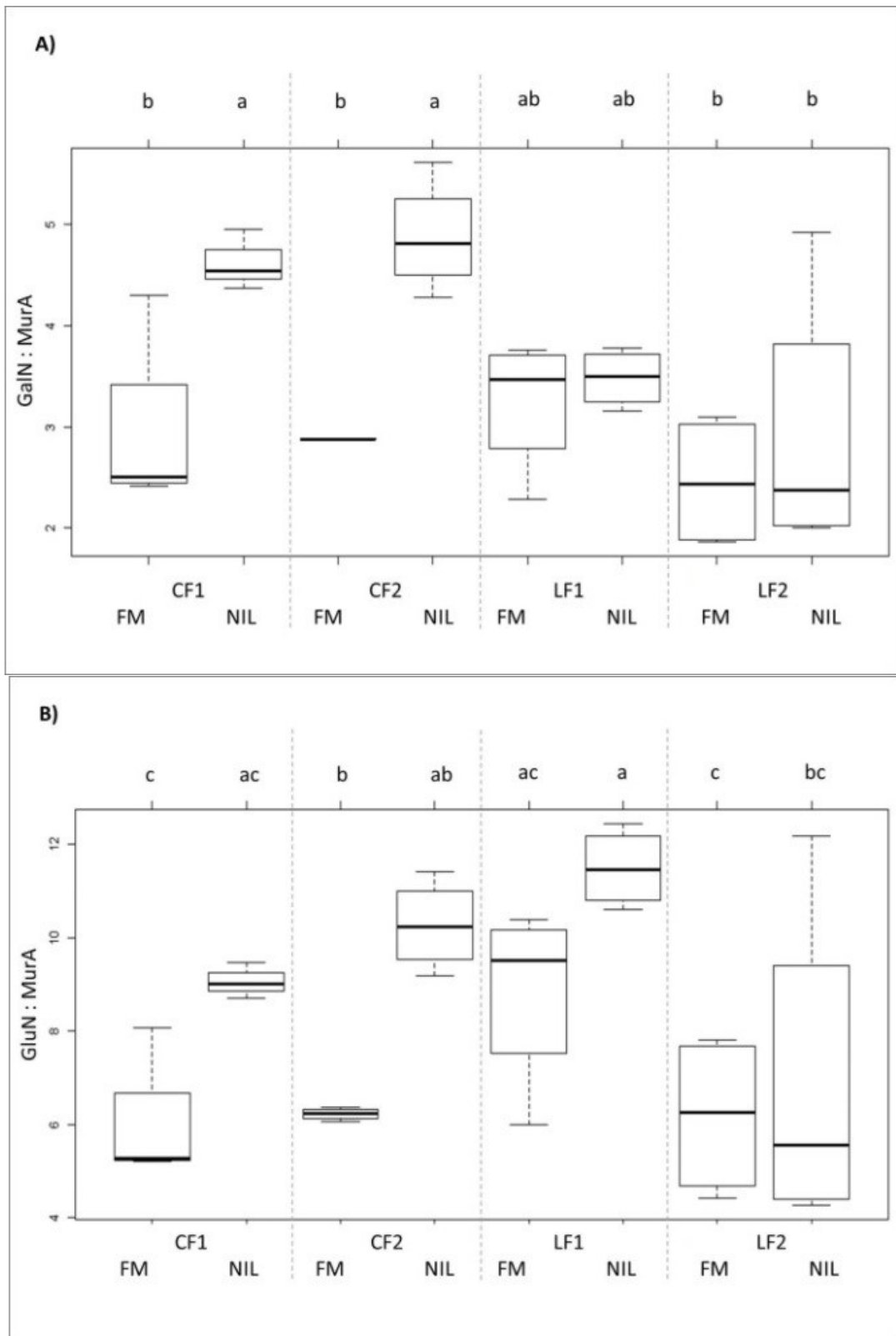
Figure 14: Ratio of A) GalN / MurA, and B) GluN / MurA of different SOM pools as affected by fertilization. Bars labelled with different letters indicate significant differences according to Tukey´s honestly significant difference test (P<0.05).

Finally, considering microbial groups represented by PLFAs, Gram-positive bacteria appeared to increase the most in response to fertilization, followed by general fungi, and Gram-negative bacteria (Tab. 18).

Table 18: Changes in the relative abundances of microbial taxa indicated by key indicator PLFA in response to fertilization. Differences between FM and NIL samples were highly significant for all taxa (p<0.001).

Fraction	Taxon	Relative abundance	
		FM [%]	NIL [%]
CF2	Gram$^+$	22.6	6.9
	GF	15.5	5.3
	Gram$^-$	11.6	4.5
	Action	7.4	3.3
	AMF	6.4	1.9
CF1	Gram$^+$	21.5	6.7
	GF	15.0	6.8
	Gram$^-$	12.6	6.0
	Action	8.8	3.7
	AMF	5.9	2.2

FM = with farmyard manure, NIL = unfertilized control, Gram$^+$ = Gram-positive bacteria, Gram$^-$ = Gram-negative bacteria, GF = general fungi, AMF = arbuscular mycorrhizal fungi, actino = actinomycetes

4.4. Discussion

Among the investigated biomarkers, monosaccharides had the highest proportion on SOC at bulk soil level, followed by amino acids, amino sugars and PLFA. The same distribution of biomarkers was found for the clay fractions, implying that bulk SOC composition is mainly shaped by the stable SOM pool. This is comprehensible, taking into account that clay associated C makes up more than 60% of the SOC, in contrast to around 30 % of SOC being present in the light fractions (Tab. 16). Clay plays a key role in the long-term sequestration of OC and its proportion in soils determines the maximum size of the stable C pool (Kool et al. 2007; Körschens et al. 1998; Six et al. 2002). While SOM in light fractions responds rapidly to management changes, clay-associated SOM is characterized by long turnover times since it is less susceptible to microbial attack. This is well reflected in our finding of higher proportions of biomarker-derived C in the total OC of the analyzed clay fractions compared to the light fractions. This finding provides field evidence for the recent advancements in understanding that microorganisms and low molecular weight microbial-derived molecules are sources and drivers of the long-term accumulation of SOC (Kallenbach et al. 2016; Miltner et al. 2011)

Being the key driver for all OC turnover processes, the soil microbial community generally benefits from any additional organic C input, responding with an increase in biomass (Docherty et al. 2012; Marschner et al. 2003; Schnürer et al. 1985). Moreover, microbial OC turnover is also controlled by the soil pore space, determining the gaseous and liquid phase transport and hence, the availability of oxygen. Other than the exclusively mineral fertilized treatments, the treatments with manure showed bulk densities that clearly decreased in comparison to the control, at the SFEBL (Eden et al. 2012). Thus, enhanced mineralization following manure application may be one reason for the observed depletion of total and labile C and N as well as the decline of E_{MS-C} in

the LF1 in response to fertilization (Kuka et al. 2007) (Tab. 17). On the other hand, an excess of N due to the application of mineral fertilizer on soil cultivated with a legume may have caused the observed enrichment of organic N, i.e. AA-N and AS-N along with in the fertilized plot (Schmidt et al. 2015).

Our results confirmed that both light fractions, in particular the LF1, represent a SOM pool of mostly fresh and less decomposed, mainly plant-derived organic material indicated by the overall high enrichment factors (Tab. 17), high C/N (Tab. 15) and low C6:C5-ratios (Fig. 13). In addition, the active, unprotected microbial biomass is generally considered to be part of the labile SOM pool represented by the light fractions (Schulz 2004; Wander 2004). Although there are no PLFA data available for the light fractions, the high proportions of AA-C imply higher living microbial biomass as compared to the clay fractions, bearing in mind that AA-C and PLFA-C, in particular, were closely related at bulk soil level (Schmidt et al. 2015). With fertilization, proportions of plant- and microbial biomass in the labile pool seemed to increase in the same way, as indicated by the comparable C6:C5 ratios of the LF1 samples from the NIL and fertilized plots. This also supports an increase in OC-turnover rates in response to fertilization, outbalancing the higher input of plant-derived OM from manure and/or root exudates by increased conversion into microbial biomass and subsequent mineralization of microbial residues.

Fertilization did not affect C6:C5 ratios of the clay fractions either, implying that stabilization of plant- and microbial-derived OM was increased to the same extent. For the stable SOM pool, however, an increase in microbial biomass and all microbial indicator groups under fertilization were supported by the PLFA results (Tab. 17 + 18) and by the shift to higher proportions of AA-C and AS-C in both clay fractions in the fertilization treatment (Fig. 12). Stimulation of microbial growth is known to enhance formation of water stable aggregates (Elmholt et al. 2008) through increased growth of

fungal hyphae, and increased production of bacterial exopolysaccharides binding the negatively charged clay minerals (Czarnes et al. 2000). Thus, the higher relative abundance of the bacterial polysaccharide galacturonic acid in the CF2 of the fertilized plot potentially reflects the microbial contribution to physical protection of SOM (Benbi et al. 1998; Czarnes et al. 2000; Norman 1950). Indeed, the microbial biomass increased most within the CF2 fraction, as indicated by E_{PLFA-C}, underpinning the importance of microorganisms for aggregate formation. The relevance of bacteria for C-stabilization is further corroborated by the significantly higher relative abundance of the desoxy-C6-sugar rhamnose within the clay fractions of the fertilized plot (Murayama 1984; Oades et al. 1970). It is noteworthy that rhamnose is particularly highly abundant in the cell-wall of Gram-positive bacteria belonging to the genera *Streptococcus*, *Enterococcus* and *Lactococcus* (Mistou et al. 2016), which suggests that besides the soil dwelling microorganisms, manure-derived bacteria may play a relevant role in soil aggregation. Finally, the significant increase in the amino acids alanine and glycine with fertilization may be associated with the increased contribution of microbial biomass from Gram-positives, since both amino acids are constituents of the peptidoglycane layer of these bacteria.

4.5.   Conclusion

This study supports former findings that management effects on SOC composition become less visible at the level of individual soil fractions, although especially clay- and light fractions differ clearly from each other. Thereby, SOC of bulk soil seems to reflect mainly the stable SOM pool. From the few significant changes, we conclude that microbial-derived molecules contribute to the stabilized pool of soil organic carbon, while Gram-positive bacteria, in particular, may support C-stabilization by promoting the

formation of microaggregates. The specific promotion of this microbial group might therefore be a tool to use for regaining decades of loss of soil C in arable lands.

Our results further imply that long-term intensive organic plus mineral fertilization causes depletion of easily decomposable C within the labile SOM pool, probably resulting from an accelerated C-turnover based on changes in soil structure. Turnover rates seem to increase in such way that the higher input of plant-derived C and its conversion into microbial biomass is outweighed, leading to proportions of plant and microbial OM in the labile SOM pool similar to those in the unfertilized control.

5. Synthesis and Conclusion

Agricultural soils are assumed to bear great potential in acting as a sink for atmospheric CO_2, provided a proper soil management. SOC stocks can be increased by either increasing the C-input and/or reducing the loss of C due to soil respiration. Both, the stabilisation and release of soil C are directly linked to the soil microbial community, standing at the centre of all nutrient cycles and driving a range of other ecological soil functions. The activity of soil microorganisms, in turn, is influenced by a number of environmental factors, such as physical- and chemical soil properties, climatic conditions, as well as the availability of energy and nutrient sources. The main objective of this thesis was to investigate the impact of long-term organic- and/or mineral fertilization treatments on the microbial mediated stabilization and turnover of organic C. Since microbial community structure is known to be significantly shaped by the plant type (Berg and Smalla 2009b; Ngosong et al. 2010), most of the investigations were performed on two strips of the SFEBL, differing in the crop type cultivated at sampling time (*alfalfa* vs. *sugar beet*) as well as in the absence or inclusion of a legume within the crop rotation. Interrelations between chemical- and physical soil properties, microbial growth and activity were elucidated by the simultaneous analysis of a set of four biomarkers. The relationships between the biomarkers and several abiotic factors were also determined. The characterization of SOM, including an analysis of the microbial community composition of both bulk soil and soil fractions further provides detailed information regarding the microbial role within functional SOM pools and hence, soil SOM dynamics.

5.1. Influence of fertilization on the stable/stabilized SOM pool

Both, the application of organic as well as of mineral fertilizers are known to directly or indirectly increase SOC levels (Powlson et al. 2011). At the SFEBL, a significant build-up of SOC was achieved by either the long-term addition of farmyard manure or the combined application of farmyard manure and NPK but not by solely application of NPK **(Chapter 2)**. The fact that even long-term addition of 30 t FYM ha^{-1} 2yrs^{-1} did not significantly increase C contents of the clay associated soil fraction CF1 **(Chapter 4)** implies, that the SOC increase at bulk soil level following fertilization **(Chapter 2)** is de facto rarely based on an increase in SOM complexed with clay. According to Dexter et al. (2008) who set the threshold for soil C-saturation at a clay:OC ratio of ≥ 10, the potential to stabilize C based on complexation with clay is exhausted in the highest fertilized treatment (clay:OC ratios = 10) (Eden et al. 2012). Schjønning et al. (2010) then extended the Dexter-threshold to ≥ 20 by considering the fraction of silt+clay (<20 μm) instead of clay only, determining the soil specific surface area which can interact with OM. Based on this, even the full-fertilized soil would have not yet reached C-saturation, which may be one reason for the observed significant increase of C within the clay associated soil fraction CF2 in response to fertilization **(Chapter 4)**.

Another explanation is given by the fact that a layer of organic molecules covering mineral particles possesses a much higher affinity to OM than the clay surface itself (Kästner et al. 2014). Thus, initially adsorbed OM promotes the further association of organo-mineral associates with each other, leading to the formation of microaggregates. The results of the present study (**Chapter 4**) support this explanation, demonstrating that exopolysaccharides and bacterial cell-wall components such as proteins and glycopolymers mediate the bacterial adhesion onto mineral surfaces and the subsequent formation of organo-mineral aggregates (Huang et al., 2015). The high abundances of

111

both, iso- and anteiso PLFA´s within the clay fractions along with significantly increased amounts of rhamnose, alanine, and glycine within the CF2 of the fertilized plot imply that Gram-positive bacteria were involved in microaggregate formation at the SFEBL in response to fertilization. Assuming a key-role of Gram-positive bacteria for C-stabilization, the question arises whether this microbial group is specifically promoted by one of the investigated fertilization treatments, i.e. farmyard manure only, NPK only, or the combined application of both types of fertilizers. The biomass of Gram-positives seem to increase more than that of Gram-negatives in response to any treatment, independent from the kind of applied fertilizer *(Giacometti et al. 2013)*. Also in the present study, biomass of Gram-positives responded more strongly to fertilization than that of Gram-negatives at least on the strip cultivated with sugar beet, where a significant increase versus the control was reached with FYM levels of 20t ha^{-1} 2yrs^{-1} or NPK only, implying that both types of fertilizers are basically equally suitable to promote the growth of Gram-positive bacteria. An even higher abundance of these microorganisms and concomitantly a crop-independent significant increase versus the control was found on the plot treated with 20t ha^{-1} 2yrs^{-1} + NPK, leading to the conclusion that the combination of both fertilizer types might enhance C-stabilization more successfully than the application of a single type of fertilizer alone. However, this seem to be valid only in case of moderate FYM levels, since the additional application of NPK did not significantly alter the biomass of Gram-positives on the 30t FYM ha^{-1} 2yrs^{-1} treatments (**Chapter 3**).

5.2. Influence of fertilization on the decomposable/labile SOM pool

Within the labile SOM pool, organic carbon is not or only loosely associated with minerals and thus, not stabilized. However, it can still be retained and accumulate within large micro- and macroaggregates, respectively, being reflected by the increase in the

proportion of the light density soil fraction LF1 in response to fertilization **(Chapter 4)**. Compartmentalization of substrate and microbial biomass along with lower oxygen levels due to reduced air permeability provide some protection from microbial attack and decrease the turnover rates of the intra-aggregate SOM (Schmidt et al. 2011; Six et al. 2002).

Sustainable application of fertilizers includes the prevention of unnecessary high releases of CO_2 and other greenhouse gases from soil in relation to SOM turnover. SOM turnover, however, is a prerequisite to maintain microbial driven soil functions, being essential also for crop growth through the subsequent release of plant-available nutrients from SOM turnover. Thus, organic fertilization of cultivated soils is always a balancing act in retaining as much C as possible within the soil on the one hand, while keeping nutrient cycles and thus, SOM turnover going on, on the other (Janzen 2006). This, however, appears much more difficult where the potential to protect SOM from microbial decay based complexation of SOM with minerals are almost exhausted and newly added C will increasingly be allocated into the labile SOM pool. In line with other studies, our results imply that on this background, turnover rates will increase proportionally to the increase of OM-input (Gulde et al. 2008). However, there is also limited potential of physical SOM protection based on aggregate formation and thus, in allegory of an overflowing barrel – excess organic C will flow off from the labile pool by respiration, resulting in a stagnation of C accumulation, becoming apparent in the observation that SOC contents show no further increase despite increased FYM levels (**Chapter 2**).

Only when soil pore space turns into anaerobic conditions, hence decreasing SOM turnover rates, labile C will again accumulate **(Chapter 2)**. Based on this, one can assume that each further increase of fertilizer input on a soil near C-saturation will promote the release of avoidable amounts of CO_2 as well as of N_2O, resulting from enhanced

denitrification in response to the ongoing oxygen limitation (Firestone and Davidson 1989; Powlson et al. 2014).

Any change in the availability of nutrients or C might affect microbial growth and could therefore have consequences for soil respiration. Beside of labile C, being supported as main driver for microbial biomass changes **(Chapter 3)**, there were strong indications that the application of NPK additionally stimulated microbial growth and respiration **(Chapter 2 & 3)**, probably being also the reason for the observed depletion of monosaccharide-C within the labile pool **(Chapter 4)**. This effect appeared to be somewhat stronger in presence of the legume, where rhizobia and mycorrhizal fungi were stabilizing N- and P-levels in the rhizosphere **(Chapter 3)**.

Recent investigations on non-legume strips at the SFEBL showed, that the activity of enzymes involved in N- and P-cycling (N-acetylglucosaminidase and phosphatase) were considerably higher in response to NPK and NPK+FYM (Francioli et al. 2016). The observed changes in enzyme production were assumed to result from a shift in microbial community composition, which was found to be distinct among the investigated fertilization treatments (NIL, NPK, 20t FYM ha^{-1} 2yrs^{-1}, 20t FYM ha^{-1} 2yrs^{-1} + NPK). This coincides with the results of the present work, showing a clear impact of fertilization on the microbial community composition with sugar beet as crop, in contrast to a very similar microbial community in presence of the legume **(Chapter 3)**. In addition, microbial biomass of Gram-positives, Gram-negatives, and actinomycetes was more strongly positively correlated to NPK additions on the strip cultivated with alfalfa than with sugar beet. Based on this one could assume that in absence of a legume, NPK addition provokes microorganisms to increase their investment in exoenzymes rather than biomass. This is only speculation, however, and would need to be tested by further investigations. Beside of this, an enhancing effect on soil respiration by mineral N cannot

be generalized as shown by the very inconsistent results of other studies. Up to now, the factors and their interactions driving the response of soil microorganisms to nutrient addition are poorly understood and need further research to mechanistically test these interactions.

5.3. Overall conclusion and research perspectives

The results of the present work basically demonstrate that both, organic and mineral fertilization are suitable measures for promoting the re-sequestration of organic C in cultivated soils. However, this study also makes clear that the success of a management strategy in mitigating global climate change can only be assessed by comparing SOC increases over the long term with losses of CO_2 and other greenhouse gases over the long-term. Finding an appropriate fertilization strategy will require land managers to take into account the soil or site specific C-saturation deficit along with a consideration of the nutrient demand of the cultivated crop type and the presence or absence of symbiotic partners, which may alter the nutrient supply of the decomposer microbiota. In this context, the results of this work imply that the determination of monosaccharides and amino acids along with SOC could be a useful tool to detect changes in SOM turnover rates within the labile SOM pool in response to management. However, due to the circumstance that all investigations were based on pseudoreplicated samples taken from only one soil type, this assumption cannot be generalized at the moment and would need further validation. Hence, results of a high OC soil will likely not apply to low OC soils, where bulk densities and thus, the availability of nutrients and oxygen for the decomposer community are determined by OC rather than the clay content (Dexter et al. 2008).

In a similar way, more research is necessary to verify whether Gram-positives actually support C-stabilization by enhancing the formation of microaggregates and hence, if

stabilization of C in cultivated soils can be supported by the specific promotion of this microbial group. An integrated analysis of the OM composition (f.ex. by using biomarkers) and microbial species diversity (f.ex. based on DNA-sequencing or at least PLFAs) in microaggregates (53–250 μm) and the silt plus clay fraction (<53 μm) in response to different fertilization strategies could bring further light to this issue.

As mentioned above, there is still a lack of knowledge regarding the effects of N availability on soil C cycling. To uncover the complex dynamics behind an inhibition or acceleration of SOM mineralization following the addition of mineral N will need to go beyond an exploration of SOM composition along with changes in microbial enzyme activities and appear to require first the development of high-resolution analysis methods (f.ex. based on DNA), providing information on both the abundance and biomass of the individual microbial species being affected.

6. Summary

Expecting a predicted world population of around nine billion people in 2050 and the increasing threat of a global climate change, agriculture is forced to develop management strategies which will ensure food security on the one hand and on the other, to promote the soils ability to act as a sink for atmospheric CO_2. The fertility of a soil strongly depends on the activity of soil microorganisms as main drivers of all nutrient cycles. At the same time, soil microorganisms also contribute to the stabilization of organic carbon (OC) by promoting aggregate formation. There is also increasing evidence that soil organic matter (SOM) is predominately of microbial origin. Although both, organic and mineral fertilization have been reported to increase soil organic carbon (SOC) stocks, it is not yet fully understood how and to which extent these different types of fertilizers affect the composition and activity of soil microorganisms and thus, either the turnover or the stabilization of SOM. The present thesis intended to contribute to answering this question by the analysis of soil samples from five different fertilization treatments and a non-fertilized control, taken from the Static Fertilization Experiment Bad Lauchstädt, established in 1902. Due to the continuous constant organic and/or mineral fertilization over a period of several decades, a dynamic equilibrium between formation and degradation of soil organic matter can be assumed, making this experimental site ideal for studying the effects of fertilization on SOM dynamics. To take into account the crop specific fertilization and the presence of plant-symbiotic relationships as additional source of mineral nutrients, most investigations were done on a strip cultivated with alfalfa (*Medicago sativa*), a host-plant for rhizobia and arbuscular mycorrhizal fungi (AMF) as well as on a strip cultivated with sugar beet *(Beta vulgaris)* as a non-host plant, respectively.

SOM is heterogeneous regarding its origin, chemical composition, physical properties, age, and bioavailability. While the chemical composition of OM is mainly determined by its source and biotic transformation processes, bioavailability and thus, its degradability, is mainly governed by the accessibility of OM to degradation enzymes, which can be hampered by entrapment of OM within aggregates or interactions with soil minerals. First and foremost, therefore, SOM can be distinguished into a decomposable/labile and a stabilized/stable pool, which, however, are not sharply delineated, but represent a continuum of different states of stabilization and associated turnover times.

In this work, the soil was fractionated according to particle-size and density and the obtained fractions subjected to further investigations in order to assess a pool of stable/stabilized (clay fractions: CF1 < 1μm, and CF2 1-2μm), intermediate (light fraction 2: LF2 1.8-2 g cm^{-3}), and decomposable/labile SOM (light fraction 1: LF1 < 1.8 g cm^{-3}), respectively. Bulk soil and fractions were characterized by abiotic parameters like OC, TN, labile OC and N. As a new approach, four biomarkers, i.e. amino acids (AA), monosaccharides (MS), amino sugars (AS), phospho- and neutrallipid fatty acids (PLFA, NLFA) were extracted from bulk soil and soil organic matter fractions to gain information about both the origin and composition of SOM as well as the composition of the microbial bio- and necromass under the different fertilization regimes.

Main aims of the thesis were:

- to test the hyptothesis that SOM of bulk soil and soil fractions differs depending on fertilization in both in their organic-chemical composition and in their proportions of plant, microbial or fungal bio- and necromass. Changes in the organic-chemical composition should be recorded by determining the proportions of the individual biomarkers (AA, MS, AS, PLFA) in the total OC content of the soil or soil fractions (SOC, SOC fraction). Structural changes in the living

microbial community should be detected by PLFA analysis. An estimate of the levels of fungal and bacterial OM in the necromass should be made by rationing galactosamine and glucosamine to muramic acid. The ratio of deoxy-hexose or hexose to pentose sugar should in turn provide information on plant or microbial contributions to SOM.

- identifying the factors being responsible for the observed changes in microbial biomarkers, and finally

- gaining information about the role that microorganisms play for C stabilization by linking the results related to the compositions of SOM and the soil microbial community at bulk soil level to that of the soil fractions.

In bulk soil up to 17.2% of the SOC and 23.9% of the TN could be explained by the investigated biomarkers, thereby MS-C and AA-C contributed most to the SOC (5.7-16.5%), followed by AS-C (0.5-2.8%) and PLFA-C (<1%). Similar distribution of the individual biomarker groups within SOC$_{-Fraction}$ was found in case of the clay- but not light fractions, implying that the SOM composition of the bulk soil is mainly determined by the stable/stabilized SOM pool. The fact that the C content of CF1 was not significantly increased by long-term fertilization is an indication that on the SFEBL the capacity of the soil for C stabilization by complexation of OM with clay is already almost exhausted on the unfertilized plot. Consequently, any significant change in the composition of bulk soil SOM can primarily be attributed to changes within the decomposable/labile pool. Among the treatments, the relative contributions of the studied biomarkers to the total SOC varied only slightly except in two cases and only with alfalfa as crop. Unusual high application rates of farmyard manure (FYM) in combination with mineral fertilizer (NPK) as well as the absence of any fertilizer in the control led to an accumulation of biomarker-C, which was hypothesized to be the final result of two

consecutive, opposite shifts in SOM turnover rates. There was some evidence that SOM degradation on both plots was initially accelerated by increased availability of labile C and mineral N, which was generally attributed to alfalfa root exudation and their symbiotic relationship with rhizobia and AMF. In addition, the very high levels of FYM along with the application of NPK played a decisive role at the highest fertilized treatment. Based on evidence of a direct correlation between the increase in labile C and the increase in microbial biomass, it was hypothesized that the oxygen content in soil pore space decreased due to the increase in microbial respiration. The increasing emergence of anaerobic areas in turn led to a slowdown in overall SOM turnover times. The fact that biomarker C was accumulated in these plots despite considerable differences in the availability of labile C might be due to differences in bulk density and the resulting soil pore volume. Thus, beside of labile C and mineral N, the soil structure was assumed as main driver for biomarker related changes in SOM composition.

Labile C and mineral N were also identified as main factors determining the composition of the microbial community at the SFEBL, while the presence of plant-symbiotic relationships was found to reduce the impact of varying inputs of mineral nutrients on the decomposer community. PLFA results support earlier findings that the composition of living microbial populations varies within a short period of time and depending on the particular crop. In contrast, the composition of the microbial necromass seems to be mainly determined by fertilization effects on the SOC-turnover. It was also confirmed that the OM associated with clay is mainly of microbial origin. High levels of iso- and anteiso-PLFAs as well as the significant increase of rhamnose, alanine and glycine within the CF2 due to fertilization suggest that Gram-positive bacteria in particular play a role in the formation of microaggregates and thus C-stabilization. The biomass of Gram-positives reacted in turn to the individual application of FYM or NPK. However, even

greater stimulation of the growth of these microorganisms by the combined use of both types of fertilizer was achieved only with moderate levels of FYM.

As a key finding of this work, both the individual and the combined use of FYM and mineral fertilizers were found to be suitable for promoting C stabilization in agricultural soils. However, the results make also clear that the capacity of a soil for C stabilization is limited and that the soil-specific C saturation deficit should be considered in the search for an appropriate fertilization strategy in addition to the plant-specific nutrient requirement.

7. Kurzzusammenfassung

Mit einer prognostizierten Weltbevölkerung von rund neun Milliarden Menschen im Jahr 2050 und der zunehmenden Bedrohung durch einen globalen Klimawandel ist die Landwirtschaft gezwungen, Managementstrategien zu entwickeln, die einerseits die Ernährungssicherheit gewährleisten und andererseits die Fähigkeit der Böden fördern, CO_2 zu speichern. Die Fruchtbarkeit eines Bodens hängt stark von der Aktivität der Bodenmikroorganismen als Hauptantrieb aller Nährstoffkreisläufe ab. Gleichzeitig tragen Bodenmikroorganismen auch zur Stabilisierung von organischem Kohlenstoff (OC) bei, indem sie die Aggregatbildung fördern. Darüber hinaus gibt es zunehmend Hinweise darauf, dass die organische Bodensubstanz (OBS) vorwiegend mikrobiellen Ursprungs ist. Obwohl es Hinweise darauf gibt, dass sowohl die organische als auch die mineralische Düngung die Lagerbestände organischer Kohlenstoffvorräte kultivierter Böden (SOC) erhöhen, ist noch nicht vollständig geklärt, wie und in welchem Umfang diese unterschiedlichen Düngemittel die Zusammensetzung und Aktivität von Bodenmikroorganismen und damit den Umsatz oder die Stabilisierung der OBS beeinflussen. Die vorliegende Arbeit soll dazu beitragen, diese Frage durch die Analyse von Bodenproben aus dem Statischen Düngungsexperiment Bad Lauchstädt zu beantworten, wobei fünf verschiedene Düngungsbehandlungen sowie die nicht gedüngten Kontrolle untersucht wurden. Aufgrund einer konstanten organischen und/oder mineralischen Düngung über mehrere Jahrzehnte hinweg kann am Versuchsstandort von einem dynamischen Gleichgewicht zwischen Bildung und Abbau der organischen Bodensubstanz ausgegangen werden. Dies bietet wiederum ideale Voraussetzungen, um den Einfluss von Düngung auf die Umwandlung/Stabilisierung der OBS zu untersuchen. Um sowohl die kulturpflanzenspezifische Düngung als auch das Vorhandensein von Symbiosen als zusätzliche Mineralstoffquelle zu berücksichtigen, wurden die Beprobung

auf zwei Schlägen durchgeführt, welche sich sowohl in der Fruchtart zum Probenahmezeitpunkt (Luzerne als Wirtspflanze für Rhizobien und arbuskuläre Mykorrhizapilze (AM) vs. Zuckerrübe als Nicht-Wirtspflanze) als auch in ihrer Fruchtfolge (mit Leguminosen vs. ohne Leguminosen) unterschieden.

Die OBS ist sowohl in ihrer Herkunft, chemischen Zusammensetzung, ihren physikalischen Eigenschaften, als auch bezüglich ihres Alters und ihrer Bioverfügbarkeit sehr heterogen. Während die chemische Zusammensetzung des organischen Materials im Boden hauptsächlich durch dessen Ursprung und bereits durchlaufene biotische Transformationsprozesse bestimmt wird, hängt seine Bioverfügbarkeit und somit Abbaubarkeit hauptsächlich davon ab, ob Abbauenzyme ungehindert auf das Substrat zugreifen können oder ob es durch Einschluss in Aggregate oder Wechselwirkungen mit der Mineralsubstanz vor mikrobiellem Angriff geschützt ist. In erster Linie kann die OBS daher in einen zersetzbaren/labilen und einen stabilisierten/stabilen Pool unterschieden werden, die jedoch nicht scharf voneinander abgegrenzt sind, sondern ein Kontinuum unterschiedlicher Stabilisierungszustände- und damit verbundener Umsatzzeiten darstellen.

In der vorliegenden Arbeit wurde der Boden nach Partikelgröße und Dichte fraktioniert und die erhaltenen Fraktionen weiteren Untersuchungen unterzogen, um stabilen/stabilisierten (Ton-Fraktionen: CF1 <1μm und CF2 1-2μm), intermediären (leichte Fraktion 2: LF2 1.8-2 g cm^{-3}) und zersetzbaren/labilen (leichte Fraktion 1: LF1 < 1.8 g cm^{-3}) OBS pool zu bewerten. Der Boden wie auch die Bodenfraktionen wurde durch abiotische Parameter wie OC, TN, labiler OC und N charakterisiert. Als neuer Ansatz wurden vier Biomarker, i.e.S. Aminosäuren (AA), Monosaccharide (MS), Aminozucker (AS), Phospho- und Neutrallipidfettsäuren (PLFA, NLFA) aus dem Gesamtboden und den organischen Bodenfraktionen extrahiert, um Informationen über den Einfluss

organisch- und/oder mineralischer Düngung auf den Ursprung und die Zusammensetzung der OBS sowie der mikrobiellen Bio- und Nekromasse zu erhalten.

Hauptziele der Arbeit waren:

- die Annahme zu überprüfen, dass die OBS des Gesamtbodens- und der Bodenfraktionen sich je nach Düngungsbehandlung sowohl in ihrer organisch-chemischen Zusammensetzung als auch hinsichtlich ihrer Anteile an pflanzlicher, mikrobieller oder pilzlicher Bio- und Nekromasse unterscheidet. Änderungen in der organisch-chemischen Zusammensetzung sollten dabei über die Bestimmung der Anteile der einzelnen Biomarker (AA, MS, AS, PLFA) am gesamten organischen Kohlenstoffgehalt des Bodens- bzw. der Bodenfraktionen (SOC, SOC-Fraktion) erfasst werden. Strukturelle Veränderungen in der lebenden mikrobiellen Gemeinschaft sollten mittels PLFA-Analyse nachgewiesen werden. Eine Abschätzung der Anteile an pilzlicher und bakterieller organischer Substanz in der Nekromasse sollte mittels Verhältnisbildung aus Galactosamin und Glucosamin zu Muraminsäure erfolgen. Das Verhältnis von Deoxy-Hexose- bzw. Hexose- zu Pentosezuckern sollte wiederum Aufschluss über pflanzliche bzw. mikrobielle Beiträge an der OBS geben.

- die Identifizierung der für die Änderungen verantwortlichen Faktoren und schließlich

- die Erlangung von Informationen bezüglich der Rolle von Mikroorganismen bei der Stabilisierung von organischem C durch Verknüpfung der Ergebnisse des Gesamtbodens mit denen Ergebnissen der Bodenfraktionen.

Für den Gesamtboden konnten bis zu 17,2% des SOC und 23,9% des TN durch die untersuchten Biomarker erklärt werden, wobei MS-C und AA-C dabei den größten Anteil ausmachten (5,7-16,5%), gefolgt von AS-C (0,5-2,8%) und PLFA-C (<1%). Eine ähnliche Verteilung der Biomarker fand sich in den beiden Ton-, jedoch nicht in den leichten Fraktionen, woraus sich schlussfolgern lässt, dass die Zusammensetzung der OBS des Gesamtbodens hauptsächlich durch den stabilen/stabilisierten OBS-Pool bestimmt wird. Dass der C-Gehalt der CF1 durch Langzeitdüngung nicht signifikant erhöht wurde, ist dabei ein Hinweis darauf, dass auf dem SFEBL die Kapazität des Bodens zur C Stabilisierung durch Komplexierung von OM mit Ton bereits auf dem ungedüngten Plot nahezu ausgeschöpft ist. Folglich kann angenommen werden, dass jede signifikante Änderung in der Zusammensetzung der OBS des Gesamtbodens in erster Linie auf Veränderungen innerhalb des zersetzbaren/labilen Pools beruht. Anders als ursprünglich vermutet, variierten die relativen Anteile der untersuchten Biomarker am SOC nur geringfügig entlang der untersuchten Plots. Nur mit Luzerne als Fruchtart kam es sowohl unter extrem hohen Stalldungapplikationen in Kombination mit Mineraldünger als auch auf der ungedüngten Variante zu einer Akkumulation von Biomarker-C, welche vermutlich aus zwei aufeinanderfolgenden Wechseln in den Abbauraten der OBS resultierte. Es gab Hinweise, dass der Abbau der OBS auf beiden Plots durch eine erhöhte Verfügbarkeit von labilem C und Mineralstickstoff (Mineral-N) zunächst beschleunigt wurde, was im Allgemeinen auf die Freisetzung an Wurzelexsudaten durch die Luzerne sowie auf deren symbiotische Beziehung zu Rhizobien und AM-Pilzen zurückgeführt wurde. Auf der höchst gedüngten Variante spielten zusätzlich dazu die sehr hohen Einträge an Stalldung und Mineralnährstoffen in Form von Mineraldünger eine entscheidende Rolle. Basierend auf dem Nachweis einer direkten Korrelation zwischen der Erhöhung an labilem C und dem Zuwachs der mikrobiellen Biomasse wurde

vermutet, dass sich der Sauerstoffgehalt im Bodenporenraum durch den Anstieg der mikrobiellen Atmung verringerte. Die zunehmende Entstehung anaerober Bereiche hatte wiederum eine Verlangsamung der OBS-Umsatzzeiten zur Folge. Dass es auf den genannten Plots trotz starker Unterschiede in der Verfügbarkeit an labilem C zu einer Akkumulation an Biomarker-C kam, könnte auf Unterschiede in der Lagerungsdichte und des sich daraus ergebenden Bodenporenvolumens zurückzuführen sein. Neben labilem C und Mineral-N ist die Bodenstruktur vermutlich ein weiterer Hauptfaktor für die mittels Biomarkeranalyse detektierten Veränderungen in der Zusammensetzung der OBS.

Labiler C und Mineral-N wurden ebenfalls als Haupteinflussfaktoren für die Zusammensetzung der mikrobiellen Gemeinschaft identifiziert. Dabei deuteten die Ergebnisse darauf hin, dass die der mikrobiellen Zersetzergemeinschaft im Boden zur Verfügung stehenden Mineralnährstofflevel durch die Symbiose zwischen Pflanze und Rhizobien bzw. AM-Pilzen kontrolliert und somit Düngungseffekte auf die mikrobielle Gemeinschaft reduziert werden.

PLFA-Ergebnisse unterstützen dabei frühere Befunde, dass die Zusammensetzung lebender mikrobielle Populationen innerhalb kurzer Zeiträume und in Abhängigkeit von der jeweiligen Fruchtart variiert. Im Gegensatz dazu scheint die Zusammensetzung der mikrobiellen Nekromasse hauptsächlich durch Düngungseffekte auf den C-Umsatz im Boden bestimmt zu werden. Es wurde ebenso bestätigt, dass die mit Ton assoziierte OBS hauptsächlich mikrobiellen Ursprungs ist. Hohe Gehalte an iso- und anteiso-PLFAs sowie die signifikante Zunahme an Rhamnose, Alanin und Glycin innerhalb des CF2 infolge von Düngung lassen dabei vermuten, dass insbesondere Gram-positive Bakterien bei der Bildung von Mikroaggregaten und damit der C-Stabilisierung eine Rolle spielen. Die Biomasse von Gram-positiven reagierte wiederum grundsätzlich auf die individuelle Anwendung von Stalldung oder NPK. Eine noch stärkere Stimulation des Wachstums

dieser Mikroorganismen durch die kombinierte Anwendung beider Düngemitteltypen wurde jedoch nur unter moderaten Mengen an Stalldung erreicht.

Die wesentliche Erkenntnis dieser Arbeit ist, dass sowohl die individuelle als auch die kombinierte Anwendung von Stallmist- und Mineraldünger geeignet sind, um die C-Stabilisierung in landwirtschaftlichen Böden zu fördern. Die Ergebnisse machen jedoch deutlich, dass die Kapazität eines Bodens zur C-Stabilisierung begrenzt ist und bei der Suche nach einer geeigneten Düngestrategie neben dem pflanzenspezifischen Nährstoffbedarf auch das bodenspezifische C-Sättigungsdefizit berücksichtigt werden sollte.

8. References

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest Global Change Biology 14:1156-1168 doi:10.1111/j.1365-2486.2008.01549.x

Amelung W (2001) Methods using amino sugars as markers for microbial residues in soil. In: Assessment Methods for Soil Carbon. Lewis Publishers, Boca Raton, pp 233-272

Amelung W (2003) Nitrogen biomarkers and their fate in soil Journal of Plant Nutrition and Soil Science 166:677-686 doi:10.1002/jpln.200321274

Amelung W, Cheshire MV, Guggenberger G (1996) Determination of neutral and acidic sugars in soil by capillary gas-liquid chromatography after trifluoroacetic acid hydrolysis Soil Biology and Biochemistry 28:1631-1639

Amelung W, Kimble JM, Samson-Liebig S, Follett RF (2001a) Restoration of microbial residues in soils of the conservation reserve program Soil Sci Soc Am J 65:1704-1709 doi:10.2136/sssaj2001.1704

Amelung W, Miltner A, Zhang X, Zech W (2001b) Fate of microbial residues during litter decomposition as affected by minerals Soil Science 166:598-606

Amelung W, Zhang X (2001) Determination of amino acid enantiomers in soils Soil Biology & Biochemistry 33:553-562

Appuhn A, Joergensen RG (2006) Microbial colonisation of roots as a function of plant species Soil Biology and Biochemistry 38:1040-1051 doi:http://dx.doi.org/10.1016/j.soilbio.2005.09.002

Bååth E (2003) The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi Microbial Ecology 45:373-383 doi:10.1007/s00248-003-2002-y

Bai Z, Bodé S, Huygens D, Zhang X, Boeckx P (2013) Kinetics of amino sugar formation from organic residues of different quality Soil Biology and Biochemistry 57:814-821 doi:http://dx.doi.org/10.1016/j.soilbio.2012.08.006

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms Annual Review of Plant Biology 57:233-266 doi:doi:10.1146/annurev.arplant.57.032905.105159

Balser TC, Treseder, K. K. , Ekenler, M. (2005) Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence Soil Biology and Biochemistry 37:601-604

Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities Soil Biology and Biochemistry 31:1471-1479 doi:http://dx.doi.org/10.1016/S0038-0717(99)00051-6

Bardgett RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS, Davies WJ (1999) Plant species and nitrogen effects on soil biological properties of temperate upland grasslands Functional Ecology 13:650-660 doi:10.1046/j.1365-2435.1999.00362.x

Batjes NH (1998) Mitigation of atmospheric CO2 concentrations by increased carbon sequestration in the soil Biology and Fertility of Soils 27:230-235 doi:10.1007/s003740050425

Benbi DK, Biswas CR, Bawa SS, Kumar K (1998) Influence of farmyard manure, inorganic fertilizers and weed control practices on some soil physical properties in a long-term experiment Soil Use and Management 14:52-54 doi:10.1111/j.1475-2743.1998.tb00610.x

Berg G, Smalla K (2009a) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere FEMS Microbiology Ecology 68:1-13 doi:10.1111/j.1574-6941.2009.00654.x

Berg G, Smalla K (2009b) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere FEMS Microbiol Ecol 68:1-13 doi:10.1111/j.1574-6941.2009.00654.x

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification Canadian Journal of Biochemistry and Physiology 37:911-917 doi:10.1139/o59-099

Boas NF (1953) Method for the determination of hexosamines in tissues Journal of Biological Chemistry 204:553-563

Böhm C, Landgraf D, Makeschin F (2010) Changes in total and labile carbon and nitrogen contents in a sandy soil after the conversion of a succession fallow to cultivated land Journal of Plant Nutrition and Soil Science 173:46-54 doi:10.1002/jpln.200625189

Böhme L, Böhme F (2006) Soil microbiological and biochemical properties affected by plant growth and different long-term fertilisation European Journal of Soil Biology 42:1-12 doi:http://dx.doi.org/10.1016/j.ejsobi.2005.08.001

Bossio DA, Scow KM, Gunapala N, Graham KJ (1998) Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles Microbial Ecology 36:1-12

Breulmann M (2011) Functional soil organic matter pools and soil organic carbon stocks in grasslands - an ecosystem perspective. Universität Hannover

Broghammer A et al. (2012) Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding Proceedings of the National Academy of Sciences 109:13859-13864 doi:10.1073/pnas.1205171109

Bronick CJ, Lal R (2005) Soil structure and management: A review Geoderma 124:3-22 doi:http://dx.doi.org/10.1016/j.geoderma.2004.03.005

Buscot F, Varma A (2005) Microorganisms in soils: roles in genesis and functions. Springer,

Campbell CA, Bowren KE, Schnitzer M, Zentner RP, Townleysmith L (1991) Effect of crop rotations and fertilization on soil organic-matter and some biochemical-properties of a thick black chernozem Canadian Journal of Soil Science 71:377-387

Caravaca F, Maboreke H, Kurth F, Herrmann S, Tarkka MT, Ruess L (2015) Synergists and antagonists in the rhizosphere modulate microbial communities and growth of Quercus robur L Soil Biology and Biochemistry 82:65-73 doi:http://dx.doi.org/10.1016/j.soilbio.2014.12.004

Chantigny MH, Angers DA, Prévost D, Vézina L-P, Chalifour F-P (1997) Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems Soil Science Society of America Journal 61:262-267 doi:10.2136/sssaj1997.03615995006100010037x

Chen R et al. (2014) Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories Global Change Biology 20:2356-2367 doi:10.1111/gcb.12475

Christensen BT (1985) Carbon and Nitrogen in Particle Size Fractions Isolated from Danish Arable Soils by Ultrasonic Dispersion and Gravity-Sedimentation Acta Agriculturae Scandinavica 35:175-187 doi:10.1080/00015128509435773

Christensen BT (2001) Physical fractionation of soil and structural and functional complexity in organic matter turnover European Journal of Soil Science 52:345-353

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure Australian Journal of Ecology 18:117-143 doi:10.1111/j.1442-9993.1993.tb00438.x

Conant RT, Paustian K, Elliott ET (2001) Grassland management and conversion into grassland: Effects on soil carbon Ecological Applications 11:343-355 doi:10.1890/1051-0761(2001)011[0343:GMACIG]2.0.CO;2

Costa R, Götz M, Mrotzek N, Lottmann J, Berg G, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds FEMS Microbiology Ecology 56:236-249 doi:10.1111/j.1574-6941.2005.00026.x

Curiel Yuste J, Baldocchi DD, Gershenson A, Goldstein A, Misson L, Wong S (2007) Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture Global Change Biology 13:2018-2035 doi:10.1111/j.1365-2486.2007.01415.x

Czarnes S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport European Journal of Soil Science 51:435-443 doi:10.1046/j.1365-2389.2000.00327.x

D´Haeze W, Holsters M (2002) Nod factor structures, responses, and perception during initiation of nodule development Glycobiology 12:79R-105R doi:10.1093/glycob/12.6.79R

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments Plant and Soil 245:35-47 doi:10.1023/a:1020809400075

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change Nature 440:165-173

Davies GM, Gray A (2015) Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring) Ecology and Evolution 5:5295-5304 doi:10.1002/ece3.1782

de Graaff M-A, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates New Phytologist 188:1055-1064 doi:10.1111/j.1469-8137.2010.03427.x

Demoling F, Figueroa D, Bååth E (2007) Comparison of factors limiting bacterial growth in different soils Soil Biology and Biochemistry 39:2485-2495 doi:http://dx.doi.org/10.1016/j.soilbio.2007.05.002

Demyan MS, Rasche F, Schulz E, Breulmann M, Müller T, Cadisch G (2012) Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem European Journal of Soil Science 63:189-199 doi:10.1111/j.1365-2389.2011.01420.x

Denef K, Plante AF, Six J (2010) Characterization of soil organic matter. In: Kutsch WL, Bahn M, Heinemeyer A (eds) Soil Carbon Dynamics: An Integrated Methodology. Cambridge University Press, Cambridge, pp 91-126. doi:10.1017/CBO9780511711794.007

Derrien D, Amelung W (2011) Computing the mean residence time of soil carbon fractions using stable isotopes: impacts of the model framework European Journal of Soil Science 62:237-252 doi:10.1111/j.1365-2389.2010.01333.x

Derrien D, Marol C, Balabane M, Balesdent J (2006) The turnover of carbohydrate carbon in a cultivated soil estimated by ^{13}C natural abundances European Journal of Soil Science 57:547-557 doi:10.1111/j.1365-2389.2006.00811.x

Dexter AR, Richard G, Arrouays D, Czyż EA, Jolivet C, Duval O (2008) Complexed organic matter controls soil physical properties Geoderma 144:620-627 doi:https://doi.org/10.1016/j.geoderma.2008.01.022

Dick RP, Rasmussen PE, Kerle EA (1988) Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system Biology and Fertility of Soils 6:159-164 doi:10.1007/bf00257667

Ding X, Han X, Zhang X (2013) Long-term impacts of manure, straw, and fertilizer on amino sugars in a silty clay loam soil under temperate conditions Biology and Fertility of Soils 49:949-954 doi:10.1007/s00374-012-0768-0

Dixon NM, Kell DB (1989a) The inhibition by CO_2 of the growth and metabolism of micro-organisms Journal of Applied Microbiology 67:109-136 doi:10.1111/j.1365-2672.1989.tb03387.x

Dixon NM, Kell DB (1989b) The inhibition by CO_2 of the growth and metabolism of micro-organisms Journal of Applied Bacteriology 67:109-136

Docherty K, Balser T, Bohannan B, Gutknecht J (2012) Soil microbial responses to fire and interacting global change factors in a California annual grassland Biogeochemistry 109:63-83 doi:10.1007/s10533-011-9654-3

Dontsova KM, Norton LD, Johnston CT (2005) Calcium and magnesium effects on ammonia adsorption by soil clays Soil Science Society of America Journal 69:1225-1232 doi:10.2136/sssaj2004.0335

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance Global Change Biology 18:1781-1796 doi:10.1111/j.1365-2486.2012.02665.x

Eden M, Moldrup P, Schjønning P, Vogel H-J, Scow KM, de Jonge LW (2012) Linking soil physical parameters along a density gradient in a loess-soil long-term experiment Soil Science 177:1-11

Elmholt S, Schjønning P, Munkholm LJ, Debosz K (2008) Soil management effects on aggregate stability and biological binding Geoderma 144:455-467

Engelking B, Flessa H, Joergensen RG (2007) Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil Soil Biology and Biochemistry 39:2111-2118

Esperschütz J, Gattinger A, Mäder P, Schloter M, Fließbach A (2007) Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations FEMS Microbiology Ecology 61:26-37 doi:10.1111/j.1574-6941.2007.00318.x

Fageria NK, Baligar VC, Jones CA (2011) Growth and mineral nutrition of field crops. Books in soils, plants, and the environment. CRC, Boca Raton, FL [etc.]

Federle TW (1986) Microbial distribution in soil: New techniques. In: Perspectives in Microbial Ecology Slovene Society for Microbiology, Ljubljana, pp 493-498

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N2O production and consumption in soil Exchange of trace gases between terrestrial ecosystems and the atmosphere 47:7-21

Fischer H, Ingwersen J, Kuzyakov Y (2010) Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil European Journal of Soil Science 61:504-513 doi:10.1111/j.1365-2389.2010.01244.x

Fließbach A, Oberholzer H-R, Gunst L, Mäder P (2007) Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming Agriculture, Ecosystems & Environment 118:273-284

Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T (2016) Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies Frontiers in Microbiology 7 doi:10.3389/fmicb.2016.01446

Friedel JK, Scheller E (2002) Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass Soil Biology and Biochemistry 34:315-325

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 doi:10.1007/bf00384433

Frostegård Å, Bååth E, Tunlid A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis Soil Biology and Biochemistry 25:723-730 doi:http://dx.doi.org/10.1016/0038-0717(93)90113-P

Frostegård Å, Tunlid A, Bååth 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

Gevao B, Semple KT, Jones KC (2000) Bound pesticide residues in soils: a review Environmental Pollution 108:3-14 doi:http://dx.doi.org/10.1016/S0269-7491(99)00197-9

Ghani A, Dexter M, Perrott KW (2003) Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilisation, grazing and cultivation Soil Biology and Biochemistry 35:1231-1243

Giacometti C, Demyan MS, Cavani L, Marzadori C, Ciavatta C, Kandeler E (2013) Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems Applied Soil Ecology 64:32-48 doi:http://dx.doi.org/10.1016/j.apsoil.2012.10.002

Glaser B, Turrion MB, Alef K (2004) Amino sugars and muramic acid - biomarkers for soil microbial community structure analysis Soil Biology & Biochemistry 36:399-407 doi:10.1016/j.soilbio.2003.10.013

Gök M, Ottow JCG (1988) Effect of cellulose and straw incorporation in soil on total denitrification and nitrogen immobilization at initially aerobic and permanent anaerobic conditions Biology and Fertility of Soils 5:317-322 doi:10.1007/bf00262139

Grant WD, West AW (1986) Measurement of ergosterol, diaminopimelic acid and glucosamine in soil: Evaluation as indicators of microbial biomass Journal of Microbiological Methods 6:47-53 doi:http://dx.doi.org/10.1016/0167-7012(86)90031-X

Gregorich EG, Beare MH, McKim UF, Skjemstad JO (2006) Chemical and Biological Characteristics of Physically Uncomplexed Organic Matter Soil Science Society of America Journal 70:975-985 doi:10.2136/sssaj2005.0116

Gryndler M, Larsen J, Hršelová H, Řezáčová V, Gryndlerová H, Kubát J (2006) Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment Mycorrhiza 16:159-166 doi:10.1007/s00572-005-0027-4

Guerrant GO, Moss CW (1984) Determination of monosaccharides as aldononitrile, O-methyloxime, alditol, and cyclitol acetate derivatives by gas chromatography Analytical Chemistry 56:633-638 doi:10.1021/ac00268a010

Guggenberger G, Christensen BT, Zech W (1994) Land-use effects on the composition of organic-matter in particle-size separates of soil, 1. Lignin and carbohydrate signature European Journal of Soil Science 45:449-458

Guggenberger G, Frey SD, Six J, Paustian K, Elliott ET (1999) Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems Soil Sci Soc Am J 63:1188-1198 doi:10.2136/sssaj1999.6351188x

Guggenberger G, Zech W, Haumaier L, Christensen B (1995) Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution 13C NMR analysis European Journal of Soil Science 46:147-158 doi:10.1111/j.1365-2389.1995.tb01821.x

Gulde S, Chung H, Amelung W, Chang C, Six J (2008) Soil carbon saturation controls labile and stable carbon pool dynamics Soil Sci Soc Am J 72:605-612 doi:10.2136/sssaj2007.0251

Hartman WH, Richardson CJ (2013) Differential nutrient limitation of soil microbial biomass and metabolic quotients ($q\mathrm{CO_2}$): Is there a biological stoichiometry of soil microbes? PLoS ONE 8:e57127

Hassink J (1997) The capacity of soils to preserve organic C and N by their association with clay and silt particles Plant and Soil 191:77-87 doi:10.1023/a:1004213929699

Hassink J, Whitmore AP, Kubát J, Ittersum MKv, Geijn SCvd (1997) Size and density fractionation of soil organic matter and the physical capacity of soils to protect organic matter. In: Developments in Crop Science, vol Volume 25. Elsevier, pp 245-255. doi:http://dx.doi.org/10.1016/S0378-519X(97)80025-6

Haynes RJ, Naidu R (1998) Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review Nutrient Cycling in Agroecosystems 51:123-137 doi:10.1023/a:1009738307837

He HB, Li XB, Zhang W, Zhang XD (2011) Differentiating the dynamics of native and newly immobilized amino sugars in soil frequently amended with inorganic nitrogen and glucose European Journal of Soil Science 62:144-151 doi:10.1111/j.1365-2389.2010.01324.x

Helfrich M, Flessa H, Mikutta R, Dreves A, Ludwig B (2007) Comparison of chemical fractionation methods for isolating stable soil organic carbon pools European Journal of Soil Science 58:1316-1329 doi:10.1111/j.1365-2389.2007.00926.x

Hoffmann S, Schulz E, Csitári G, Bankó L (2006) Influence of mineral and organic fertilizers on soil organic carbon pools - Einfluss von organischer und mineralischer Düngung auf C-Pools organischer Bodensubstanz Archives of Agronomy and Soil Science 52:627 - 635

Huggins DR et al. (1998) Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management Soil and Tillage Research 47:219-234 doi:http://dx.doi.org/10.1016/S0167-1987(98)00108-1

Hurlbert SH (1984) Pseudoreplication and the Design of Ecological Field Experiments Ecological Monographs 54:187-211 doi:10.2307/1942661

IPCC IPoCC (2007) Agriculture. In: B. Metz ORD, P.R. Bosch, R. Dave, L.A. Meyer (ed) Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA,

Jangid K et al. (2008) Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems Soil Biology and Biochemistry 40:2843-2853 doi:http://dx.doi.org/10.1016/j.soilbio.2008.07.030

Janzen HH (2005) Soil carbon: A measure of ecosystem response in a changing world? Canadian Journal of Soil Science 85:467-480 doi:10.4141/s04-081

Janzen HH (2006) The soil carbon dilemma: Shall we hoard it or use it? Soil Biology and Biochemistry 38:419-424 doi:https://doi.org/10.1016/j.soilbio.2005.10.008

Jarecki MK, Lal R (2003) Crop management for soil carbon sequestration Critical Reviews in Plant Sciences 22:471-502 doi:10.1080/713608318

Jastrow JD, Amonette JE, Bailey VL (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration Clim Change 80:5-23 doi:10.1007/s10584-006-9178-3

Jenkinson D, Ladd J (1981) Microbial biomass in soil: measurement and turnover Soil biochemistry

Jenkinson DS, Poulton PR, Bryant C (2008) The turnover of organic carbon in subsoils. Part 1. Natural and bomb radiocarbon in soil profiles from the Rothamsted long-term field experiments European Journal of Soil Science 59:391-399 doi:10.1111/j.1365-2389.2008.01025.x

Joergensen R, Mäder P, Fließbach A (2010) Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism Biology and Fertility of Soils 46:303-307 doi:10.1007/s00374-009-0433-4

Joergensen R, Meyer B (1990) Chemical change in organic matter decomposing in and on a forest Rendzina under beech (Fagus sylvatica L.) European Journal of Soil Science 41:17-21

Joergensen RG, Wichern F (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil Soil Biology and Biochemistry 40:2977-2991

Kalbitz K, Angers D, Kaiser K, Chantigny M (2007) Extraction and Characterization of Dissolved Organic Matter. In: Soil Sampling and Methods of Analysis, Second Edition. CRC Press. doi:doi:10.1201/9781420005271.ch48

10.1201/9781420005271.ch48

Kalbitz K, Solinger S, Park J-H, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: A review Soil Science 165:277-304

Kallenbach CM, Frey SD, Grandy AS (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls Nature communications 7:13630

Kandeler E, Stemmer M, Klimanek E-M (1999) Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management Soil Biology and Biochemistry 31:261-273 doi:http://dx.doi.org/10.1016/S0038-0717(98)00115-1

Kästner M, Nowak KM, Miltner A, Trapp S, Schäffer A (2014) Classification and Modelling of Nonextractable Residue (NER) Formation of Xenobiotics in Soil – A Synthesis Critical Reviews in Environmental Science and Technology 44:2107-2171 doi:10.1080/10643389.2013.828270

Kästner M, Streibich S, Beyrer M, Richnow HH, Fritsche W (1999) Formation of bound residues during microbial degradation of [14C]anthracene in soil Appl Environ Microbiol 65:1834-1842

Kenne LK, Lindburg B (1983) Bacterial polysaccharides. In: In: The Polysaccharides, vol 2. Academic Press, New York, pp 287-353

Kiem R, Kögel-Knabner I (2003) Contribution of lignin and polysaccharides to the refractory carbon pool in C-depleted arable soils Soil Biology and Biochemistry 35:101-118 doi:http://dx.doi.org/10.1016/S0038-0717(02)00242-0

Kindler R, Miltner A, Richnow H-H, Kästner M (2006) Fate of gram-negative bacterial biomass in soil—mineralization and contribution to SOM Soil Biology and Biochemistry 38:2860-2870 doi:http://dx.doi.org/10.1016/j.soilbio.2006.04.047

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 doi:http://dx.doi.org/10.1016/j.orggeochem.2008.09.005

Kleber M (2010) What is recalcitrant soil organic matter? Environmental Chemistry 7:320-332

Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil carbon turnover to warming Nature 433:298-301

Kong AY, Six J, Bryant DC, Denison RF, Van Kessel C (2005) The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems Soil Science Society of America Journal 69:1078-1085

Kool DM, Chung HG, Tate KR, Ross DJ, Newton PCD, Six J (2007) Hierarchical saturation of soil carbon pools near a natural CO_2 spring Global Change Biology 13:1282-1293 doi:10.1111/j.1365-2486.2007.01362.x

Körschens M (2002) 100 Jahre Statischer Düngungsversuch Bad Lauchstädt. UFZ Umweltforschungszentrum Leipzig-Halle GmbH,

Körschens M, Pfefferkorn A (1998) Der Statische Düngungsversuch und andere Feldversuche: Bad Lauchstädt. Umweltforschungszentrum Leipzig-Halle,

Körschens M, Weigel A, Schulz E (1998) Turnover of soil organic matter (SOM) and long-term balances - Tools for evaluating sustainable productivity of soils Zeitschrift für Pflanzenernährung und Bodenkunde 161:409-424

Kuka K, Franko U, Rühlmann J (2007) Modelling the impact of pore space distribution on carbon turnover Ecological Modelling 208:295-306

Lal R (2004) Soil carbon sequestration impacts on global climate change and food security science 304:1623-1627

Lal R (2010) Enhancing eco-efficiency in agro-ecosystems through soil carbon sequestration Crop Sci 50:S-120-S-131 doi:10.2135/cropsci2010.01.0012

Lal R, Follett RF, Kimble JM (2003) Achieving soil carbon sequestration in the United States: A challenge to the policy makers Soil Science 168:827-845

Lal R, Follett RF, Stewart BA, Kimble JM (2007) Soil carbon sequestration to mitigate climate change and advance food security Soil Science 172:943-956 910.1097/ss.1090b1013e31815cc31498

Lambers H, Mougel C, Jaillard B, Hinsinger P (2009) Plant-microbe-soil interactions in the rhizosphere: An evolutionary perspective Plant and Soil 321:83-115

Landgraf D, Leinweber P, Makeschin F (2006) Cold and hot water–extractable organic matter as indicators of litter decomposition in forest soils Journal of Plant Nutrition and Soil Science 169:76-82 doi:10.1002/jpln.200521711

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota – A review Soil Biology and Biochemistry 43:1812-1836 doi:http://dx.doi.org/10.1016/j.soilbio.2011.04.022

Leigh J, Hodge A, Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material New Phytologist 181:199-207 doi:10.1111/j.1469-8137.2008.02630.x

Leinweber P, Jandl G, Baum C, Eckhardt KU, Kandeler E (2008) Stability and composition of soil organic matter control respiration and soil enzyme activities Soil Biology & Biochemistry 40:1496-1505 doi:10.1016/j.soilbio.2008.01.003

Liang C, Balser TC (2012) Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool Nat Commun 3:1222

Liang C, Cheng G, Wixon D, Balser T (2011) An Absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization Biogeochemistry 106:303-309 doi:10.1007/s10533-010-9525-3

Liang C, Fujinuma R, Balser TC (2008) Comparing PLFA and amino sugars for microbial analysis in an Upper Michigan old growth forest Soil Biology and Biochemistry 40:2063-2065

Liang C, Gutknecht JLM, Balser TC (2015) Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland Frontiers in Microbiology 6:385 doi:10.3389/fmicb.2015.00385

Liang C, Zhang X, Balser T (2007a) Net microbial amino sugar accumulation process in soil as influenced by different plant material inputs Biology and Fertility of Soils 44:1-7 doi:10.1007/s00374-007-0170-5

Liang C, Zhang X, Rubert K, Balser T (2007b) Effect of plant materials on microbial transformation of amino sugars in three soil microcosms Biology and Fertility of Soils 43:631-639 doi:10.1007/s00374-006-0142-1

Loonen H et al. (1999) Prediction of biodegradability from chemical structure: Modeling of ready biodegradation test data Environmental Toxicology and Chemistry 18:1763-1768 doi:10.1002/etc.5620180822

Lützow Mv, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions – a review European Journal of Soil Science 57:426-445 doi:10.1111/j.1365-2389.2006.00809.x

Macko SA, Uhle ME, Engel MH, Andrusevich V (1997) Stable nitrogen isotope analysis of amino acid enantiomers by gas chromatography/combustion/isotope ratio mass spectrometry Analytical Chemistry 69:926-929 doi:10.1021/ac960956l

Marschner HMPS (2012) Marschner's mineral nutrition of higher plants. Academic Press. http://www.sciencedirect.com/science/book/9780123849052.

Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment Soil Biology and Biochemistry 35:453-461

Martens DA, Reedy TE, Lewis DT (2004) Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements Global Change Biology 10:65-78 doi:10.1046/j.1529-8817.2003.00722.x

McCune B, Grace JB, Urban DL (2002) Analysis of ecological communities vol 28. MjM software design Gleneden Beach, Oregon,

McDowell WH (2003) Dissolved organic matter in soils - future directions and unanswered questions Geoderma 113:179-186

Meidute S, Demoling F, Bååth E (2008) Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources Soil Biology and Biochemistry 40:2334-2343 doi:http://dx.doi.org/10.1016/j.soilbio.2008.05.011

Merbach I, Schulz E (2012) Long-term fertilization effects on crop yields, soil fertility and sustainability in the Static Fertilization Experiment Bad Lauchstädt under climatic conditions 2001–2010 Archives of Agronomy and Soil Science 59:1041-1057 doi:10.1080/03650340.2012.702895

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M (2011) SOM genesis: microbial biomass as a significant source Biogeochemistry:1-15 doi:10.1007/s10533-011-9658-z

Miltner A, Kindler R, Knicker H, Richnow H-H, Kästner M (2009) Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter Organic Geochemistry 40:978-985

Mistou M-Y, Sutcliffe IC, van Sorge NM (2016) Bacterial glycobiology: rhamnose-containing cell wall polysaccharides in Gram-positive bacteria FEMS Microbiology Reviews 40:464-479 doi:10.1093/femsre/fuw006

Monreal CM (2015) Labile Organic Matter in Soil Solution: I. Metabolites of Chemical Signaling Pathways from Plant–Microbe Interactions. In: Labile Organic Matter—Chemical Compositions, Function, and Significance in Soil and the Environment. SSSA Special Publication, vol 62. Soil Science Society of America, Inc., Madison, WI, pp 157-172. doi:10.2136/sssaspecpub62.2014.0073

Monreal CM, Schnitzer MI (2015) Labile Organic Matter in Soil Solution: II. Separation and Identification of Metabolites from Plant–Microbial Communication in Soil Solutions of Wheat Rhizospheres. In: Labile Organic Matter—Chemical Compositions, Function, and Significance in Soil and the Environment. SSSA Special Publication, vol 62. Soil Science Society of America, Inc., Madison, WI, pp 173-194. doi:10.2136/sssaspecpub62.2014.0074

Moore JM, Klose S, Tabatabai MA (2000) Soil microbial biomass carbon and nitrogen as affected by cropping systems Biology and Fertility of Soils 31:200-210 doi:10.1007/s003740050646

Murayama S (1984) Changes in the monosaccharide composition during the decomposition of straws under field conditions Soil Science and Plant Nutrition 30:367-381 doi:10.1080/00380768.1984.10434702

Mylona P, Pawlowski K, Bisseling T (1995) Symbiotic nitrogen fixation The Plant Cell 7:869

Nannipieri P, Badalucco L (2003) Biological processes. In: Benbi DK, Nieder R (eds) Handbook of Processes and Modelling in the Soil–Plant System. Haworth Press, Binghamton, NY, pp pp. 57-82

Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon Nature 419:915 doi:10.1038/nature01136

Neidhardt F, Ingraham, J. & Schaechter, M. (1990) Physiology of the Bacterial Cell: A Molecular Approach. Sinauer Associates, MA, U.S.A

Ngosong C, Jarosch M, Raupp J, Neumann E, Ruess L (2010) The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization Applied Soil Ecology 46:134-142

Norman AG (1950) Advances in Agronomy. vol Bd. 7. Elsevier Science,

O'Donnell A, Seasman M, Macrae A, Waite I, Davies J (2001) Plants and fertilisers as drivers of change in microbial community structure and function in soils Plant and Soil 232:135-145 doi:10.1023/a:1010394221729

Oades J (1984) Soil organic matter and structural stability: Mechanisms and implications for management Plant and Soil 76:319-337 doi:10.1007/bf02205590

Oades JM, Kirkman MA, Wagner GH (1970) The use of gas-liquid chromatography for the determination of sugars extracted from soils by sulfuric acid Soil Sci Soc Am J 34:230-235 doi:10.2136/sssaj1970.03615995003400020017x

Vegan: Community Ecology Package." R package version XXX: pp 117e118. (2011) http://CRANRprojectorg/.

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activityby N and P availability Biogeochemistry 49:175-191 doi:10.1023/a:1006316117817

Olsson PA, Bååth E, Jakobsen I (1997) Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of Fatty Acid signatures Appl Environ Microbiol 63:3531-3538

Pan B, Smith DL (2000) Preincubation of B. japonicum cells with genistein reduces the inhibitory effects of mineral nitrogen on soybean nodulation and nitrogen fixation under field conditions Plant and Soil 223:237-244 doi:10.1023/a:1004832422665

Paterson E, Midwood AJ, Millard P (2009) Through the eye of the needle: A review of isotope approaches to quantify microbial processes mediating soil carbon balance New Phytologist 184:19-33

Paustian K et al. (1997a) Agricultural soils as a sink to mitigate CO_2 emissions Soil Use and Management 13:230-244 doi:10.1111/j.1475-2743.1997.tb00594.x

Paustian K, Collins HP, Paul EA (1997b) Management controls on soil carbon. In: al. EAPe (ed) Soil organic matter in temperate agroecosystems: Long-term experiments in North America. CRC Press, Boca Raton, FL, pp 15-49

Peacock GA, Goosey MW (1989) Separation of fungal sterols by normal-phase high-performance liquid chromatography : Application to the evaluation of ergosterol biosynthesis inhibitors Journal of Chromatography A 469:293-304 doi:http://dx.doi.org/10.1016/S0021-9673(01)96464-7

Poirier N, Sohi SP, Gaunt JL, Mahieu N, Randall EW, Powlson DS, Evershed RP (2005) The chemical composition of measurable soil organic matter pools Organic Geochemistry 36:1174-1189

Powlson DS, Stirling CM, Jat ML, Gerard BG, Palm CA, Sanchez PA, Cassman KG (2014) Limited potential of no-till agriculture for climate change mitigation Nature Climate Change 4:678 doi:10.1038/nclimate2292

Powlson DS, Whitmore AP, Goulding KWT (2011) Soil carbon sequestration to mitigate climate change: A critical re-examination to identify the true and the false European Journal of Soil Science 62:42-55 doi:10.1111/j.1365-2389.2010.01342.x

RCoreTeam (2012) R: A language and environment for statistical computing. Vienna, Austria

Richnow HH, Eschenbach A, Mahro B, Kästner M, Annweiler E, Seifert R, Michaelis W (1999) Formation of Nonextractable Soil Residues: A Stable Isotope Approach Environmental Science & Technology 33:3761-3767 doi:10.1021/es980927n

Rinaudi L, Fujishige NA, Hirsch AM, Banchio E, Zorreguieta A, Giordano W (2006) Effects of nutritional and environmental conditions on *Sinorhizobium meliloti* biofilm formation Research in Microbiology 157:867-875 doi:http://dx.doi.org/10.1016/j.resmic.2006.06.002

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization Appl Environ Microbiol 75:1589-1596 doi:10.1128/aem.02775-08

Rousk J, Brookes PC, Bååth E (2010) The microbial PLFA composition as affected by pH in an arable soil Soil Biology and Biochemistry 42:516-520 doi:http://dx.doi.org/10.1016/j.soilbio.2009.11.026

Rüde E, Goebel WF (1962) Colicine K: V. The somatic antigen of a non-colicinogenic variant of *E. Coli* K235 The Journal of Experimental Medicine 116:73-107 doi:10.1084/jem.116.1.73

Russell NJ, Fukunaga N (1990) A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria FEMS Microbiology Letters 75:171-182 doi:10.1111/j.1574-6968.1990.tb04093.x

Said-Pullicino D, Kaiser K, Guggenberger G, Gigliotti G (2007) Changes in the chemical composition of water-extractable organic matter during composting: Distribution between stable and labile organic matter pools Chemosphere 66:2166-2176 doi:http://dx.doi.org/10.1016/j.chemosphere.2006.09.010

Schieber A, Brückner H, Ling JR (1999) GC–MS analysis of diaminopimelic acid stereoisomers and amino acid enantiomers in rumen bacteria Biomedical Chromatography 13:46-50 doi:10.1002/(sici)1099-0801(199902)13:1<46::aid-bmc808>3.0.co;2-4

Schjønning P, de Jonge LW, Moldrup P, Christensen BT, Olesen JE Searching the critical soil organic carbon threshold for satisfactory tilth conditions–test of the Dexter clay: carbon hypothesis. In: Proceedings of the 1st international conference and exploratory workshop on soil architecture and physico-chemical functions" Cesar", 2010. Aarhus University, Faculty of Agricultural Sciences, pp 341-346

Schmidt J, Schulz E, Michalzik B, Buscot F, Gutknecht JLM (2015) Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon Soil Biology and Biochemistry 85:39-50 doi:http://dx.doi.org/10.1016/j.soilbio.2015.02.024

Schmidt L, Warnstorff K, Dörfel H, Leinweber P, Lange H, Merbach W (2000) The influence of fertilization and rotation on soil organic matter and plant yields in the long-term Eternal Rye trial in Halle (Saale), Germany Journal of Plant Nutrition and Soil Science 163:639-648 doi:10.1002/1522-2624(200012)163:6<639::aid-jpln639>3.0.co;2-l

Schmidt MW et al. (2011) Persistence of soil organic matter as an ecosystem property Nature 478:49

Schnürer J, Clarholm M, Rosswall T (1985) Microbial biomass and activity in an agricultural soil with different organic matter contents Soil Biology and Biochemistry 17:611-618 doi:http://dx.doi.org/10.1016/0038-0717(85)90036-7

Schulz E (2002) Influence of extreme management on decomposable soil organic matter pool Archives of Agronomy and Soil Science 48:101 - 105

Schulz E (2004) Influence of site conditions and management on different soil organic matter (SOM) pools Archives of Agronomy and Soil Science 50:33 - 47

Schulz E, Breulmann M, Boettger T, Wang KR, Neue HU (2011) Effect of organic matter input on functional pools of soil organic carbon in a long-term double rice crop experiment in China European Journal of Soil Science 62:134-143 doi:10.1111/j.1365-2389.2010.01330.x

Schulz E, Körschens M (1998) Characterization of the decomposable part of soil organic matter (SOM) and transformation processes by hot water extraction Eurasian Soil Science 31:809-813

Senesi N (1992) Binding mechanisms of pesticides to soil humic substances Science of The Total Environment 123:63-76 doi:http://dx.doi.org/10.1016/0048-9697(92)90133-D

Senwo ZN, Tabatabai MA (1998) Amino acid composition of soil organic matter Biology and Fertility of Soils 26:235-242 doi:10.1007/s003740050373

Sharma S, Aneja MK, Mayer J, Munch JC, Schloter M (2005) Characterization of bacterial community structure in rhizosphere soil of grain legumes Microbial Ecology 49:407-415 doi:10.1007/s00248-004-0041-7

Shaymukhametov MS, Titova NA, Travnikova LS, Labenets YM (1985) Use of physical fractionation methods to characterize soil organic matter Pochvovedeniye 8:131–141

Shen S, Tu S-I, Kemper WD (1997) Equilibrium and kinetic study of ammonium adsorption and fixation in sodium-treated vermiculite Soil Science Society of America Journal 61:1611-1618

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics Soil and Tillage Research 79:7-31 doi:http://dx.doi.org/10.1016/j.still.2004.03.008

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils Plant and Soil 241:155-176

Smith J, Paul E, Bollag J, Stotzky G (1990) The significance of soil microbial biomass estimations Soil biochemistry Volume 6:357-396

Smith P et al. (2014) Agriculture, Forestry and Other Land Use (AFOLU). In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA,

Smith P et al. (2008) Greenhouse gas mitigation in agriculture Philosophical Transactions of the Royal Society B: Biological Sciences 363:789-813 doi:10.1098/rstb.2007.2184

Smol´kina ON, Shishonkova NS, Yurasov NA, Ignatov VV (2012) Capsular and extracellular polysaccharides of the diazotrophic rhizobacterium *Herbaspirillum seropedicae* Z78 Microbiology 81:317-323 doi:10.1134/s0026261712030113

Smolander A, Kurka A, Kitunen V, Mälkönen E (1994) Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N-and P-fertilized Norway spruce stands Soil Biology and Biochemistry 26:957-962 doi:http://dx.doi.org/10.1016/0038-0717(94)90109-0

Solberg EDN, M.; Izairralde, R.C.; Malhi, S.S.; Jansen, H.H.; Molina-Ayala, M. (1998) Carbon storage in soils under continuous cereal grain cropping: N fertilizer and straw. In: Management of carbon sequestration in soil. CRC Press Inc., Boca Raton, pp 235-254

Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter: Mechanisms and controls Geoderma 74:65-105 doi:http://dx.doi.org/10.1016/S0016-7061(96)00036-5

Solomon D, Lehmann J, Zech W (2001) Land use effects on amino sugar signature of chromic Luvisol in the semi-arid part of northern Tanzania Biology and Fertility of Soils 33:33-40

Sparling G, Vojvodic-Vukovic M, Schipper L (1998) Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C Soil Biology and Biochemistry 30

Spohn M, Giani L (2010) Water-stable aggregates, glomalin-related soil protein, and carbohydrates in a chronosequence of sandy hydromorphic soils Soil Biology and Biochemistry 42:1505-1511

Staehelin C, Schultze M, Kondorosi E, Kondorosi A (1995) Lipo-chitooligosaccharide nodulation signals from *Rhizobium meliloti* induce their rapid degradation by the host plant alfalfa Plant Physiology 108:1607-1614 doi:10.1104/pp.108.4.1607

Stahl PD, Klug MJ (1996) Characterization and differentiation of filamentous fungi based on fatty acid composition Appl Environ Microbiol 62:4136-4146

Stapleton LM, Crout NMJ, Säwström C, Marshall WA, Poulton PR, Tye AM, Laybourn-Parry J (2005) Microbial carbon dynamics in nitrogen amended Arctic tundra soil: Measurement and model testing Soil Biology and Biochemistry 37:2088-2098 doi:http://dx.doi.org/10.1016/j.soilbio.2005.03.016

Stewart CE, Plante AF, Paustian K, Conant RT, Six J (2008) Soil carbon saturation: Linking concept and measurable carbon pools Soil Sci Soc Am J 72:379-392 doi:10.2136/sssaj2007.0104

Talbot JM, Treseder KK (2010) Controls over mycorrhizal uptake of organic nitrogen Pedobiologia 53:169-179 doi:http://dx.doi.org/10.1016/j.pedobi.2009.12.001

Tiedje JM, Sexstone AJ, Parkin TB, Revsbech NP (1984) Anaerobic processes in soil Plant and Soil 76:197-212 doi:10.1007/bf02205580

Totsche KU et al. (2018) Microaggregates in soils Journal of Plant Nutrition and Soil Science 181:104-136 doi:doi:10.1002/jpln.201600451

Treseder KK (2008) Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies Ecology Letters 11:1111-1120 doi:10.1111/j.1461-0248.2008.01230.x

Trujillo ME, Alonso-Vega P, Rodriguez R, Carro L, Cerda E, Alonso P, Martinez-Molina E (2010) The genus Micromonospora is widespread in legume root nodules: The example of *Lupinus angustifolius* ISME J 4:1265-1281 doi:http://www.nature.com/ismej/journal/v4/n10/suppinfo/ismej201055s1.html

Ueda K, Morgan SL, Fox A, Gilbart J, Sonesson A, Larsson L, Odham G (1989) D-Alanine as a chemical marker for the determination of streptococcal cell wall levels in mammalian tissues by gas chromatography/negative ion chemical ionization mass spectrometry Analytical Chemistry 61:265-270 doi:10.1021/ac00178a015

Van Wesemael B, Paustian K, Meersmans J, Goidts E, Barancikova G, Easter M (2010) Agricultural management explains historic changes in regional soil carbon stocks Proceedings of the National Academy of Sciences 107:14926-14930

Vasse J, de Billy F, Truchet G (1993) Abortion of infection during the *Rhizobium meliloti*—alfalfa symbiotic interaction is accompanied by a hypersensitive reaction The Plant Journal 4:555-566 doi:10.1046/j.1365-313X.1993.04030555.x

Veen JA, Kuikman PJ (1990) Soil structural aspects of decomposition of organic matter by micro-organisms Biogeochemistry 11:213-233 doi:10.1007/bf00004497

Vestal JR, White DC (1989) Lipid analysis in microbial ecology BioScience 39:535-541

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi Appl Environ Microbiol 64:5004-5007

von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms Soil Biology and Biochemistry 39:2183-2207 doi:http://dx.doi.org/10.1016/j.soilbio.2007.03.007

Wander M (2004) Soil organic matter fractions and their relevance to soil function. In: Soil Organic Matter in Sustainable Agriculture. Advances in Agroecology. CRC Press. doi:doi:10.1201/9780203496374.ch3

Wang Y, Hollingsworth RI (1994) The structure of the O-antigenic chain of the lipopolysaccharide of *Rhizobium trifolii* 4s Carbohydrate Research 260:305-317 doi:http://dx.doi.org/10.1016/0008-6215(94)84048-2

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, Van Der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota Science 304:1629-1633

Wei X, Shao M, Gale W, Li L (2014) Global pattern of soil carbon losses due to the conversion of forests to agricultural land Scientific reports 4

Weigel A, Kubat J, Körschens M, Powlson DS, Mercik S (1998) Determination of the decomposable part of soil organic matter in arable soils Archives of Agronomy and Soil Science 43:123-143 doi:10.1080/03650349809366031

Wendisch VF (2007) Amino acid biosynthesis - pathways, regulation and metabolic engineering vol 5. Microbiology Monographs. Springer Berlin Heidelberg. doi:10.1007/978-3-540-48596-4

White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Determination of the sedimentary microbial biomass by extractible lipid phosphate Oecologia 40:51-62 doi:10.1007/BF00388810

Whiteside MD, Digman MA, Gratton E, Treseder KK (2012a) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest Soil Biology and Biochemistry 55:7-13 doi:http://dx.doi.org/10.1016/j.soilbio.2012.06.001

Whiteside MD, Garcia MO, Treseder KK (2012b) Amino Acid Uptake in Arbuscular Mycorrhizal Plants PLoS ONE 7:e47643

Whiteside MD, Treseder KK, Atsatt PR (2009) The brighter side of soils: Quantum dots track organic nitrogen through fungi and plants Ecology 90:100-108 doi:10.1890/07-2115.1

Widmer F, Rasche F, Hartmann M, Fliessbach A (2006) Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment Applied Soil Ecology 33:294-307 doi:http://dx.doi.org/10.1016/j.apsoil.2005.09.007

Wiesmeier M et al. (2014) Carbon sequestration potential of soils in southeast Germany derived from stable soil organic carbon saturation Global Change Biology 20:653-665 doi:10.1111/gcb.12384

Wilkinson SC, Anderson JM, Scardelis SP, 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

Wilkinson SG (1988) Gram-negative bacteria. In: In: Microbial Lipids, vol 1. Academic Press, London, pp 299-457

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments Ecology Letters 12:452-461 doi:10.1111/j.1461-0248.2009.01303.x

Witter E, Mårtensson A, Garcia F (1993) Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures Soil Biology and Biochemistry 25:659-669

Wixon DL, Balser TC (2013) Toward conceptual clarity: PLFA in warmed soils Soil Biology and Biochemistry 57:769-774

WorldBank (2015) Agricultural land (% of land area).

Zelles L (1999) 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 doi:10.1007/s003740050533

Zelles L, Bai QY, 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 doi:http://dx.doi.org/10.1016/0038-0717(92)90191-Y

Zhang B, Drury CF, Yang X, Reynolds D, Zhang X (2014) Crop rotation, red clover and cropping history influences microbial amino sugars of a clay loam soil Soil Science Society of America Journal 78 doi:10.2136/sssaj2013.03.0098

Zhang B, Liang C, He H, Zhang X (2013) Variations in soil microbial communities and residues along an altitude gradient on the northern slope of Changbai Mountain, China PLoS ONE 8:e66184 doi:10.1371/journal.pone.0066184

Zhang W, He H, Zhang X (2007) Determination of neutral sugars in soil by capillary gas chromatography after derivatization to aldononitrile acetates Soil Biology and Biochemistry 39:2665-2669

Zhang X, Amelung W, Yuan Y, Samson-Liebig S, Brown L, Zech W (1999) Land-use effects on amino sugars in particle size fractions of an Argiudoll Applied Soil Ecology 11:271-275

Zhang XD, Amelung W (1996) Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils Soil Biology & Biochemistry 28:1201-1206

Zhang XD, Amelung W, Ying YA, Zech W (1998) Amino sugar signature of particle-size fractions in soils of the native prairie as affected by climate Soil Science 163:220-229

Zsolnay Á (1996) Dissolved humus in soil waters. In: In: Humic substances in terrestrial ecosystems. Elsevier, Amsterdam, pp 171-224

9. Author Contributions

Erklärung zu den Eigenanteilen der Promovendin sowie der Koautoren an den Publikationen und Zweitpublikationsrechten bei einer kumulativen Dissertation

Für alle in dieser kumulativen Dissertation verwendeten Manuskripte liegen die notwendigen Genehmigungen der Verlage ("Reprint permissions") für die Zweitpublikation vor.

Die Co-Autoren der in dieser kumulativen Dissertation verwendeten Manuskripte sind sowohl über die Nutzung, als auch über die oben angegebenen Eigenanteile informiert und stimmen dem zu. Die Anteile der Promovendin sowie der Co-Autoren an den Publikationen und Zweitpublikationsrechten sind im Folgenden aufgeführt.

Jana Schmidt   26.07.2018   Halle/Saale

"Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon." *Soil Biology and Biochemistry* 85 (2015): 39-50.					
	Jana Schmidt First author	**Dr. Elke Schulz** Co-Author	**Prof. Dr. Beate Michalzik** Co-Author	**Prof. Dr. François Buscot** Co-Author	**Dr. Jessica Gutknecht** Co-Author
Konzeption	X	x	x	x	x
Planung der Untersuchungen	X	x	x		x
Datenerhebung	X	x (C, N, HWC, HWN, pH, NO_3^-, NH_4^+)		x	
Datenanalyse- und interpretation	X				x
Schreiben des Manuskripts	X				
Korrekturlesen des Manuskripts		x	x	x	x
Vorschlag Anrechnung Publikationsäquivalente	1.0				

"Effects of plant-symbiotic relationships on the living soil microbial community and microbial necromass in a long-term agro-ecosystem." *Science of the Total Environment* 581 (2017): 756-765.						
	Jana Schmidt First author	Dr. Thomas Fester Co-Author	Dr. Elke Schulz Co-Author	Prof. Dr. Beate Michalzik Co-Author	Prof. Dr. François Buscot Co-Author	Dr. Jessica Gutknecht Co-Author
Konzeption	X	x	x	x	x	x
Planung der Untersuchungen	X	x	x		x	x
Datenerhebung	x	x (root nodules and AMF-colonization rates)	x (C, N, HWC, HWN)			
Datenanalyse- und interpretation	x	x				
Schreiben des Manuskripts	x					
Korrekturlesen des Manuskripts		x	x	x	x	x
Vorschlag Anrechnung Publikationsäquivalente	1.0					

" Biomarker levels of soil organic matter fractions indicate long-term fertilization effects on C stabilization and turnover" (submitted)					
	Jana Schmidt First author	Dr. Jessica Gutknecht Co-Author	Prof. Dr. Beate Michalzik Co-Author	Prof. Dr. François Buscot Co-Author	Dr. Elke Schulz Co-Author
Konzeption	x		x		x
Planung der Untersuchungen	x				x
Datenerhebung	x				x (C, N, HWC, HWN)
Datenanalyse- und interpretation	x				x
Schreiben des Manuskripts	x				
Korrekturlesen des Manuskripts		x		x	x
Vorschlag Anrechnung Publikationsäquivalente	1.0				

10. Erklärungen

Selbständigkeitserklärung

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

Halle, 26.07.2018

Jana Schmidt