Crop residue decomposition and stabilization in soil organic matter

Dissertation

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Submitted by

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To my parents

and my son

Arham Shahbaz

Preface

The German Academic Exchange Service (DAAD) provided a scholarship for the doctoral study at the Georg-August University of Göttignen. Subsequent research work was funded by Deutsche Forschungsgemeinschaft (DFG). The dissertation is being submitted to the Faculty of Agricultural Sciences under the doctoral degree program of Graduate School in Forest and Agriculture Sciences (GFA), to fulfill the requirements for the acquisition of the doctoral degree of agricultural sciences 'Doctor scientiarum agrariarum' (Dr. sc. agr.).

The presented dissertation is cumulative based on three papers as the first author, which are published in the international refereed journals, and on an additional study (study 4, in preparation). The published manuscripts are included in chapters 3.

The focus of the general introduction (chapter 1) is on the theoretical background of the soil organic matter and the need of work. Chapter 2 presents the objectives, study site, methods and summary of the main results. Whereas specific introductions on the effects of crop residue decomposition and stabilization in soil organic matter are given in chapter 3 comprising following manuscripts.

Chapter 3.1

Shahbaz M, Kuzyakov Y, Heitkamp F: Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls. *Geoderma* (2016). doi: org/10.1016/j.geoderma.2016.05.019

Chapter 3.2

Shahbaz M, Kuzyakov Y, Sanaullah M, Heitkamp F, Zelenev V, Kumar A, Blagodatskaya E (2016) Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. *Biology and Fertility of Soils* (2016). doi: 10.1007/s00374-016-1174-9

Chapter 3.3

Shahbaz M, Kuzyakov Y, Maqsood M, Wendland M, Heitkamp F: Decadal nitrogen fertilization decreases mineral-associated and subsoil carbon: a 32 years study. *Land degradation and development* (2016). doi: 10.1002/ldr.2667

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V List of abbreviations

ANOVA	Analysis of variance
At%	Atom%
C	Carbon
^{13}C	Stable carbon isotope with atomic mass 13
^{14}C	Radioactive carbon isotope with atomic mass 14
CO_2	Carbon dioxide
C/N	Carbon to nitrogen ratio
EA-IRMS	Elemental analysis - isotope ratio mass spectrometry
f-LF	Free light fraction (ρ <1.6 g cm^{-3})
ha	Hectare
HF	Mineral-associated heavy fraction (ρ >1.6 g cm^{-3})
IUSS-WRB	International Union of Soil Sciences-World Reference Base
K	Potassium
LAP	Leucine aminopeptidase
MB	Microbial biomass
N	Nitrogen
o-LF	Occluded light fraction (ρ <1.6 g cm^{-3})
P	Phosphorus
PE	Priming effect
Res_C_{MB}	Residue originated microbial biomass carbon
rpm	rotation per minute
SE	Standard error
SOM	Soil organic matter
WHC	Water holding capacity
y^{-1}/yr^{-1}	Per year
ρ	Density

VI Summary

Cropland soils may be sources or sinks for atmospheric CO_2. In general, it is assumed that C input into the soil and soil organic matter (SOM) levels are linearly related. This gives rise to environmental concerns regarding the removal of crop residue. In recent years, it has been shown that residue incorporation increases SOM levels to only a small extent, and high C input is not directly beneficial for SOM stabilization. Similar observations have been reported from a well-documented long-term field experiment at Puch, Germany, which contradicted the predicted (linear) relationship between C inputs (1-5 Mg C ha^{-1} y^{-1}) and SOM changes. Several factors have been suggested to explain the relationship between high C inputs and small observed increases of SOM: (i) alteration of soil physical properties, affecting residue mineralization and protection; (ii) differences in residue input quality, recalcitrant belowground versus labile aboveground inputs; (iii) decomposition of native SOM through priming effects of incorporated residues; (iv) partitioning of residue C between protected and less protected SOM fraction; and (v) translocation of part of the unprotected C to the subsoil. The aim of this thesis was to ascertain whether these factors can really explain the limited increases of SOM often observed in the context of increasing crop residue inputs.

In order to quantify the effect of crop residue quality and quantity on soil physical structure and SOM stabilization, ^{13}C-labeled wheat residues with variable quality (leaves, stems, roots) and quantity were added to the soil and incubated for 2 months. Soil aggregation generally increased with higher residue additions, but the proportion of residue C protected within aggregates decreased. The protection of aboveground biomass residues (leaves and stems) was more reduced than belowground (root) residues at high additions. However, regardless of residue type, SOM decomposition increased with higher crop residue addition. The decrease of residue protection within aggregates and the increase of SOM mineralization led to a decrease in the rate of C stabilization within SOM by higher residue additions.

To explore the mechanisms how crop residue quality (leaves, stems, roots) and quantity effect residue and SOM mineralization, with a special focus on the priming effect, an incubation study was conducted over a period of 4 months. The added C was traced in CO_2 and in microbial biomass, and enzyme activities were measured. Roots were least decomposed and the mineralization of aboveground biomass residue disproportionally increased with higher residue additions. However, roots caused much higher SOM priming than leaves and stems. The C

source partitioning and enzyme activities revealed that SOM priming was mainly controlled by residue-feeding microorganisms. To quantify the relationship between residue decomposition (i.e. quality effect), input levels, and priming, a new unifying model (logistic & power functions) was proposed. The model enabled the estimation of threshold values for mineralization of low and high residue additions above which incremental priming was maximal: i.e. ca. 20% for roots, 29-44% for stems and 39-51% for leaves. SOM priming depended on residue quality and decreased with increasing C additions. Nonetheless, priming was a power function of residue mineralization, whereby the threshold for strong increases in priming was lower for root decomposition than for aboveground residues.

In order to determine the effect of long-term C inputs (straw- or root-dominated) on changes in SOM contents and partitioning of added C between SOM fractions, the soil was sampled (top- and subsoil) from a field experiment started in 1983. Where, five organic amendments (either with straw or root dominated C inputs) were combined with different N fertilization rates. C input driven by straw incorporation was highest and increased with N fertilization. The density fractionation approach was used to separate topsoil SOM fractions. Total SOM content showed an increase with C inputs, which was mainly explained by the free light fraction of SOM. Despite high inputs, straw contributed little to the free light fraction, but prevented C losses from the mineral-associated SOM fraction (ρ >1.6 g cm^{-3}), which were observed in the absence of straw addition. In contrast to topsoil, subsoil SOM contents decreased with N fertilization, thus also with C input. Above- (straw) and belowground (root) residues showed opposite effects on SOM fractions. Root C remained longer in the light fractions and was responsible for topsoil SOM increase with N fertilization. Straw decomposed rapidly (from light fractions), and sustained the most stable mineral-associated SOM fraction.

Overall, results from incubation studies and the field experiment reveal that increasing amounts of aboveground residue addition improve soil aggregation. However, low physical protection and disproportionally increased residue mineralization decreases residue stabilization in SOM. Roots are recalcitrant to decomposition, but cause stronger and higher priming effects than aboveground residues. Nevertheless, high aboveground residue mineralization protects C in the most stable mineral-associated SOM fraction. Low root mineralization indicates that root litter can mainly stay in the unprotected free light SOM fraction, but roots can increase SOM losses through priming effects. The often described minor increase of SOM after organic matter input reflects the opposing behaviors of root and aboveground residues in SOM stabilization.

1 General introduction

1.1 Global carbon cycle

In the last few decades, the soil has been intensively cultivated to meet the global demands of the growing population for food, fodder, fibers and biofuel (Garnett et al. 2013; Keating et al. 2014). Simultaneously, it is important that any change in land management must maintain soil quality and while not increasing its negative impacts on the environment, such as greenhouse gas emissions. Compared to soil carbon (C), the global distribution of phytomass C and its relation to the environment is relatively well researched (Harris et al. 2012). The soil C exceeds the amounts which is stored in both plants and atmosphere (Scharlemann et al. 2014). Despite a great deal of research, estimates of global soil C sources and resources are still uncertain, and C emissions due to land use changes remain the least-understood component of the C cycle (Scharlemann et al. 2014).

Globally, approximately 1500 Petagrams of C is stored in the soil (Pg C, Scharlemann et al. 2014; Schlesinger 1984). To understand the background of increasing atmospheric CO_2 levels, there is a much interest in knowing whether the soil acts as a net source or sink for C (IPCC 2007; Krull et al. 2003). Since 1850, after the industrial revolution, it is estimated that ca. 108 to 188 Pg C has been lost from the terrestrial ecosystem due to the rapid increase in population and associated land use changes (Houghton 2012). Although there are considerable disagreements between estimates of global C pool sizes, however, it is accepted that approximately 68-100 gigatons C per year is released to the atmosphere from various ecosystems.

Within this C loss pool, the contribution of C evolved from agriculture (i.e. due to land use changes) ranged from approximately 25-50% (Houghton 2012). These losses due to land use changes represents the second largest anthropogenic C source released into the atmosphere, after fossil fuel combustion (Lal 2004; Post and Kwon 2000). Perhaps as late as the 1950s, land use changes accounted for higher levels of anthropogenic CO_2 emissions than fossil fuel combustion (Lal et al. 2012).

Soil C losses can theoretically be mitigated by adopting recommended management practices (Lal et al. 2012; Sauerbeck 2001). Smith et al. (2013) estimated that, until 2050, agriculture holds the potential to mitigate the release of up to 4.3 gigatons yr^{-1} of CO_2 to the atmosphere by adopting proper mitigation options. However, some mitigation options are in direct competition

with each other, e.g., use of crop residue for bioenergy *versus* crop residue incorporation into the soil for increasing and maintaining SOM levels.

In cropland soils, the main source of biomass is crop residues (Lal 2012). Crop residue is mainly incorporated into the soil with the aim of improving SOM and soil quality. A fraction of added crop residues can be stabilized as SOM by various mechanisms, while the other fraction will be lost to the environment *via* microbial decomposition. The effectiveness of SOM stabilization depends on the quality and quantity of biomass returned to the soil. Therefore, it is important to investigate the effect of crop residue management on SOM stabilization and the potential losses of soil C (as CO_2) following residue addition for sustaining both soil and environmental quality.

1.2 Soil organic matter stabilization

SOM has beneficial effects on soil physical, chemical and biological properties, which in turn influence the productive capacity of soils. SOM is also a major contributor of N, P and other nutrients to plants. Soil microbial communities are dependent on SOM as a C source for their metabolic activities, which in turn affects soil structure and nutrient fluxes. A majority of models used to predict SOM dynamics assume that the increase of SOM is linearly proportional to the amount of C input (Six et al. 2002). Thus, SOM levels can theoretically increase without limits, given that C inputs correspondingly increase without limit. Such predictions of SOM content dynamics are acceptable for soils possessing low to moderate C contents. However, changes in SOM content resulting from, C inputs usually depends on the amount and nature of inputs (C availability), soil physicochemical properties, management practices and native SOM conditions (e.g. Powlson and Glendining 2011; Heitkamp et al. 2012). Native SOM contents reflect the balance of C inputs and its losses under specific conditions that do not necessarily represent the maximum ability of a soil to stabilize SOM. The relationship between soil structure and its ability to stabilize C is a key element for understanding SOM protection (Six et al. 2002). There is a distinction between SOM which is protected against decomposition by various mechanisms from that which is not protected from decomposition.

The protected SOM pools mainly represent the contents of SOM (affected by long-term inputs) and are often characterized by three main protection mechanisms: (i) physical protection, (ii) chemical stabilization, and (iii) biochemical stabilization (Christensen 1996; Six et al. 2002; Six and Paustian 2014). Physical protection by soil aggregates (especially microaggregates) is indicated by the positive influence of aggregation on SOM accumulation (Elliott 1986; Tisdall

and Oades 1982; Six et al. 2002). Aggregates protect SOM by forming physical barriers between microbes, enzymes and their substrate's diffusion, as well as by controlling food web interactions and consequently SOM decomposition (Six et al. 2000). Chemical stabilization of SOM is known to be the result of the chemical or physicochemical binding of SOM with soil mineral surfaces (i.e. clay and silt particles). The relationship of organic C stabilization with soils mineral surfaces is often defined as mineral-associated SOM, which has long mean residence times and comprises a large proportion of SOM in cropland soils (Hassink 1997). Biochemical stabilization is understood as the stabilization of SOM due to its own chemical composition (e.g. recalcitrant compounds such as polyphenols and lignin) and through chemical association processes (e.g. condensation reactions) in soil. This mechanism is mostly referred to as biochemical stabilization of SOM through selective protection of recalcitrant SOM compounds. However, all of these mechanisms can only stabilize SOM up to a certain limit. Thereafter, increasing C inputs may lead to high losses of added organic compounds through mineralization processes. Nevertheless, environmental conditions, management practices and soil physicochemical characteristics may have a strong impact on SOM stabilization by affecting those mechanisms which can limit C sequestration under high C inputs.

1.3 Impact of long-term C inputs on SOM

Under long-term field conditions, in contrary to general assumptions, SOM does not increase linearly with increasing C inputs (primarily crop residues) (Heitkamp et al. 2012; Stewart et al. 2007) (e.g. Fig. 1-1). This means that, while SOM may continue to increase with increasing C input, the efficiency of the C input to SOM conversion decreases (i.e. increase in SOM is smaller per unit of C input). According to specific conditions, there are several factors which can affect SOM stabilization that need to be addressed. For example, Freibauer et al. (2004) reported that, for the EU-15 countries, the addition of 1 to 3.7 Mg C ha^{-1} yr^{-1} (by farmyard manure and straw inputs) resulted in increases in SOM stocks of 0.4 and 0.7 Mg C ha^{-1} yr^{-1} relative to non-amended cropland soils. However, Powlson and Glendining (2011) reported that in most of their 23 long-term experiments, straw incorporation (as compared to removal) had minor effects on SOM storage. Likewise, Stewart et al. (2007) tested C predictions models and found that an asymptotic relationship can best predict the change in SOM against C inputs within their dataset of 14 experimental sites located in Canada and the US (duration: 12-50 years). More recently, Steinmann et al. (2016) found a loss of SOM ca. 0.6 t C ha^{-1} yr^{-1} (at 60 cm depth) even under balanced C input (from 20 to 133 kg C ha^{-1} yr^{-1}) across 268 sites in 8

years. In general, these findings primarily indicate that increasing the amount of C input does not result in proportional increases in SOM content, as predicted in several studies (Fig. 1-1).

Similarly, Heitkamp et al. (2012) also observed a quadratic relationship between the change in SOM stocks and C-input in a long-term cereal-based crop rotation experiment in Puch, Germany. The organic additions ranged from 1-5 Mg C ha^{-1} yr^{-1}, where C inputs driven by animal manure were fixed, while those by straw incorporation were increased with N fertilization levels. Their findings were in agreement with previous works (Paustian et al. 2000; Lorenz and Lal 2012), demonstrating a serious decline in SOM increase when annual C input exceeded 2.4 Mg C ha^{-1}.

Overall, the authors could only speculate about the rational of this relationship (see below), but it was clear that increasing amounts of C input (e.g. from straw) were not efficient in increasing SOM levels (Fig. 1-1). This could give rise to the idea that, e.g., energetic use of crop residue would be a more efficient option in mitigating greenhouse gas (CO_2) emissions (because of less C stabilization and high mineralization) compared to residue incorporation in soil.

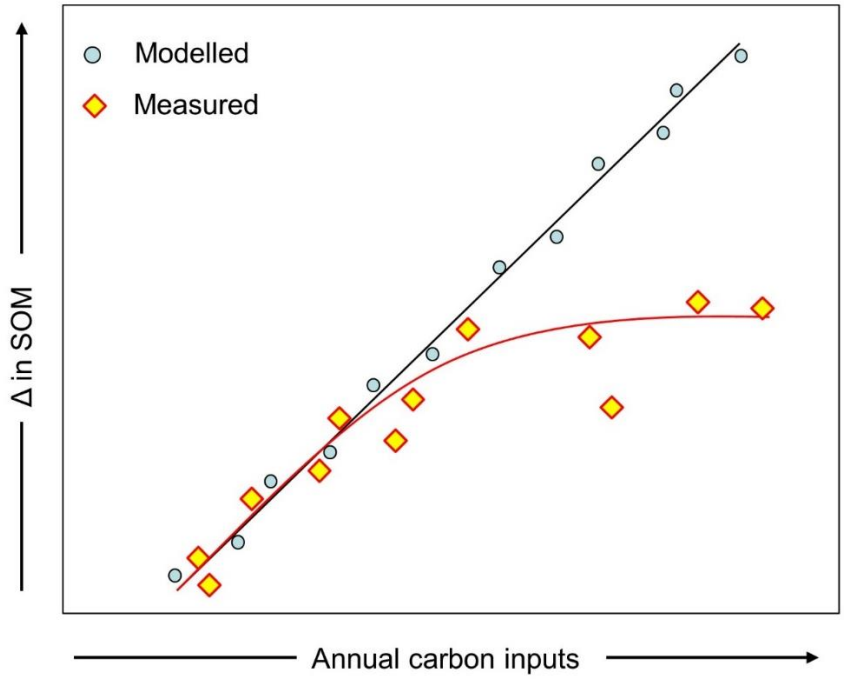

Figure 1-1: Hypothetical relationship between annual carbon inputs and measured (diamond symbols) or modelled (round symbols) long-term changes in soil organic matter (SOM) contents as proposed by Heitkamp et al. (2012a), Powlson et al. (2011) and Stewart et al. (2007).

Although it is agreed that increasing C inputs (mainly through straw) is not automatically beneficial for SOM (Fig. 1-1), the reasons for these findings are not completely understood. Potential reasons for these findings could be: (i) alteration of soil physical properties, resulting in conditions favoring high decomposition and less accumulation of SOM in aggregates; (ii) a shift from recalcitrant belowground to labile aboveground residue inputs, (due to high above ground biomass production under high N fertilization) which may result in higher decomposition and less C stabilization from crop residue (iii) acceleration of native SOM mineralization through priming effect of incorporated residues (iv) lower partitioning of added C in stable SOM fractions (i.e. mineral-associated) due to their limited capacity to stabilize SOM, and (v) translocation of a portion of unprotected SOM to the subsoil.

1.3.1 Alteration of soil physical properties

The soil structure refers to the arrangement of soil particles into units called aggregates. Well-stabilized and aggregated soil is an important indicator of soil quality and workability. In general, most soil physical properties are related to SOM contents within a given texture (Haynes und Naidu 1998; Bronick und Lal 2005). Soil aggregates vary in size (e.g. macro- and microaggregates) and are strongly influenced by the quality and quantity of added organics, as they influence soil processes involved in aggregation (Abiven et al. 2009; Majumder and Kuzyakov 2010). The addition of readily-decomposable substrates improves aggregation process more so than recalcitrant substrates. During substrate decomposition, microorganisms excrete substances which act as cementing agents (e.g. glomalin, polysaccharides) that bind soil particles together. In addition, fungi hyphae and roots can also act as binding agents. In the presence of organics, binding of soil particles results in microaggregate (< 250 µm) formation and, thereafter, microaggragates bind with SOM and silt clay particles to form macroaggreagtes (> 250 µm) (Tisdall and Oades 1982). However, microaggregates may form inside macroaggregates and can release during macroaggregates turnover (Six et al. 2000).

Aggregates physically protect SOM from microbial decomposition through spatial inaccessibility of degrading microorganisms and their enzymes (Angers et al. 1997; Kögel-Knabner et al. 2008). However, aggregates have limited capacity to stabilize SOM. After reaching a threshold level, most of the added organics remain unprotected (Andruschkewitsch et al. 2014; Shahbaz et al. 2016; Stewart et al. 2008). The physically unprotected fraction can serve as a favorable substrate for microorganisms due to its high accessibility, typically resulting in rapid decomposition. An increase in the proportion of macroaggregates is an

indicator of high soil physical quality (e.g. aeration, water movements, nutrients exchange) , as such conditions offer favorable conditions for microbial growth and activities (Schjønning et al. 1999; Jäger et al. 2011). Under such situations (low physical protection and high microbial activity), increased mineralization of unprotected residues may lead to a high residue C loss to stabilization ratio. This would explain the shape of observed (measured) relationships (Fig. 1-1) between C input and SOM change under increasing C additions. Nevertheless, residue quality can have a stronger effect on aggregation because it directly affects microbial functioning.

1.3.2 Changes in crop residue quality

Crop residue quality is an essential factor controlling SOM formation, stabilization and dynamics. Crop residues vary in their structural and chemical composition (Adair et al. 2008). Residues are often classified on the basis of C/N ratio and the contents of a recalcitrant substance such as phenols, tannins, or lignin (Stewart et al. 2015; Wang et al. 2015a). Residue decomposition rates are generally negatively related to the amount of recalcitrant compounds present in their biomass (Bertrand et al. 2006; Castellano et al. 2015). Aboveground residues (e.g. leaves and stems) are considered high quality because they contain less recalcitrant compounds and lower C/N ratios than belowground residues (e.g. roots) (Bertrand et al. 2006; Lian et. 2016; Rasse et al. 2005). The application of N fertilization generally improves crop residue quality by increasing nutrient contents and lowering C/N ratio (Schmidt et al. 2015; Silveira et al. 2013).

The role of residue quality in SOM formation is currently under debate. The common view on recalcitrant root residues, which are decomposed slowly and therefore contribute significantly to SOM content (Berg and McClaugherty 2014; Johnson et al. 2014; Rasse et al. 2005), contradicts the view of the large contribution of high quality (easily decomposable) residues to stable SOM formation (Castellano et al. 2015; Cotrufo et al. 2013; Lehmann and Kleber 2015). The latter concept is mainly associated with microbial by-products, which are released and stabilized in the mineral-associated SOM fraction during microbially-mediated plant residue decomposition (Cotrufo et al. 2013; Lehmann and Kleber 2015). Therefore, compared to recalcitrant residues, easily-decomposable residues can greatly contribute to the stable SOM fraction. However, the fast mineralization of easily-decomposable residues (disproportionally increase with addition level) may increase C losses more than stabilization within SOM fractions (Xiao et al. 2015).

1.3.3 Residue partitioning within SOM fractions

SOM is usually classified into its three main fractions on the basis of density. On the basis of residue decomposability, added crop residues partition into various SOM fractions. The fraction which is least decomposed (fresh input) and remains unprotected by physical mechanisms is referred as the free light fraction of SOM. The SOM fraction that partially decomposes and is protected by physical mechanisms, e.g., inside aggregates, reflects the occluded light fraction of SOM. The third SOM fraction is the heavy fraction, which is highly decomposed and considered stable for decades because of its strong association with mineral particles. The heavy fraction of SOM generally depends on microbial by-products, which are released during crop residue decomposition (Cotrufo et al. 2013; Schrumpf et al. 2013) or microbial turnover (Miltner et al. 2012). On the basis of its formation mechanism, the heavy SOM fraction is highly affected by long-term management practices.

A non-linear increase of SOM levels (as shown in Fig 1-1) under increasing C inputs is primarily linked to the saturation (or less increase per unit of C input) of SOM fractions (Hassink and Whitmore 1997; Six et al. 2002). This means that, besides the unprotected fraction, SOM fractions have only a limited capacity to stabilize SOM. Within SOM fractions, the saturation of mineral-associated fractions is more important, as it represents the major pool (up to 80%) of SOM in cropland soils (Stewart et al. 2007). It has been observed that the unprotected light fraction of SOM increases proportionally to the C input, however, mineral-associated or aggregate protected fractions can stabilize SOM only up to a certain limit (Gulde et al. 2008; Stewart et al. 2008). This may explain why Gong et al. (2009) found increases in heavy fraction C (and also total SOM) with increasing C input in a soil depleted in SOM, while Heitkamp et al. (2011) did not report any effect in a sandy soil (due to a lower proportion of the heavy SOM fraction). When the heavy or occluded fraction reaches its effective capacity, a higher portion of C input will be partitioned to a less-protected labile fraction (e.g., light fraction). The light fraction can rapidly mineralize, resulting in greater losses (as CO_2) or leaching of unprotected SOM into the subsoil (Stewart et al. 2008).

1.3.4 Stabilization in subsoil

The importance of management effects on SOM is mostly considered for topsoil (Ap horizon, plough layer), while information for subsoil is scarce (Gregory et al. 2014; Ogle et al. 2005). Subsoil SOM stabilization mostly occurs through dissolved organic C (leaching from topsoil), bioturbation and root growth (Rumpel and Kögel-Knabner 2011). The subsoil SOM

stabilization is linked with the soil texture and topsoil management practices (Hobley and Wilson 2016; Hobley et al. 2016). Apparent differences in SOM stocks between land-uses or management practices turned out to be primarily due to redistribution of SOM into deeper soil layers (Don et al. 2009). If translocation of SOM happens only at high rates of C inputs, the relationship observed between input and SOM changes in the topsoil can be similar to that in Fig. 1-1 (nonlinear measured relation). SOM stabilization in subsoil is considered more effective because it generally contains higher clay contents (e.g. Luvisols), which are mostly C-deficient and is less exposed to these changes (Hobley and Wilson 2016; Kögel-Knabner et al. 2008). However, in contrast to the general assumption that subsoil is less affected by management, Khan et al. (2007) showed more serious losses of SOM below the plough layer in a silty-loam soil. A possible explanation of subsoil C loss can be due to priming, which normally occurs after an input of "fresh C" to the soil (Fontaine et al. 2007).

1.3.5 Soil priming effect

Soil priming effect (PE) is the short-term change in native SOM mineralization caused by substrate addition (Fig. 1-2, Kuzyakov et al. 2000). The PE is a natural process that is induced by pulses or continuous inputs of fresh organics (Kuzyakov et al. 2000). Soil microorganisms are frequently C limited, thus, the input of C-rich crop residues stimulates microbial decomposition of SOM, resulting in PE (Blagodatskaya et al. 2011). The size of PE increases with the amount of substrate addition. Following substrate addition SOM mineralization typically increases, which is defined as a positive PE. However, if SOM decomposition slows following substrate additions then it reflects a negative PE (Fig. 1-2). For instance, if the added substrate is labile, then microorganisms would mainly rely on the added substrate and may decrease their dependence on recalcitrant SOM (Fontaine et al. 2003). This ultimately may result in a negative PE due to high substrate availability (Blagodatskaya and Kuzyakov 2008). On the other hand, if the added substrate stimulates the inactive or dormant soil microflora by providing energy, this would accelerate SOM mineralization (in parallel to substrate decomposition), thus resulting in a positive PE. The quality and amount of substrate is the most important factor that can affect soil PE.

The increase in the number of studies on PE during the last decade reflects the interest in biotic mechanisms of carbon turnover in soil, which is still poorly understood (Blagodatskaya and Kuzyakov 2008; Chen et al. 2014; Fontaine et al. 2003; Wang et al. 2015b; Xiao et al. 2015). Most of the investigations on PE are performed with glucose additions as substrate, because

most plant polymers are rapidly decomposed to monosaccharides (Gunina and Kuzyakov 2015). The few available studies which have determined the impact of plant residue additions on SOM dynamics primarily found a positive PE following application (Guenet et al. 2010; Moreno-Cornejo et al. 2015; Xiao et al. 2015). There may be several mechanisms explaining the variations in PE under contrasting substrate quality, as substrate quality directly affects microbial activity (Blagodatskaya et al. 2014; Fontaine et al. 2003; Kuzyakov et al. 2000). However, regardless of the specific mechanisms, any positive increase in SOM decomposition following substrate (residue) addition may lead to severe C losses, which would decrease the overall rate of SOM stabilization under high C inputs.

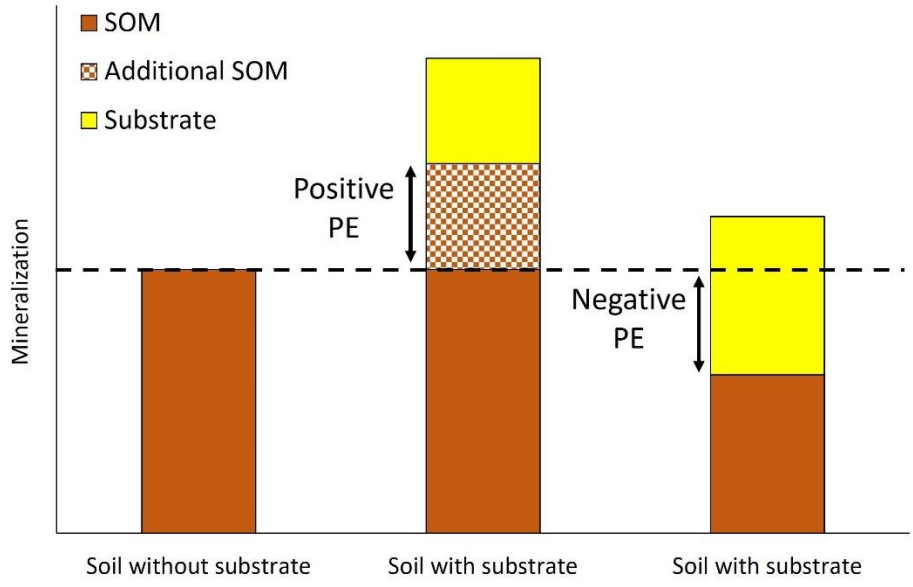

Figure 1-2: Schematic diagram of the influence of organic substrate addition on soil organic matter (SOM) mineralization, i.e. priming effect (PE). The increase of SOM mineralization represents positive PE, while decrease of SOM mineralization reflects negative PE (modified after Kuzyakov et al. 2000).

2 Objectives and Methods

2.1 Objectives

To contribute to knowledge needed, the thesis aim is to explain an unexplained observations from long-term experiment Puch (near to Munich) Germany: where increasing amounts of C-input with straw did not increase SOM stocks efficiently (Fig.1-1, Heitkamp et al. 2012a). The idea was to test whether soil structure, crop residue quality or quantity, C partitioning between SOM fraction, C-translocation and SOM priming would explain the pattern of SOM stabilization, which was observed under long-term experiment started in 1983.

The specific study objectives were as following:

(1) To determine the effect of crop residue quality and quantity on soil aggregates formation and SOM physical protection (Chapter 3.1) by hypothesizing that;

(i) regardless of addition level, belowground residues will be mineralized slower than aboveground crop residues;

(ii) the aggregate formation will increase with the residue addition level;

(iii) the percentage of residue derived C stabilized within aggregates will decrease with the addition level.

(2) To investigate the mechanisms and thresholds levels of wheat residue quality and quantity (^{13}C-labeled) inducing SOM priming (Chapter 3.2) by hypothesizing that;

(i) the intensity of SOM decomposition will be affected by the residue mineralization rates, i.e. SOM decomposition will be dependent on residue type;

(ii) regardless of residue type, the intensity of PE will decrease with increasing C addition;

(iii) we assumed that microorganisms decomposing added residues will represent the most active fraction of soil microflora. Therefore, we further hypothesized that the PE will be the main function of the microbial fraction feeding on residues and of its enzymes activities.

(3) To explain and compare the integrated effect of long-term C inputs (along N fertilization rates), either straw or root dominated, on topsoil SOM contents, partitioning

of C within topsoil SOM fractions and accumulation of SOM in the subsoil (Chapter 3.3). In particular, the specific goals of this study are;

(i) to estimate and compare the changes in topsoil SOM levels due to C inputs (variable organics) and N fertilization over the study period, i.e. 32 years;
(ii) to analyze the effects of topsoil managements on SOM accumulation in the subsoil;
(iii) to quantify and compare the effects of C inputs and N fertilization on the partitioning of C among topsoil SOM fractions (f-LF, o-LF and HF), and the overall impact of these fractions on SOM formation.

2.2 Methods

The findings from controlled but short-term laboratory experiments and outcomes from the long-term field experiment will be correlated to test and quantify the relevance of SOM stabilization factors (discussed above). The long-term field experiment at Puch is well documented and designed, represents a common soil type (silt loam texture) in central Europe (Luvisol derived from loess) and covers a wide range of management options in a widespread cereal-based crop rotation.

Knowing the reasons for the observed SOM changes versus C inputs is very important: if, e.g., soil limited physical capacity or increasing soil respiration (including priming) would explain low efficiency of SOM stabilization under high C inputs, then the alternative use of straw can be justified. This can suggest that energetic use of residues would be more efficient e.g. regarding mitigation of greenhouse gas emissions. However, soil physical properties cannot be disregarded in this respect, because it is important for the sustainable use of croplands and soil quality.

2.2.1 Study site and layout

The site is located in Puch, Germany close to the Munich (48°11’ N, 11°12’ E). The mean annual temperature and precipitation from 1984 to 2009 was 8.4 °C and 868 mm yr-1, respectively. The soil is classified as Luvisol (IUSS-WRB 2015) derived from loess sediments (clay: 18%, silt: 73%, sand: 9%) overlying glacial moraine deposits. The pH value declined from 6.5 to 6.1 during the study period. Before the initiation of the experiment the site was used as cropland probably for decades or centuries.

The crop rotation is silage maize (*Zea mays* L) – winter wheat (*Triticum aestivum* L) – winter barley (*Hordeum vulgare* L). In 1983 the experiment was laid out as a full-factorial strip-design with two factors (n = 3). Factor one is organic additions and the second N-fertilization rates (Table 2-1). Application of P and K was equal in all treatments but varied between years according to plant needs (Hege and Offenberger, 2006). Five levels of organic amendments are considered here: (i) – Control: no amendment, straw (wheat and barley) removed; (ii) – Slurry: slurry application, straw removed; (iii) – Manure: application of farmyard manure every third year, straw removed; (iv) – Straw: straw incorporated; and (v) – Straw with slurry: slurry application, straw incorporated.

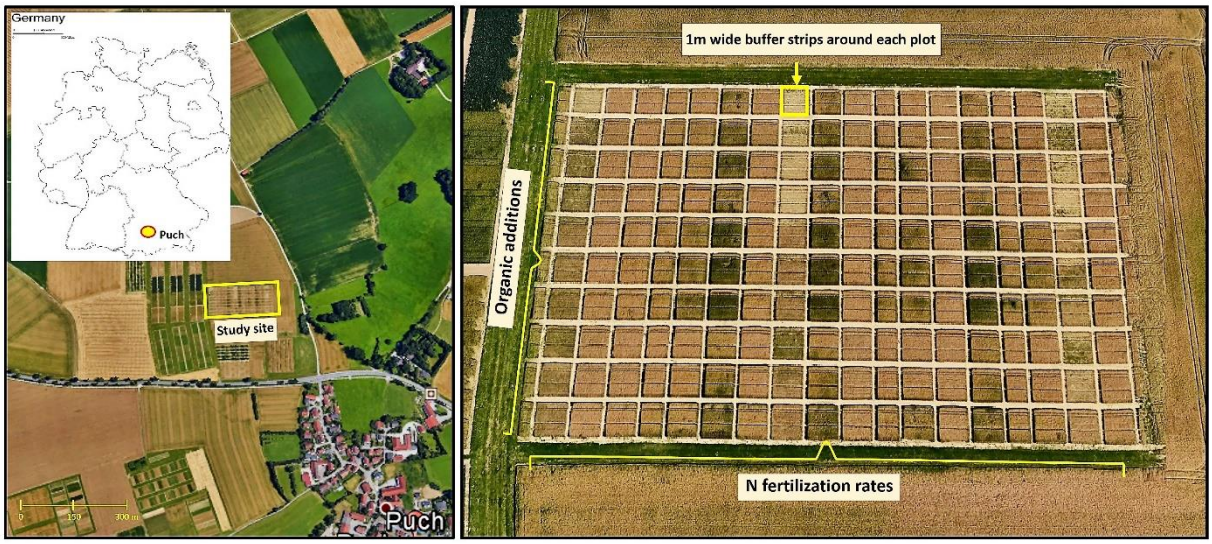

Figure 2-3 An aerial view of the study site located at Puch, close to Munich

C input by farmyard manure, slurry and straw addition was fixed and measured before the addition. While inputs by crop residues (stubbles and roots) were estimated and were increased with N fertilization (Heitkamp et al. 2012a). Since already measured C-inputs showed a large gradient of C-input especially with straw and straw plus slurry additions, which make the experiment well suited for the proposed study. Rates of N-fertilizer (three levels, N0, N2 and N4) varied between crops and since 1999 the amounts and frequency of N fertilization given to winter wheat and winter barley was changed (Table 2-1).

Table 2-1: Rates of N-fertilizer application and annual C additions.

N-addition (kg ha^{-1} yr^{-1})	N0	N2	N4
1983-1998			
barley	0	60	80/40
wheat	0	50/30*	70/50/40
maize	0	100	120/80
Since 1999			
barley	0	50/30	80/40/40
wheat	0	50/20/30	80/60/60
maize	0	100	120/80
C-addition (Mg ha^{-1} yr^{-1})			
Control	1.17	1.45	1.58
Slurry	2.09	2.34	2.44
Manure	2.23	2.49	2.64
Straw	1.83	2.87	3.73
Straw+Slurry	3.16	4.27	4.86

*N amounts divided by slash indicate split applications

2.2.2 Soil sampling

To estimate the effects of crop residue quality and quantity inputs on SOM stabilization under controlled conditions the soil (Luvisol) was sampled from the Ap horizon (0-25 cm) of an experimental-field located in the North West of Göttingen, Germany (51°33′36.8″ N, 9°53′46.9″ E). The sampled soil characteristics were similar to the long-term field experiment located at Puch. The soil had silt-loam texture with following chemical characteristics: the organic C (with standard error) content of 12.6 (0.4) g kg^{-1}, a C/N ratio of 9.7 and pH ($CaCl_2$) of 6.0.

To estimate the long-term management effects on SOM stabilization and to correlate them with the findings of incubation studies, the soil samples were taken from a depth of 0-25 cm (topsoil) and 25-50 cm (subsoil) from the Puch field site. The sampled topsoil depth (0-25 cm) represents the plough layer which is annually mixed by tillage. While the purpose of subsoil (25-50 cm) sampling was to investigate the impact of long-term C inputs on subsoil SOM accumulation. For each organic additions, the soil was sampled in three field replicates within each selected N fertilization rate. Three levels of N fertilization were selected i.e. no- (N0); medium- (N2) and high-fertilization (N4) (Table 2-1).

2.3 Summary of experiment and main results

Table 2-2: Summary of the experiments: objectives, methods and main results.

Objectives/Aims	Methods	Main results
1. To determine the effect of crop residue quality and quantity on soil aggregates formation and SOM protection	^{13}C-labeled wheat residue varying in quality (leaf, stem, root) and quantity were used to trace plant residue C in various pools. Aggregates size fractionations (macro-, microaggregates and silt plus clay) was done by wet sieving method. C sources were partitioned in CO_2 efflux, microbial biomass and aggregates.	Aggregate formation increased generally with addition level. Decrease of residue occlusion with increasing inputs Aboveground C retention in aggregates decreased at a high level of addition. Soil priming mainly depended on the level of addition. Increased mineralization and less residue physical protection decreased SOM stabilization.
2. To investigate the mechanisms and thresholds levels of wheat residue quality and quantity (^{13}C-labeled) inducing SOM priming	Partitioning of C sources in CO_2 and in microbial biomass at different sampling periods over a 120 days incubation period. Measurements of enzyme activities involved in C, N and P cycles at different sampling periods. Estimation of threshold values of residue quality for SOM priming by developing a new unifying logistic model.	Root residue induced stronger and higher SOM priming effect than aboveground crop residues. Microbial-residues served as SOM primer. Priming effect was a power function of crop residue mineralization rate. The microbial fraction (and their enzyme activities) feeding on crop residues served as an active players of SOM priming. Aboveground residues decomposition disproportionally increased with the addition level.
3. To determine the impact of long-term C inputs (along N fertilization rates), either root or straw dominated, on SOM stabilization and C	Estimation of topsoil (0-25 cm) SOM changes occurred since the initiation of the experiment (32 years). Separation of SOM pools into free light fraction, occluded light fraction and mineral-	Topsoil SOM contents increased with input (also by N fertilization), mainly because of the C in the free light fraction. The topsoil SOM lost up to 15% under no organic additions during 32 years.

partitioning within topsoil SOM fractions, and to estimate the subsoil SOM contents	associated SOM fraction by density fractionation using sodium polytungstate (p <1.6 g cm^{-3}). Estimation of subsoil (25-60 cm) SOM contents.	Straw contributed little to the f-LF but prevented C losses from the mineral-associated SOM fraction, which observed without straw additions. Root C retained longer in the light-fractions and were responsible for SOM increase with N fertilization.
4. Additional studies: To explore the responses of SOM versus residue mineralization in response to glucose addition over an incubation period of 3 months in a soil having one month partially decomposed wheat residues (leaves, root)	A three-source partitioning approach was applied using dual isotopic labels ($^{13}C/^{14}C$) to partition the decomposition of glucose, residue and SOM. Residues were preincubated in soil (for 30 days) to obtain partially decomposed wheat residues and there after glucose was added. Glucose priming effect both on SOM and residues was distinguished. Source partitioning was done for CO_2 and microbial biomass.	Glucose addition caused negative priming effect on residues (predominantly leaves) and strong positive priming effect on SOM. Increased SOM derived C (compared to residue derived) in microbial biomass suggested that glucose caused preferential microbial utilization of SOM over plant residue. Priming induced by glucose was mainly due to SOM decomposing microorganisms. The priming effects of residue on SOM changed by the presence of glucose.

3 Publications and Manuscripts

3.1 Study 1

Decrease of soil organic matter stabilization with increasing inputs: mechanisms and controls

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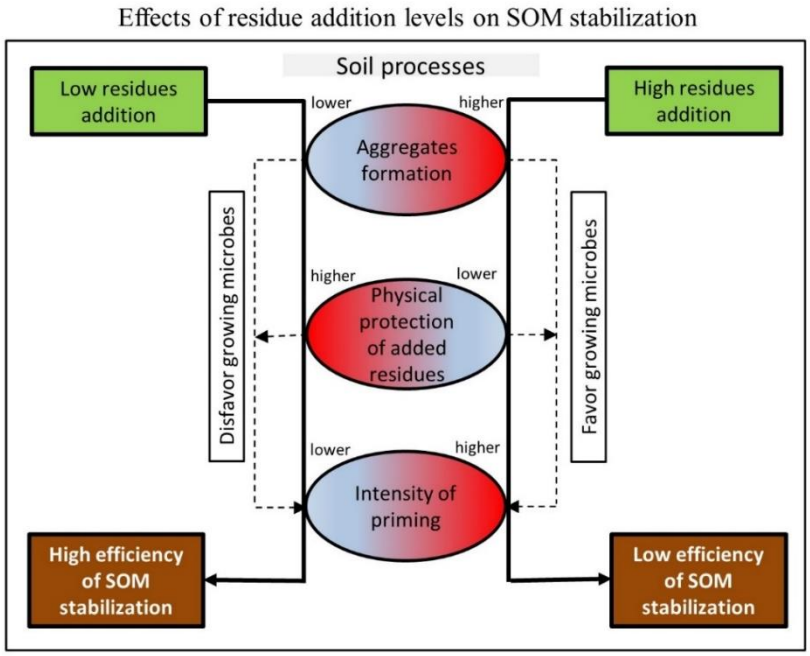
Highlights

- Aggregate formation increased generally with addition level.
- Decrease of residue occlusion with increasing inputs
- Aboveground C retention in aggregates decreased at a high level of addition.
- Soil priming mainly depended on the level of addition.
- Increased mineralization and less residue physical protection decreased SOM stabilization.

Graphical abstract



Conceptual diagram of the effects of levels of residue addition on the efficiency of soil organic matter stabilization (SOM) by three processes: Aggregate formation, Physical protection of added residues, and Priming intensity. The red color represents increasing process intensity. The dashed lines indicate the conditions affecting growing fraction of microbial biomass (important for priming) as influenced by the three soil processes.

3.1.1 Abstract

Crop residue addition is a way to increase soil organic matter (SOM) level in croplands. However, organic matter input and SOM stocks are not linearly related. Consequently, adding high amounts of residues, such as straw, may increase SOM to only a small extent, and an alternative use of the residues may be justified. The objective of this study was to test how the level and type (above- or belowground) of residue addition affect SOM stabilization. We hypothesize that (1) root residues will be mineralized slower than leaf and stalk residues, (2) soil aggregate formation will increase with high additions, and (3) wheat residue addition will induce positive priming, with the magnitude depending on the residue level and type. Homogeneously ^{13}C-labeled wheat residues (leaves, stalks, roots) were added to a silt-loam soil at levels of 1.40 and 5.04 g DM kg^{-1} and CO_2 release and $\delta^{13}C$ signature were measured over 64 days at 20 °C. Water-stable macroaggregates (> 250 µm), microaggregates (53-250 µm) and silt plus clay size fractions (<53 µm) were separated and ^{13}C incorporation from residue was quantified in each fraction after 64 days. Aggregate formation generally increased with added residue amount, but the proportion of residues occluded within aggregates decreased with increasing addition level. The occlusion of residues from aboveground biomass was more reduced with addition level than that of roots. Residue mineralization increased with the addition level, but this increase was less for roots compared to stalks and leaves. Priming effects were similar between residue types and mainly depended on the added amount: SOM mineralization increased by 50% and 90% at low and high addition levels, respectively. We conclude that the proportion of residues physically protected within aggregates decreases and priming effects increase with increasing C input leading to decreasing rate of long-term C stabilization within SOM by increasing residue addition.

Keywords: root mineralization, straw residue, soil organic matter, carbon sequestration, priming effect, water stable aggregates

3.1.2 Introduction

Globally, anthropogenic loss of carbon (C) from terrestrial ecosystems is estimated from 48 to 114 Pg before the industrial revolution (Houghton, 2012). Since 1850, another 108 to 188 Pg C has been lost, which mostly stems from biomass but about 25% of this loss is contributed by soil organic matter (SOM) mineralization (Houghton, 2012; Lal, 2004). The soil C losses can be mitigated by recarbonization using recommended management practices thereby increasing food security (Lorenz and Lal, 2012; Sauerbeck, 2001). However, some mitigation options in agriculture are in direct competition with each other, e.g., use of crop residue for 2nd generation bioenergy crops versus residue incorporation into the soil for maintenance or build-up of SOM.

The incorporation of crop residues, such as cereal straw, is an important measure to maintain or increase SOM levels under cropland (Lugato et al., 2014). Recent studies on long-term field experiments, however, show that incorporation of cereal straw is often not very effective in terms of SOM increases (Heitkamp et al., 2012b; Poeplau et al., 2015; Powlson et al., 2011). Resultantly, the efficiency (i.e. the increase of SOM per unit of input) of residue incorporation decreases with the amount added, as shown in a long-term experiment on a silt-loam Luvisol (Heitkamp et al., 2012b). Reasons for this may be (1) a lower proportion of belowground plant biomass, which is supposed to be more recalcitrant and have longer mean residence time in soil (Rasse et al., 2005), (2) a finite capacity of aggregates, which provide physical protection of SOM against mineralisation , and (3) priming of SOM by incorporation of plant residues.

The biochemical composition and physical structure of crop residue affect mineralization (Prescott, 2010). Plant parts differ in chemical composition and physical structure, especially roots are more recalcitrant and so, have a longer mean residence time in soil (Heitkamp et al., 2012a; Rasse et al., 2005). For instance, a meta-analysis showed that roots of herbaceous species decompose 1.8 times slower than leaves (Freschet et al., 2013). Therefore, increasing aboveground input by crop residue shifts the input away from below-ground sources and can decrease the average litter mean residence time in soil. Occlusion within aggregates is another important mechanism to protect litter from mineralization (Six et al., 2004; von Lützow et al., 2008).

Aggregates, which protect SOM by physical occlusion, are formed by biological and physicochemical processes (Six et al., 2004). Aggregates are often classified according to stability (e.g. resistance against slaking) and size. The addition of residue forms hotspots of

microbial activity triggering the formation of aggregates. The amount and type of organic matter input having differential decomposition rates can affect aggregate dynamics (Gunina et al., 2015). However, due to the limited capacity of storage, some studies showed that residue addition levels had little effect on aggregate C contents (Andruschkewitsch et al., 2014; Stewart et al., 2008). In consequence, a higher proportion of crop residue would remain physically unprotected when incorporation of residues is increased. In contrast Poirier et al. (2014) observed macroaggregates formation was leveled off at increasing residue input, however, residue kept accumulating in aggregates due to occlusion and adsorption mechanisms.

In the soil, labile substances can cause positive priming, i.e. additional (compared to without substrate addition soil) CO_2 release by accelerated SOM mineralization. Many experiments on priming were performed with glucose because the most plant polymers will be decomposed to monosaccharides rapidly (Gunina and Kuzyakov, 2015). Only a few studies investigated priming effects of crop residue on SOM (Guenet et al., 2010; Moreno-Cornejo et al., 2015). These studies show contrasting results: whereas Guenet et al. (2010) reported that priming of SOM by wheat residues is a non-linear function which saturates with the addition of 2.2 g straw kg^{-1} soil, Poirier et al. (2013) showed an almost linear increase up to 40 g maize residue C kg^{-1} soil. Xiao et al. (2015) suggested that priming increases linearly with litter addition upon the response of enhanced microbial biomass and activity. Residues with lower C/N ratio or mineral N addition decreased the priming effect slightly (Guenet et al., 2010; Moreno-Cornejo et al., 2015; Wang et al., 2015).

Summarizing, with increasing levels of residue incorporation the increase of SOM per unit of input may decrease 1) due to a shift from recalcitrant below to labile aboveground input, 2) by a lower proportion of fresh residues protected within aggregates or 3) by inducing positive priming of SOM. In a controlled experiment, we tested these three possibilities by incorporation of ^{13}C labeled wheat plant parts (leaves, stalks and roots) at two levels into a silt-loam soil during 64 days of incubation. We hypothesise that (1) regardless of addition level, root residue will be mineralised slower than leaves and stalk residue, (2) aggregate formation will increase with addition level, but the proportion of residue C stabilized within aggregates will decrease, and (3) wheat residue addition will induce positive priming, with its magnitude depending on the level of addition and the type of residue.

3.1.3 Materials and methods

3.1.3.1 Soil and wheat residue

The soil (Haplic Luvisol) samples were taken from the Ap horizon (0-25 cm) of an experimental field, located on a terrace plain of the river Leine in the North West of Goettingen, Germany (51°33′36.8″ N, 9°53′46.9″ E). The soil had silt-loam texture (clay: 7.0%, silt: 87.2%, sand: 5.8%) and was carbonate-free with a mean organic C (with standard error) content of 12.6 (0.4) g kg^{-1}, a C/N ratio of 9.7 and pH ($CaCl_2$) of 6.0. Since more than 25 years the field has been cultivated with annual C3 crops (predominantly wheat; Kramer et al., 2012). The soil was air dried after sampling. Larger clods were crushed with mortar and pestle, sieved (< 2 mm) and fine roots and other visible plant debris were carefully removed.

The wheat (*Triticum aestivum* L.) plants were labeled with ^{13}C every week after emergence for at least 8 hours in a growth chamber. Seeds were planted into pots filled with quartz sand, were watered regularly and once a week Hoagland's nutrient solution (N: 210, K 235, Ca 200, P 31, S64, Mg: 48 ppm plus micronutrients) was added. Labeled (99 Atom%) $NaH^{13}CO_3$ was injected into H_2SO_4 positioned in the chamber. In the night (dark period) the chamber was left closed and was opened in the morning after respired CO_2 was taken up again. Further details are presented by Bromand et al. (2001). Plants were harvested after senescence, where roots were washed free from the sand with tap water. Wheat biomass was carefully separated into leaves, stalks and roots. Each part was chopped and sieved (< 2 mm) to achieve more homogeneous mixing with soil for incubation. The content of C, N and ^{13}C Atom % (At%) was measured with an isotope ratio spectrometer coupled to an elemental analyzer (Delta plus, EA-IRMS, see detail section 3.1.3.5). The mean C concentrations of leaves, stalk and roots were in the order: 391.9 ±6.1 (C/N: 17.2±0.3), 409.6 ±8.7 (C/N: 21.5 ±1.17) and 278.3 ±5.9 (C/N: 15.5±0.5) g kg^{-1}, respectively. The At%^{13}C values for the residue types were 1.55 ±0.00 (leaves), 1.34 ±0.01 (stalks) and 1.51 ±0.03 (roots).

3.1.3.2 Incubation and sampling

Maximum water holding capacity (WHC) of the soil was determined by soaking for 24 hours, subsequent free drain for 1 hour and weighing in the wet and dry state. A hundred grams of sieved and dried soil was weighed into 750-ml incubation jars. The soil was then preincubated at 50% of its WHC for seven days, because rewetting and sieving affect the availability of SOM for microorganisms and may cause a respiration flush (Blagodatskaya and Anderson, 1999).

The pre-incubated soil was amended with labeled wheat leaves, stalks or roots with low or high amounts and one control were left without residue addition (n = 4). The added residues were thoroughly mixed with incubated soil. Water contents were then adjusted to 70% of WHC before starting the incubation for 64 days. Residues were added at rates of 1.4 and 5.04 g DM kg^{-1} as low and high addition level, respectively. These amounts correspond to 5 and 18 Mg ha^{-1} of residues under field conditions assuming 25 cm depth and a bulk density of 1.5 g cm^{-3}. We added residues on a dry matter base, however, C input by roots with lower C-contents corresponds to ca. 70% of the C amount added with leaves or stalks.

3.1.3.2 CO_2 efflux

Released CO_2 was trapped in small bottles with 10 mL of 1 M NaOH placed in the incubation jars (including 4 controls without soil) which were closed air-tight. The NaOH traps were replaced after 2, 6, 11, 17, 27, 51 and 64 days. Therefore, jars were not closed longer than 14 days and the capacity of NaOH was never used up to more than 60%. To quantify respired CO_2, NaOH was titrated with 0.1 M HCl until pH 8.2 using phenolphthalein as indicator. Excess 0.5 M $BaCl_2$ was added to precipitate CO_3^{2-} before titration. Another Aliquot of NaOH was mixed with 1 M $SrCl_2$ in a 15 ml centrifugation tube and centrifuged for 5 min at 2000 rpm (Blagodatskaya et al., 2011). The centrifugation process was repeated until the pH level of the aliquot reached 7. The $SrCO_3$ pellets were dried at 60 °C and stored for $\delta^{13}C$ analysis.

3.1.3.3 Fractionation of soil aggregates

Water stable aggregates were separated at the end of incubation. The soil was oven-dried at 40°C for 24 h. Then, 70 g of dry soil was placed on a 250 µm sieve and submerged in ca. 1.5 L distilled water for 5 min to allow slaking (Six et al., 1998). Thereafter, the sieve was moved up and down into the water with 50 repetitions in 2 min. Water-stable aggregates remaining on the mesh (macroaggregates >250 µm) were collected in pre-weighed aluminum foil then dried and weighed. Aggregates which passed the 250 µm-sieve were poured onto the next smaller mesh size (microaggregates: 53-250 µm) and the fractionation-procedure was continued as described above. Finally, the silt and clay size fraction together with the finest microaggregates <53 µm was collected in a pre-weighed container, dried and weighed.

3.1.3.4 Microbial biomass

The fumigation extraction method was used to measure microbial biomass C, as described by Vance et al. (1987). Briefly, 10 g of moist soil was divided and one subsample was fumigated

for 24 h at 25 °C with ethanol-free $CHCl_3$. Both subsamples were shaken for 1 h at 175 rev. min^{-1} with 20 mL of 0.05 M K_2SO_4. The obtained extracts were kept cold (< 4 ºC) and analyzed the next day for total C concentration (Multi N/C 2100, Analytik Jena, Germany). Microbial biomass C was calculated as EC/K_{EC}, where EC = (organic C from fumigated soils) – (organic C from non-fumigated soils) and K_{EC} = 0.45 (Wu et al., 1990).

3.1.3.5 Isotopic analysis and calculations

At the end of incubation period, soil aggregates size classes were ground to a fine powder using a ball mill for 3 minutes and then analyzed for carbon concentration as well as $^{13}C/^{12}C$ ratios. The analyses were performed at the Centre for Stable Isotope Research and Analysis (KOSI) University of Goettingen, Germany, using an isotope ratio mass spectrometer (Delta plus, IRMS; Thermo Fisher Scientific, Bremen, Germany), coupled to an elemental analyzer (NC 2500; CE Instruments, Milano, Itlay). The values were calibrated with reference to the international VPDB (Vienna Peedee Belemnite) standard. For $^{13}C/^{12}C$ ratio measurements in microbial biomass, the extracts from fumigated and nonfumigated samples were freeze-dried and weighed in capsules. As incorporated wheat residues were highly enriched, residue derived C in all pools was calculated by using At%^{13}C values. At%^{13}C values originated from the incubated soil were calculated according to the following Eq. (1):

$$At\%\ ^{13}C\ = [number\ of\ ^{13}C\ atoms/(numberof(^{12}C + ^{13}C)\ atoms] * 100 \quad (1)$$

In the various pools, the fraction of total C (f C) derived from residues was calculated using Eq. (2):

$$fC = [(At_{tr} - At_c)/(At_r - At_c)] \quad (2)$$

Where At_{tr} represents At%^{13}C values of, aggregate size fractions, CO_2-C trapped in NaOH, extracted C, derived from the residues amended soil. While At_r represents At%^{13}C values of initially incorporated wheat residues (leaves, stalk or roots), At_c represents At%^{13}C values of each corresponding pool coming from the unamended sample. Thus, the amount of residue derived C ($C_{res\text{-}derived}$) in various pools, was computed using Eq. (3) (Poirier et al., 2013).

$$C_{res-derived} = fC * [A] \quad (3)$$

Where [A] represent either total organic C in aggregates size classes (g kg^{-1} soil) measured by a dry combustion method, total respired CO_2 (mg C kg^{-1}) measured by titration method, C contents of fumigated or non-fumigated K_2SO_4 extract (mg kg^{-1}).

Similarly, the amount of SOM derived C ($C_{SOM\text{-}derived}$) was calculated by subtracting $C_{res\text{-}derived}$ from total C of the corresponding pool. The amount of priming effect (PE, mg C kg^{-1}) was calculated according to the following Eq. (Blagodatskaya et al., 2011).

$$PE = (CO_{2\ total} - CO_{2\ res-derived}) - CO_{2\ control} \quad (4)$$

For the estimation of total residues derived C incorporation in microbial biomass, firstly residues derived C was calculated separately from fumigated and non-fumigated samples by using Eq. 3, thereafter values of the non-fumigated sample were subtracted from fumigated.

3.1.3.6 Statistical analysis

The experiment was laid out as a full-factorial, fully randomized design. The factor "type" had three levels (leaves, stalks, roots) and the factor "level" had three (no addition, 1.4. and 5.04 g kg^{-1}) or two levels. Two levels were used when comparing residue-derived C in fractions, where inclusion of "no addition" was not suitable. Statistical analyses were performed with SPSS 11 using a two-way ANOVA with "level" and "type" as fixed effects. When significant ($p \leq 0.05$) effects were found, post hoc comparisons of means were performed using Fisher's Least Significant Difference (Webster, 2007). A students t-test was used to test whether the increase in mineralization was different from the increase in addition within the different residue types. Assumptions of a normal distribution were tested by the Kolmogorov–Smirnov test while homoscedasticity was checked using Levene's test. When assumptions were not met, a logarithmic transformation was used. The results are presented as means of 4 replicates for non-isotopic, and 3 replicates for isotopic measurements.

3.1.4 Results

3.1.4.1 Effect of residue addition on aggregates and C distribution

The distribution of the water-stable macroaggregates (>250 µm) was strongly affected by both residue level ($p \leq 0.001$) and type ($p \leq 0.001$). The interaction of level and type showed a strong tendency ($p = 0.068$) to affect macroaggregate distribution, meaning that the effect of residue type tended to be more pronounced at high level (Fig. S1-1). At high addition level, the proportion of macroaggregates decreased with residue type in the order: leaves (45± 2.9%), stalk (37.3± 3.8%) and roots (28.2 ± 2.4%). Correspondingly, the proportion of microaggregates increased in the same sequence (Fig. S1-1). The proportion of macroaggregates (17-23%) at

low addition level did not differ from unamended soil. Proportions of microaggregates were inversely related to macroaggregates.

The formation of aggregates was accompanied by incorporation of wheat residues. Up to 58% of the residue C was incorporated in all aggregate fractions and about 37% was protected in macroaggregates (Table S1-1, Fig. S1-2). A much lower portion of residue derived C was observed in the microaggregates (7-15%) and in the silt plus clay fraction (1.5-2.7%, Fig. S1-2). Absolute amounts of residue C were higher at high level throughout all size classes. However the portion of residue derived C (% of initial input) incorporated into aggregates was smaller at high addition level in macro- and microaggregates (Fig. S1-2). Moreover, the portion of root-derived C in microaggregates was significantly higher compared to stalk and leaves (Fig. S1-2).

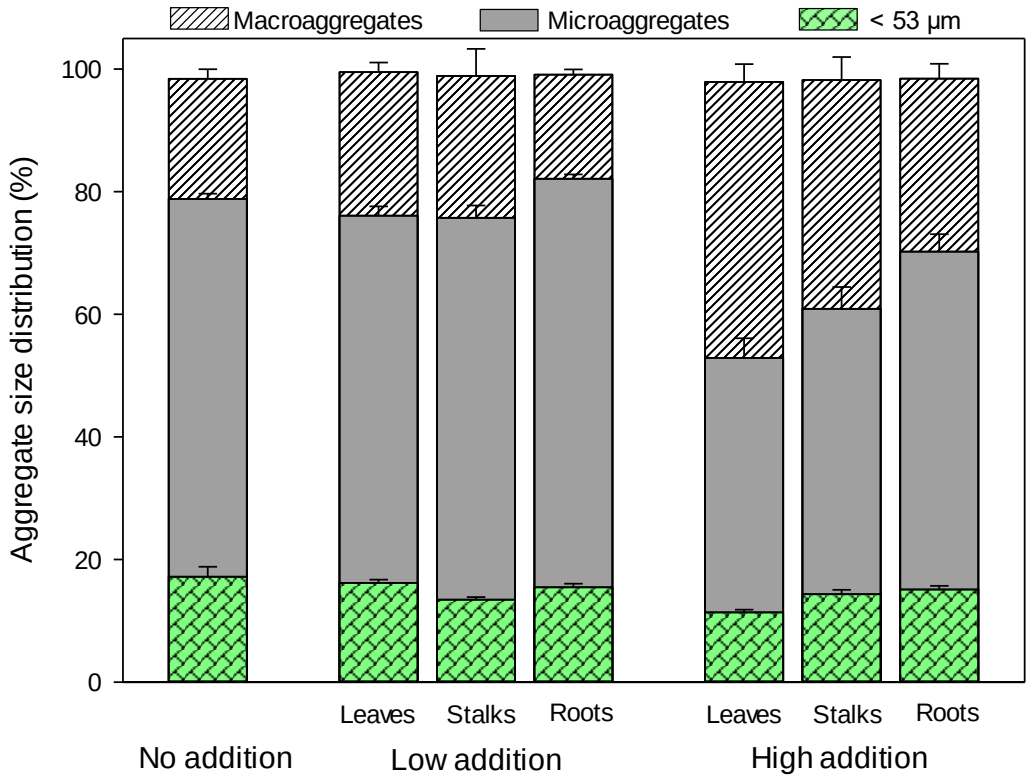


Figure S1-1 The relative distribution of aggregate size classes (Macro >250 µm, Micro 53-250 µm and silt plus clay <53 µm) after 64 days of incubation depending on type and level of crop residue additions. Means and standard errors (n=4). The probability levels of the ANOVA for accepting the null hypothesis that the factors have no effect are as follows: macroaggregates (type < 0.001; level < 0.001; type × level = 0.068), microaggregates (type < 0.001; level = 0.001; type × level = 0.349), < 53 mm (type = 0.034; level = 0.116; type × level = 0.003).

Table S1-1. The contribution of residue-C (as % of initial input) protected in different soil aggregate fractions (macroaggregates >250 µm, microaggregates 53-250 µm and silt plus clay <53 µm), and mineralized as CO_2, in total recovery of added residue after 64 days of incubation, depending on the level and type of addition. Unrecovered plant residues were not incorporated into aggregates and removed from samples. Numbers in the brackets represent SE (n=3).

Treatment	Residue C (% of initial input)		Total recovery (%)
	Aggregate classes	CO_2	
Low addition level			
Leaf	47.3 (3.9)	42.6 (0.8)	89.9 (4.7)
Stalk	55.2 (1.5)	34.7 (2.2)	89.8 (3.5)
Root	58.6 (0.9)	37.1 (1.4)	95.7 (2.3)
High addition level			
Leaf	34.0 (0.3)	40.2 (0.9)	74.2 (0.9)
Stalk	38.2 (0.7)	39.0 (1.6)	77.2 (2.2)
Root	47.62 (5.2)	25.6 (1.4)	73.2 (4.3)
ANOVA results(*p* values)			
Type	0.003	<0.001	0.758
Level	<0.001	0.018	<0.001
Level x Type	0.567	0.001	0.332

3.1.4.2 Microbial biomass

Residue-derived C in microbial biomass was affected both by type ($p = 0.001$) and level ($p < 0.001$) of addition. More C was incorporated at high level of all residue types (2-3 times), and incorporation was highest from leaves followed by stalks and roots (Fig. S1-3).

Microbial biomass C derived from SOM was affected by the interaction of residue type and level ($p = 0.001$, Fig. S1-3). Addition with leaves and stalks decreased C contents of microbial biomass by 24 and 45 mg kg^{-1} at high compared to low addition level, respectively.

3.1.4.3 Mineralisation

Residue mineralization (Fig. S1- 4) after 64 days depended on the type and level of the addition (for both $p < 0.001$). For instance, residue mineralization at high addition level was 3.4 times higher for leaves (230 and 790 mg CO_2-C kg^{-1}, low and high level, respectively) and 4.1 times higher for stalks (200 and 820 mg CO_2-C kg^{-1}, low and high level, respectively) than at low level. Therefore, the increase in mineralisation was in the same magnitude as to the increase in addition level, which was 3.6 times higher (t-values 1.4 and 2.1 for leaves and stalks, respectively, critical t-value: 4.3). CO_2 efflux derived from roots was lower (150 and 370 mg kg^{-1}, low and high level, respectively; $p < 0.001$) as compared to leaves or stalks (Fig. S1-4).

CO_2 evolution at low addition level was 65 to 75% compared to leaves and stalks and is fully explained by the lower C content of roots. At high level, however, mineralization of roots is less than 50% of leave and stalks. The increase of mineralization from low to high level was only 2.4 times (significantly different from 3.6, t-value: 7.0). Therefore, the type of residues was more important at high input level (type × level $p < 0.001$).

Mineralisation of SOM (Fig. S1-4) increased ($p < 0.001$) with the level of residue addition. Consequently, SOM mineralisation was 50 to 90% increased due to the addition of field-equivalent amounts of 5 and 18 Mg ha^{-1} crop residue (Fig. S1-4).

3.1.5 Discussion

Overall, results confirmed, at least in parts, all of our hypotheses. Our first hypothesis assumed that root residue mineralisation will be lower than of stalk and leaf. This was confirmed at least at high addition level (Table S1-1, Fig. S1-4) and is corroborated by previous work (Bertrand et al., 2006; Freschet et al., 2013; Rasse et al., 2005). The lower root mineralisation is generally explained by biochemical composition (more lignin, suberin, and less N) of roots being more recalcitrant (Bertrand et al., 2006; Rasse et al., 2005). It is, however, noteworthy that residue-derived CO_2-C efflux increased less with addition level for roots as compared to stalks and leaves (Fig. S1-4). Whereas residue input increased 3.6 fold, mineralisation of leaves increased 3.4 fold, of stalks 4.1 fold and of roots only 2.4 fold. The mechanisms explaining the microbial activity with root input at high level cannot be elucidated unequivocally from our experiment. On the one hand, some compounds in roots may directly affect microbial activity negatively (e.g. phenolic compounds, Bertrand et al., 2006). On the other hand, interactions with the mineral soil matrix, such as aggregation, could protect residues from mineralisation. For instance, the proportion of root-derived C in microaggregates and the fraction < 53 µm is significantly higher for roots than for stalks and leaves (Fig. S1-2).

The second hypothesis assumed that formation of aggregates will increase with the residue input level, but the proportion of residue-C incorporated within aggregates will decrease. The increase in macroaggregate formation was strikingly demonstrated (Fig. S1-1), as reported before in other studies using various organic additions (Abiven et al., 2009; Andruschkewitsch et al., 2014; Helfrich et al., 2008; Six et al., 2004). Correlation of microbial respiration with macroaggregate portion (Andruschkewitsch et al., 2014) confirms the contribution of active

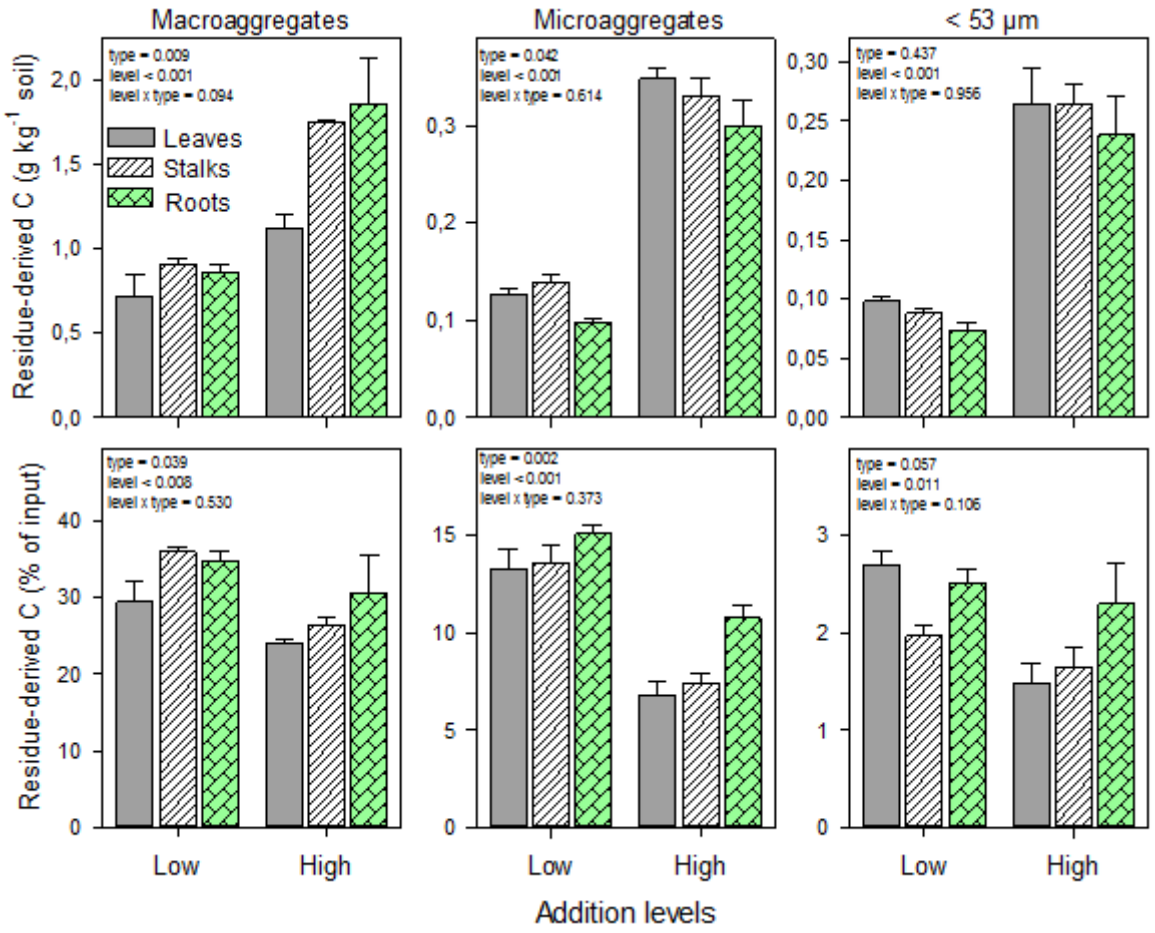


Figure S1-2 Residue-derived C in the soil aggregate size classes (Macro >250 µm, Micro 53-250 µm and silt plus clay <53 µm). Upper subfigures present total aggregate protected C in soil and lower subfigures show protected C portion of initially added residue-C. Means and standard errors (n = 3). The p-values calculated by an ANOVA show probability levels for accepting the null hypothesis that the factors have no effect.

microorganisms to aggregate formation. The correlation of the mass of macroaggregates with the CO_2 release was better with residue-derived CO_2 (r = 0.8) than with SOM-derived CO_2 (r = 0.5). The proportion of protected residue-derived C was smaller at high addition level for all types of residue (Table S1-1, Fig. S1-2). Thus, increasing addition level promotes macroaggregate formation. However, the low proportion of physically protected residues at high addition levels leads a decreasing C-stabilization rate within SOM. Only in case of high addition with roots, however, we found a potentially protecting effect of occlusion within aggregates. For instance, if occlusion within aggregates protects residues from mineralisation (Six et al., 2002) then residue mineralization (as a proportion of total input) should be lower when aggregate occlusion is higher. Table S1-1 clearly shows that this was only the case when

roots were added at high level, whereas there was no significant difference for any other treatment in the proportion of mineralised residue. Therefore, physical protection did not play a marked role in C stabilization of aboveground residues. Although there are widespread assumptions that aggregates protect organic matter from mineralisation, this may not necessarily apply to freshly incorporated aboveground residues within macroaggregates (Andruschkewitsch et al., 2014). Microaggregates may be more effective in stabilising C (von Lützow et al., 2008) because sorption instead of physical occlusion may be the prevailing process (Lehmann et al., 2007). At high addition level of roots, we found not only a lower proportion of mineralisation (Table S1-1) but also a higher association of root C with microaggregates and the < 53 µm fraction (Fig. S1-2). Overall there was no evidence for physical short-term stabilisation of aboveground plant parts and higher association of large amounts of roots may indicate preferential long-term stabilisation under field conditions (Baldock and Skjemstad, 2000; Six et al., 2002; von Lützow et al., 2008).

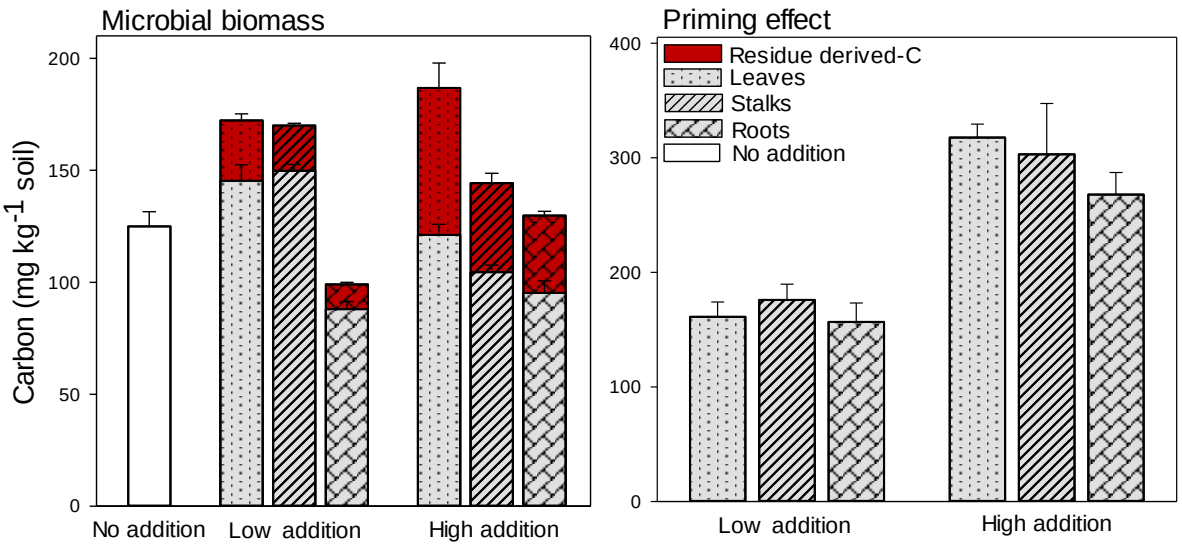


Figure S1-3 The contribution of residue derived and soil organic matter (SOM) derived C to microbial biomass (left) and the amount of primed C due to low and high level of crop residue addition (right). Means with standard errors (n=3). The probability levels of the ANOVA for accepting the null hypothesis that the factors have no effect are as follows: SOM derived C (type < 0.001; level = 0.001; level × type = 0.001), residue derived C (type = 0.001; level <0.001; level × type = 0.118), primed C (type = 0.413; level <0.001; level × type = 0.613).

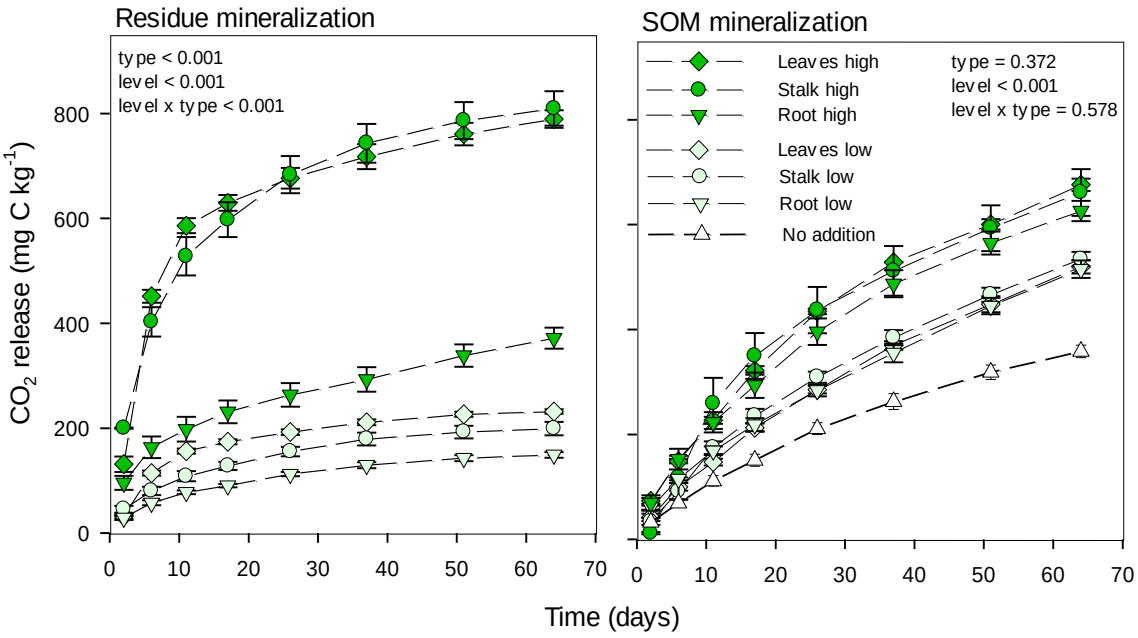


Figure S1-4 Cumulative CO2-C release during 64 days of incubation depending on type and level of crop residue additions. Left: release from crop residues; right: release from soil organic matter (SOM). Mean values with standard errors (n = 3). The p-values calculated by an ANOVA show probability levels for accepting the null hypothesis that the factors have no effect.

Our third hypothesis assumed that the incorporation of wheat residue will induce positive priming of SOM, with its magnitude depending on the level of addition and the type of residue. The priming of added residues was evident from increased mineralization of SOM which mainly depended upon the amount of addition. (Fig. S1-4) Regardless of residue type, mineralization of SOM increased up to from 50 to 90% due to addition of low and high levels, respectively, whereas residue addition was increased 3.6 times. Therefore, the amount of primed CO_2 decreased per unit of applied residue. This was also reported by Guenet et al. (2010) and Xiao et al. (2015). Generally, the addition of substrates activates microbial biomass, whose enhanced production of extra-cellular enzymes causes priming (Kuzyakov et al. 2009; Loeppmann et al. 2016; Wu et al. 1993). This is shown in our study by a growing fraction of microbial biomass which preferentially used residue C instead of SOM (Fig. S1-3; Xiao et al. 2015). Indeed, primed C is related to residue-derived microbial C (R^2 = 0.47) and also to residue-derived C in microaggregates (R^2 = 0.80). We conclude that the intimate contact of residue and soil in microaggregates promotes diffusion of enzymes between the substrates

(SOM and residues). Due to the smaller proportion of residues in aggregates with high addition level (Fig. S1-2, Table S1-1), the priming effect on a per-input-base levels off. This may also explain the lack of an effect of residue type on priming. We expected that the different mineralization of residue types would be reflected in the intensity of priming. Roots at high level showed least mineralization, but similar priming. Both findings can be linked to the higher incorporation of root residues into aggregates.

3.1.6 Conclusions

Our initial hypotheses were not all fully confirmed. Firstly, we hypothesised that mineralization of root residues will be lower regardless of addition level. Root residues at the low addition level were mineralised to a similar extent as leaves and stalks, and root mineralisation was lower only at high addition levels. Secondly, the portion of residue-C as percent of initial input incorporated into macro- and microaggregates was decreased with increasing input level, as we hypothesised. Roots at the high addition level, however, were incorporated into aggregates more effectively than leaves and stalks. Our third hypothesis assumed that priming would depend on the type and addition level of residues. Mineralisation of SOM was accelerated by 50 to 90% and increased with residue addition levels. Contrary to our hypothesis, the type of residue showed no effect on priming. Overall, SOM stabilization decreased with increase in addition level. However, at the high addition level a higher portion of roots, compared to stalk and leaves, was incorporated into aggregates, which was accompanied by decreased mineralisation. Priming induced by freshly incorporated residues should be further investigated in aggregates with a special focus on dynamics and enzyme activities. Feedbacks between incorporation of fresh residues into aggregates and priming may be important under field conditions. We conclude that the proportion of residues physically protected within aggregates decreases and priming effects increase with increasing C input leading to decreasing rate of long-term C stabilization within SOM by increasing residue addition.

In order to sustain sufficient SOM levels in arable soils, an efficient crop residue management under specific field conditions is rquired. Our findings highlight the necessity to connect the quantity and quality of crop residues for better predicting mineralization and stabilization of SOM. Specifically, this may also help to resolve the global implications to characterize and identify key soil and residue parameters for modeling of greenhouse gas emissions from soil.

3.1.7 Acknowledgements

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3.2 Study 2

Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds

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3.2.1 Abstract

Crop residue quality and quantity have contrasting effects on soil organic matter (SOM) decomposition, but the mechanisms explaining such priming effect (PE) are still elusive. To reveal the role of residue quality and quantity in SOM priming, we applied two rates (5.4-10.8 g kg^{-1}) of ^{13}C-labeled wheat residues (separately: leaves, stems, roots) to soil and incubated for 120 days. To distinguish PE mechanisms, labeled C was traced in CO_2 efflux and in microbial biomass and enzyme activities (involved in C, N and P cycles) were measured during the incubation period. Regardless of residue type, PE intensity declined with increasing C additions. Roots were least mineralized but caused up to 60% higher PE compared to leaves or stems. During intensive residue mineralization (first 2-3 weeks), the low or negative PE resulted from pool-substitution. Thereafter (15-60 days), a large decline in microbial biomass along with increased enzyme activity suggested that microbial-necromass served as SOM primer. Finally, incorporation of SOM-derived C into remaining microbial biomass corresponded to increased enzyme activity, which is indicative of SOM co-metabolism. Both PE and enzyme activities were primarily correlated with residue-metabolizing soil microorganisms. A unifying model demonstrated that PE was a function of residue mineralization, with thresholds for strong PE increase of up to 20% root, 44% stem and 51% leaf mineralization. Thus, root mineralization has the lowest threshold for a strong PE increase. Our study emphasizes the role of residue-feeding microorganisms as active players in the PE, which are mediated by quality and quantity of crop residue additions.

Keywords: ^{13}C-labeled crop residues, Enzyme activities, Litter quality, Microbial necromass, Priming effect, Soil organic matter

3.2.2 Introduction

Soil organic matter (SOM) is primarily formed through the partial degradation and transformation of crop residues by microorganisms (Castellano et al. 2015). The quality and quantity of crop residues influence the microbial decomposition processes, which may affect residue and SOM mineralization rates, leading to a priming effect (PE). The PE represents the changes of native SOM decomposition as a result of exogenous substrate inputs such as crop residues (Jenkinson et al. 1985; Kuzyakov 2010). As soil microorganisms are mostly C-limited, input and/or high availability of substrates may alter their activities, resulting in a PE (Blagodatskaya and Kuzyakov 2008). While increasing amounts of substrate addition can decrease PE, the effect, however, depends on the substrate quality (Guenet et al. 2010). If substrate composition and availability are heterogeneous (e.g. crop residues), then a cascade of apparent (due to microbial turnover) and real (due to SOM decomposition) PE can be induced over time at various stages of substrate decomposition (Kuzyakov 2010; Xu et al. 2011).

Crop residues vary in their structural and chemical composition (Adair et al. 2008; Aber and Melillo 1982). Residue decomposition rates are generally negatively related to the amount of recalcitrant compounds present in their biomass, such as lignin, phenols, tannins etc. (Bertrand et al. 2006; Castellano et al. 2015; Aber and Melillo 1982). Aboveground crop residues (e.g. leaves and stems) are considered high quality compared with belowground residues, which are relatively recalcitrant to decomposition e.g. roots (Bertrand et al. 2006; Rasse et al. 2005). The role of residue quality in SOM formation is currently under debate. The common view on recalcitrant root residues, which are decomposed slowly and therefore contribute largely to SOM (Berg and McClaugherty 2014; Johnson et al. 2014; Rasse et al. 2005), contradicts the view of the great contribution of easily decomposable residues in SOM formation (Cotrufo et al. 2013; Lehmann and Kleber 2015). The latter concept is mainly associated with the microbial by-products, which are released and stabilized in the soil mineral fraction during crop residue decomposition (Cotrufo et al. 2013; Ladd et al. 1996; Shahbaz et al. 2016b) and microbial-turnover (Ladd et al. 1996; Miltner et al. 2012).

The soil microbial turnover depends on substrate quality and availability (Leifeld and von Lützow 2014; Nguyen and Marschner 2016). If substrate availability or input is interrupted, microorganisms may respond by a switch to dormancy or their biomass decreases after microbial cell death. Such a relatively fast decrease of substrate dependent microbial biomass (MB) was already detected in several laboratory incubation experiments (e.g. Blagodatskaya et

al. 2011a; Jiang-shan et al. 2005; Tian et al. 2015; Wang et al. 2016). The decrease in MB results in accumulation of microbial-necromass (after their cell death), which is already considered as an important source for stable SOM (Miltner et al. 2009, 2012; Wagner 1968). Due to its heterogeneous nature, microbial-necromass may serve as labile substrates for living microorganisms and its re-utilization can represent SOM priming.

The PE has often been explained by the microbial activation hypothesis (Chen et al. 2014). Instead of total microorganisms, the active microbial fraction is considered to be more important (Blagodatskaya and Kuzyakov 2013). The active fraction mostly consists of the growing portion of microorganisms, which respond rapidly to substrate addition, e.g. by producing enzymes (Blagodatskaya and Kuzyakov 2013; Fontaine et al. 2007). Depending on substrate quality (i.e. labile substance or C/N ratio), the active microbial fraction produces enzymes either to degrade added organics or to decompose SOM to meet their nutritional demands (Schnecker et al. 2014; Wang et al. 2015). This, again, may result in variable successions of PE over time. The changes in microbial activity due to substrate decomposition (rapidly or slowly) can therefore be recognized, for example by the changes in enzyme activities (Burns et al. 2013; Nannipieri et al. 2002, 2012; Schnecker et al. 2014).

Most of the studies investigating PE used labeled low molecular weight substances such as glucose and amino acids (e.g. Blagodatskaya et al. 2011a; Hoyle et al. 2008; Tian et al. 2015). Only a few studies have distinguished PE using labeled crop residues, mostly with contrasting results depending on the residue type. PE proves to be a linear function of MB but is also a saturation function of the substrate's C amount (Guenet et al. 2010; Xiao et al. 2015). Although the effect of residue quality on PE is not obvious, the role of residue-decomposing microbial fractions in PE is highlighted based on the contrasting quality of substrate additions (Wang et al. 2015). We lack information explaining the mechanisms of PE under contrasting quality and quantity of crop residue C based on the active residue-feeding microbial fraction.

The present study is designed to explain the mechanisms of PE induced by crop residues varying in their amount and quality. Here, we used homogeneously ^{13}C-labeled wheat (*Triticum aestivum* L.) biomass to partition residue- and SOM-derived C within total CO_2 and MB. We added contrasting quality wheat residues from both aboveground (leaves, stems) and belowground (roots) parts to soil at two levels. We hypothesized that: i) the intensity of SOM decomposition will be affected by the residue mineralization rates, i.e. SOM decomposition will be dependent on residue type; ii) regardless of residue type, the intensity of PE will decrease

with increasing C addition. We assumed that microorganisms decomposing added residues will represent the most active fraction of soil microflora. Therefore, we further hypothesized that; (iii) the PE will be the main function of the soil microorganisms feeding on residues and of its enzymes activities

3.2.3 Materials and methods

3.2.3.1 Study area and soil

The soil used for the incubation was sampled from the Ap horizon (0-25 cm) of an experimental field located on a terrace plain of the Leine River north-west of Goettingen, Germany (51°33′36.8″ N, 9°53′46.9″ E). Since more than 25 years the field has been cultivated with annual C3 crops, predominantly wheat (Kramer et al. 2012).The soil was classified as Luvisol and had a silt-loam texture (6% sand, 87% silt, 7% clay). The pH and a test with 10% hydrochloric acid indicated the absence of carbonates. The carbonate-free soil had the following characteristics: MB 0.40 ±0.0 g C kg^{-1} soil, organic C 12.8 ±0.4 g kg^{-1}; total N 1.3±0.0 g kg^{-1}, pH ($CaCl_2$) 6.0; $\delta^{13}C$ -26.8‰. After sampling, the soil was air dried, sieved (< 2 mm) and fine roots and other visible crop debris and small stones were carefully removed.

3.2.3.2 Production of ^{13}C-labeled crop residues

Wheat plants were grown to produce ^{13}C-labeled residues as described in detail by Bromand et al. (2001). Briefly, wheat (*Triticum aestivum* L.) seeds were grown in pots filled with quartz sand and were watered regularly once a week with Hoagland nutrient solution (N: 210, K: 235, Ca: 200, P: 31, S: 64, Mg: 48 mg L^{-1} plus micronutrients). Following seedling emergence (11 days after seeding), plants were placed inside a transparent closed chamber (120 cm wide × 104 cm high × 60-cm deep) enclosed within a climate-controlled growth cabinet with the following conditions: 16/8 h photoperiod, light intensity at approximately 600 µmol m^{-2} s^{-1}, mid day and night temperatures of 25°C and 15°C, respectively. The plants were labeled (for at least 8 h) continuously with ^{13}C every week until harvesting. The intended enrichment of CO_2 in the chamber was ~99 atom% ^{13}C. To accomplish this, the CO_2 concentration was first allowed to fall to 327 ppmv in the chamber. Thereafter, ^{13}C-labeled CO_2 was generated by injecting $NaH^{13}CO_3$ (as ~99 atom% $^{13}CO_2$ source) solution through a septum into a generation flask containing 1M H_2SO_4. The evolved CO_2 was swept into the sealed chamber with a small pump through a closed loop of tubing, and a fan circulated the CO_2 inside the chamber. Wheat plants

continued to grow in the chamber until maturity (120 days of growth). Thereafter, the plants were harvested and roots were gently washed to remove the sand particles.

To avoid any preferred decomposition of above- and belowground parts of the wheat biomass, plant residues were carefully separated into leaves, stems and roots. For homogeneous mixing of residues within the soil, each part of the residues was chopped and sieved (2 mm). Carbon and N contents of applied residues varied between residue types, whereby leaves, stems and roots had C contents of 391.9 ±6.1 (C/N: 17.2±0.3), 409.6 ±8.7 (C/N: 21.5 ±1.17) and 298.3 ±5.9 (C/N: 15.5±0.5) g kg^{-1}, respectively. The atom% ^{13}C values were measured with an isotope ratio spectrometer coupled to an elemental analyzer (Delta plus, EA-IRMS, see detail section 3.2.3.7) (Table S2-1).

3.2.3.3 Incubation and sampling

For incubation, 50 g soil (dry weight basis) was weighed into 250-ml incubation bottles. The soil was then pre-incubated (at 22 °C) at 50% water holding capacity (WHC) for one week. Thereafter, the 120-day full-factorial incubation experiment with two factors – wheat residue type and residue addition level – was designed. Accordingly, eight treatments were included: three ^{13}C-labeled wheat residue types (leaves, stem, roots), two addition levels (low and high, respectively 5.4 and 10.8 g dry mass kg^{-1} soil) and one control without residue addition. A reference treatment with the decomposition of crop residues in autoclaved sand was also conducted to consider isotopic fractionation during incubation. All treatments were set with three replicates. The added residues were thoroughly mixed in the soil, and the water contents were then adjusted to 70% WHC. Note, the control soils (without residue addition) also treated in the same way as those with residue addition. The residues were added on a dry matter basis. Accordingly, the C input by roots (with lower C content) corresponds to ca. 70% of the C amount added with leaves or stems.

To estimate MB and enzyme activities, samples were destructively harvested at day 15, 30, 60 and 120 of incubation.

To prove that crop residue were homogeneously labeled, we sampled the partially decomposed residues during the incubation period (destructive sampling). A portion of the incubated soil (ca. 20 g, having residues) was submerged into distilled water; thereafter, the floating material was collected, dried (60°C) and analyzed for ^{13}C values at IRMS (section 3.2.3.7). The isotopic signature of partially decomposed residues revealed that after 2-weeks (intensive residue

mineralization), the differences in ^{13}C values for the rest of incubation period did not exceed 0.03 atom% in all treatments (Table 1). This indicated that possible error due to the inhomogeneous (non-uniform) labeling of the residues was minimal and that it did not exceed the variation in ^{13}C between the replicates. The isotopic signature was used as the residue reference material for the mass balance equation.

3.2.3.4 CO_2 efflux

In order to measure soil respiration, CO_2 was trapped in 5 mL 1M NaOH trap solution. The trap solution was replaced with fresh 1M NaOH aliquot at day 2, 4, 7, 11, 23, 30, 36, 46, 60, 81, 101 and 120 of incubation. Therefore, incubation bottles were not closed longer than 21 days and the capacity of NaOH was never used up by more than 60%. An aliquot of sampled NaOH was immediately used to measure total soil and residue-derived CO_2. The total amount of CO_2 trapped in the NaOH solution was determined by titration with 0.05 M HCl against phenolphthalein, after addition of 0.5 M $BaCl_2$ solution.

3.2.3.5 Microbial biomass

To determine the C content in MB at all destructive sampling periods, the chloroform fumigation-extraction method was used as already described by Makarov et al. (2015) and Vance et al. (1987). Briefly, 6 g (moist) soil were extracted with 24 ml of 0.05M K_2SO_4 for one hour. The other 6 g soil was firstly fumigated with ethanol-free $CHCl_3$ for 24 h at 22˚C and then extracted in the same way. The obtained extract were analyzed for total C content using a TOC/TIC analyzer (Multi N/C 2100, Analytik Jena, Germany). The MB-C (K_2SO_4 extractable) was calculated as E_C/K_{EC}, where E_C = is the difference between extracted organic C of fumigated and non-fumigated soils and K_{EC} = 0.45 (Wu et al. 1990).

3.2.3.6 Enzyme assays

The enzyme activities at all sampling periods (15, 30, 60 and 120 days) were measured using fluorogenically labeled substrates (Pritsch et al. 2004; Sanaullah et al. 2016). Five fluorogenic enzyme substrates based on 5- methylumbelliferone (MUF) were used: MUF- β -D-cellobiohydrolase (MUF-C; EC 3.2.1) for cellobiohydrolase, MUF- β -D-xylopyranoside (MUF-C; EC 3.2.1) for xylanase, MUF-N-acetyl- β -D-glucosaminide dehydrate (MUF-NAG; EC 3.2.1.14) for chitinase, MUF- β -D-glucopyranoside (MUF-G; EC 3.2.1.21) for β-glucosidase and MUF-phosphate monoester (EC 3.1.3.2) for acid phosphomonoesterase (Nannipieri et al. 2011). l-Leucine-7-amino-4-methylcoumarin (AMC) substrate was used to

estimate L-leucine aminopeptidase (LAP) activity. All enzyme substrates were purchased from Sigma (Germany).

Briefly, 0.5 g of soil (dry weight basis) were dispersed in 50 ml of deionized water for 2 min using low energy sonication (40 J s^{-1}). Then, 50 µl of the suspension were pipetted into 150 µl specific enzyme substrate solution (containing 50 µl of MES or Trizma buffer for MUF or AMC substrates, respectively) having a final concentration of 200 µmol g^{-1} soil. Fluorescence was measured by incubations of soil suspension (for 2 h at 22 °C) in 96-well microplates (puregrade, Germany) with fluorogenic substrates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm, with a Victor R^3 1420 Multilabel Counter (Perkin Elmer, Waltham USA).

3.2.3.7 Isotopic analysis

Since we used ^{13}C-enriched plant material, the CO_2-trapped NaOH samples were specifically prepared for isotopic analysis. For this, 3 ml of CO_2-trapped NaOH solution was precipitated with an equal volume of 1 M $SrCl_2$ solution. The NaOH solution containing $SrCO_3$ precipitates was then centrifuged for 5 min at 2680 × g. The process was repeated with distilled water to remove excess NaOH and to reduce pH to 7. After removing water, $SrCO_3$ pellets were dried at 60°C and stored for ^{13}C analysis by an isotope ratio mass spectrometer (Delta plus, IRMS; Thermo Fisher Scientific, Bremen, Germany). The ^{13}C values were expressed as atom%. The estimations were calibrated with reference to the international VPDB (Vienna Peedee Belemnite) standard. For ^{13}C measurement of MB, an aliquot (ca. 10 mL) of the K_2SO_4 extract was freeze-dried and thereof solid material was analyzed.

3.2.3.8 Calculations

To partition residue- and SOM-derived C in total CO_2 and microbial C, calculations were done step by step as suggested earlier (Blagodatskaya et al. 2011a; Poirier et al. 2013)

Firstly, the ^{13}C values (atom%) were calculated according to the following equation.

$$Atom\%\ ^{13}C = [number\ of\ ^{13}C\ atoms / number of (^{12}C + ^{13}C)\ atoms] \cdot 100 \qquad (1)$$

Then the fraction of residue-derived C ($f \cdot C_{res}$) was calculated according to the mass balance equation (Hayes 2004):

$$f \cdot C_{res} = (At_{mix} - At_{con})/(At_{res} - At_{con}) \qquad (2)$$

Where At_{mix} represents ^{13}C atom% values of the residues-amended soil evolved as CO_2 (trapped in NaOH), or present in fumigated or non-fumigated K_2SO_4 extracts. At_{res} represents specific ^{13}C atom% values of the corresponding residue source (i.e. leaves, stems, roots). At_{con} shows ^{13}C atom% values of each corresponding pool of soil without residue addition.

Finally, the amount of residue-derived C ($C_{res,}$ g kg^{-1}) was calculated according to equation (3):

$$C_{res} = f \cdot C_{res} \cdot [TC] \quad (3)$$

Where [*TC*] represents the total C amount of the corresponding pool (i.e. CO_2, fumigated and non-fumigated K_2SO_4 extract)

The amount of SOM-derived C ($C_{SOM,}$ g kg^{-1}) was simply calculated by subtracting C_{res} from the total C of the corresponding pool.

The amount of primed C released as total CO_2, i.e. PE (g C kg^{-1}), was calculated according to the following equation.

$$PE = CO_2 \cdot C_{total} - CO_2 \cdot C_{res} - CO_2 \cdot C_{control} \quad (4)$$

To estimate the residue-derived C fraction present as MB (Res_C_{MB}) in each destructive sampling and at the end of incubation, firstly residue-derived C was calculated separately for fumigated and non-fumigated samples using equation 3. Then calculations were done according to following equation.

$$Res\_C_{MB} = (f \cdot C_{res} - nf \cdot C_{res})/(f \cdot C - nf \cdot C) \quad (5)$$

Where $f{\cdot}C_{res}$ and $nf{\cdot}C_{res}$ are the C_{res} values of fumigated and non-fumigated samples, respectively, calculated according to Eq. 3. f·C and nf·C are the amounts of total C in fumigated and non-fumigated samples, respectively, determined as discussed in section 3.2.3.5.

The relationship of both CO_2 efflux and enzyme activities were highly positively correlated with the Res_C_{MB} instead of total MB-C. Therefore, to estimate specific PE and enzyme activities (in relation to Res_C_{MB}), the absolute amounts of PE (calculated according to Eq. 4) and enzyme activities (as described in section 3.2.3.6) were divided by the total amounts of Res_C_{MB}, which was calculated according to Eq. 5.

3.2.3.9 Threshold values for PE increase

The relationship between the fraction of mineralized residue, x (as % of initial input) and specific priming effect (PE), was best explained and fitted by a unifying model (combining logistic and power functions):

$$PE(x) = a \cdot x^2 + b/\left[1 + \exp\left(-c \cdot (x - d)\right)\right] + e \quad (6)$$

where PE(x) represents the specific PE, x the value of mineralized fraction of crop residues, a the residue quality coefficient, b the maximal PE value, c the residue-specific maximal rate of PE increment, d the mineralized fraction of residues at maximal rate of PE increment, and e the minimal PE value. Model parameters were optimized for best fitting of the model output to experimental data. All fits were done with Excel Solver facilities. For all the types of crop residues, the model demonstrated an excellent goodness of fit with r^2 above 0.98. The model enabled estimation of the threshold value of the mineralized fraction of crop residues when maximal changes in PE increment occur. That corresponds to the point where the second derivative of the function (6) has its maximum.

3.2.3.10 Statistical analysis

The experiment was carried out as a full factorial, completely randomized design. The factor "type" had three levels (leaves, stems, roots) and the factor "addition" had two or three levels (no, low and high addition). Time was considered as a random factor where applicable. Two addition levels were used when comparing residue-derived C where inclusion of "no addition" was not suitable. Statistical analyses were performed with SPSS 11 using a two-way ANOVA (when the time was not considered) and three-way ANOVA with "addition level", "type" as fixed effects and "time" as a random effect. When significant ($p \leq 0.05$) effects were found, post hoc comparisons of means were performed using Fisher's Least Significant Difference test (Webster 2007). The error propagation was calculated when the mean values were used for determining PE (Meyer 1975). Correlations (r) between MB (derived from residue and SOM) values and CO_2 or potential enzyme activities in soils were analyzed by Pearson's correlation method.

3.2.4 Results

3.2.4.1 ^{13}C in crop residues during incubation

The ^{13}C values of incorporated residues were specific to residue type i.e. leaves, stem and root had 1.54, 1.36 and 1.51 ^{13}C atom%, respectively. During the incubation period, the ^{13}C values

of partially decomposed residues mainly declined until day 15, and thereafter remained nearly constant for the rest of incubation period, indicating that residues were homogeneously labeled (Table S2-1). The averaged (±SD) ^{13}C (atom%) values of partially decomposed residues of leaves, stem and roots across all sampling period were 1.41±0.01, 1.32±0.01, 1.44±0.03, respectively. Accordingly, a very similar ^{13}C values of the CO_2 evolved from reference treatments with sand were detected. The average (±SD) values of ^{13}C (atom%) across sampling periods were 1.41±0.03, 1.30±0.02, and 1.42±0.01 for leaves, stems and roots, respectively.

Table S2-1: The ^{13}C values (atom%) of leaves, stems and roots of wheat residues at different decomposition stages over the incubation period at days 0, 15, 30, 60 and 120. Numbers in brackets: ± SE of mean.

Residue type	0 day	15 day	30 day	60 day	120 day
Leaves (^{13}C atom%)	1.54 (0.00)	1.41 (0.01)	1.43 (0.00)	1.40 (0.02)	1.41 (0.01)
Stems (^{13}C atom%)	1.36 (0.00)	1.32 (0.00)	1.33 (0.00)	1.31 (0.00)	1.31 (0.01)
Roots (^{13}C atom%)	1.51 (0.00)	1.46 (0.00)	1.47 (0.01)	1.40 (0.04)	1.45 (0.01)

3.2.4.2 Residue and soil organic matter mineralization

Residue addition caused a significant increase in total soil CO_2 efflux compared to the control without additions. At low additions, the amount of total CO_2 efflux was higher in leaves and stems (for both, up to 1.9 g C kg^{-1}) than in roots (1.5 g C kg^{-1}) (Fig. S2-S1 supplementary material). At the high residue addition level, absolute CO_2 efflux also increased. Similar to low additions, no differences in total efflux between leaves and stems (3.3 g C kg^{-1}) were also found at high additions.

The total amount of SOM-mineralized C in the control was 0.65 g C kg^{-1} soil over 120 days of incubation. Mineralization of SOM significantly increased with residue addition depending on the type and amount of residue (Fig. S2-1a). At the doubled amount of residue addition, the cumulative SOM mineralization remained similar between low and high addition levels of leaves (up to 0.9 g C kg^{-1}) and stems (1.1 g C kg^{-1}). In contrast, SOM mineralization under high root addition increased up to 15% compared with low additions (Fig. S2-1a).

The residue mineralization rate was significantly affected by both type and level of additions. Total mineralization was highest in leaves, lowest in roots (Fig. S2-1b). Depending on the quality and on the added amount, two distinct residue mineralization phases (intensive and slow) were observed. Remarkably, during the intensive phase, the residue decomposition was proportional to the added amount (for leaves and stems ca. 2-3 weeks) (Fig. S2-1b). During the slow phase (after 2-3 weeks), residue mineralization was disproportionally stronger for high versus the low amount of leaves and stems, but not for roots.

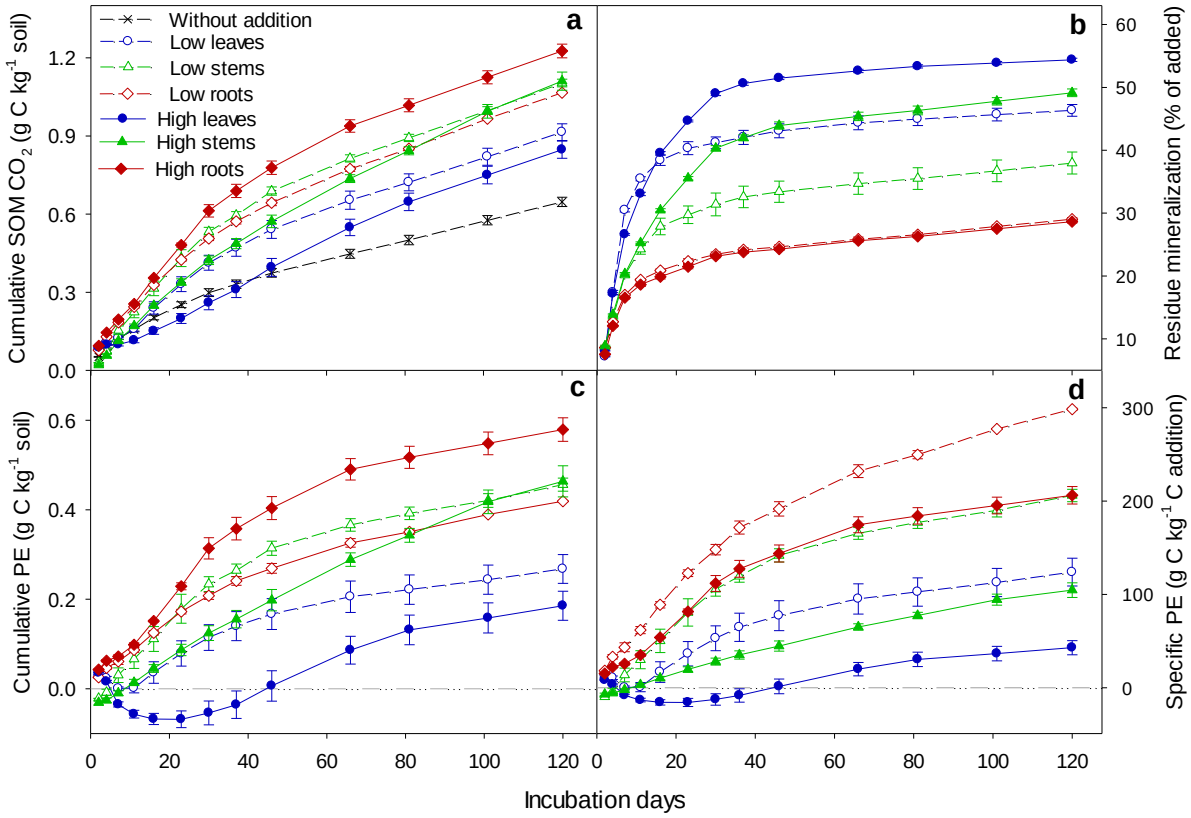

Figure S2-1. Cumulative CO2 release originated from soil organic matter (SOM, a), crop residue decomposition (% of initial addition, b), total priming effect (PE, c), and specific PE (d) over 120 days of incubation, depending on the residue type and addition level. Mean values with standard errors (n = 3). The p-values of the ANOVA showing the effect of different factors for all; cumulative CO2 release originated from SOM (a); residue decomposition (% of initial addition, b); total PE (c) and; specific PE (d) are as follows: type < 0.001, level <0.001 and their interactions; level × type < 0.001.

Therefore, at the end of the experiment, at low addition, the leaf-, stem- and root-derived CO_2 reached 1.0, 0.9 and 0.4 g C kg^{-1}, corresponding to 46, 38 and 29% of their initial additions, respectively (Fig. S2-1b). Relative root mineralization after intensive phase was similar at high

and low addition (i.e. 29% of initial input), whereas the leaf and stem mineralization rate were up to 17 and 30% faster at high than at low additions, respectively (Fig. S2-1b).

3.2.4.3 Priming effect

A significant increase in the SOM-originated CO_2 efflux after residue addition caused a positive PE, but the PE intensity was strongly affected by residue quality and amount (Fig. S2-1c). The maximum PE was recorded for root (increase with the addition level: 0.42 to 0.58 g C kg^{-1}) and lowest (even negative) for leaf addition (0.20 to 0.27 g C kg^{-1}). Remarkably, larger PE was observed at low versus the high amount of stems and leaves during the intensive phase of decomposition. Furthermore, the cumulative PE remained negative under high leaf addition during the initial ca. 6 weeks of incubation (Fig. S2-1c).

As the added residues varied in their C contents (see above), the specific PE was calculated based on C addition (Fig. 1d). The amount of specific PE (per unit of C) in root treatments was significantly higher than that of leaves and stems at both residue addition levels (Fig. S2-1d). The total amounts of specific PE in root treatments were 300 and 200 g C kg^{-1} at low and high root-C addition, respectively.

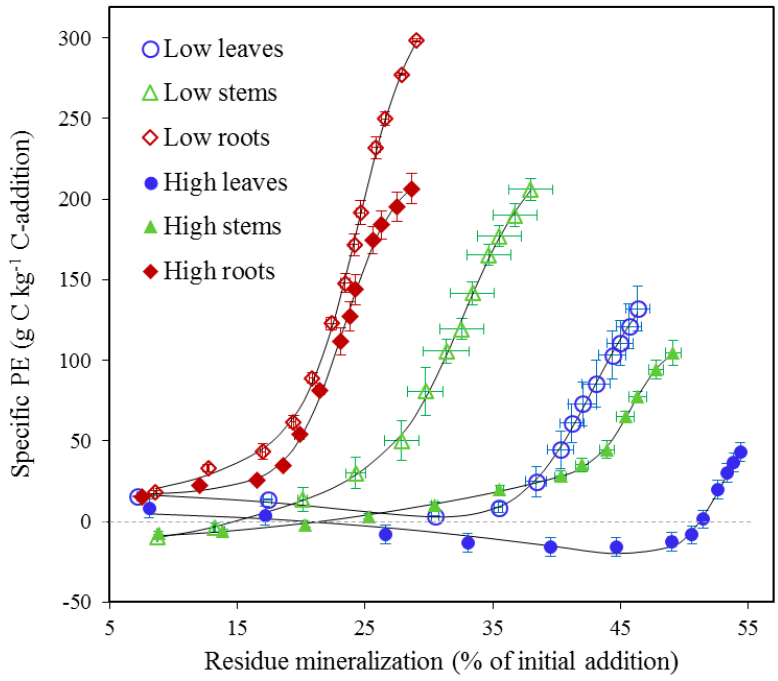

Figure S2-2. The relationship between the fraction of mineralized residue, x (as % of initial input) and soil specific priming effect (PE), was best explained and fitted by a unifying model (combined with logistic and power functions): $PE(x) = a \cdot x^2 + b/\left[1 + \exp\left(-c \cdot (x - d)\right)\right] + e$. Means and standard error (n=3).

Regardless of residue type, specific PE decreased at high versus low C addition (Fig. S2-1d), suggesting that the PE depended on the amount of decomposed residues. To clarify this relationship, we plotted the cumulative specific PE against residue-originated CO_2 (Fig. S2-2). After a lag-period, the PE increased strongly over the course of residue decomposition. This demonstrates that during the initial intensive phase the decomposition rate of residues exceeded the PE. During the slow phase of residue decomposition, however, the PE increased drastically. A threshold (calculated by the second derivative, see section 3.2.3.9) of the decomposition function indicated that such a drastic increase in PE occurred when ca. 20, 29-44 and 39-51% of (low and high) roots, stems and leaves were decomposed, respectively (Fig. S2-2).

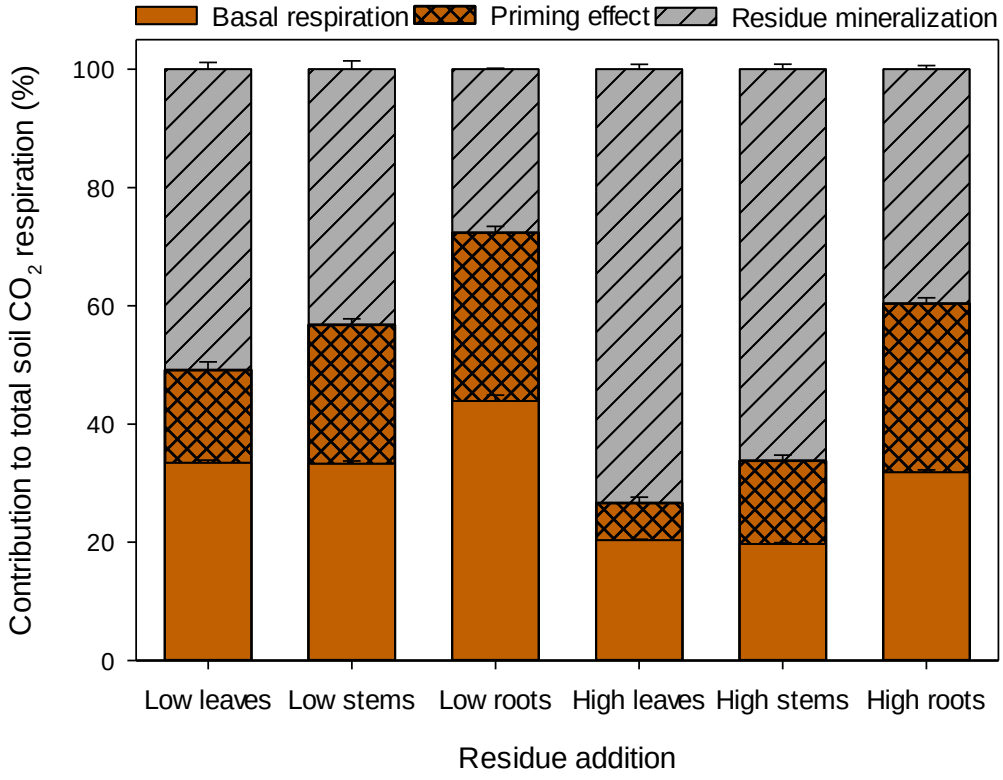

Figure S2-3. The relative contribution (%) of carbon (C) sources from basal respiration, priming effect and residue mineralization) to their corresponding total soil CO_2 efflux after 120 days of incubation, depending on the residue type and addition level. Basal respiration (without addition) was assumed to be constant for all residue treated soils. Means and standard errors (n = 3). The p-values from the ANOVA showing the factors effect is as: basal respiration (type < 0.001; level < 0.001; and their interactions: type × level = 0.081), and for both; priming effect and residue mineralization is as (type < 0.001; level < 0.001; and their interactions: type × level < 0.001).

To differentiate the contribution of various sources of CO_2 efflux from soil (within residue additions), respired from soil over 120 days, total respiration was partitioned (% contribution)

into basal respiration, residue originated, and PE (Fig. S2-3). The basal respiration (from the control 0.65 g C kg^{-1}) was assumed to be constant for all residue additions. At the end of the experiment, 53-73%, 43-66% and 28-40% of added leaves-, stems- and roots-originated CO_2 contributed to their corresponding total CO_2 efflux. At high addition, the percentage of primed C in total CO_2 efflux was lower in the leaves and stems versus their low addition level. At high additions, root-induced primed C percentage of total respiration remained the same as at its low addition levels, but the contribution of basal respiration was reduced, which was substituted by residue-originated C (Fig. S2-3).

3.2.4.4 Microbial biomass

Consistent with the CO_2 efflux, adding residues significantly (28-85%) increased MB-C compared with control. This highlights the microbial demands for C and nutrients. The MB-C significantly increased (compared to the control) during the intensive decomposition phase of the residues (during the first two weeks), with an average of 42-85, 42-53 and 28-54% due to leaf, stem and root addition, respectively (Fig. S2-4a). Remarkably, the increase of MB-C was solely (intensive phase) or mainly (slow phase) due to residue-feeding microorganisms, because the differences in SOM-decomposed biomass were insignificant (except at day 120). The amount of residue-derived C present as MB (Res_C_{MB}) was the highest under leaves (0.07 to 0.37 g C kg^{-1} soil) and the lowest under root additions (0.02-0.17 g C kg^{-1} soil) across all sampling periods (Fig. S2-4a). Despite a major overall drop (15-60 days) in biomass during the slow decomposition phase, the MB in residue-treated soil still remained higher than in the control (exception: low root treatment at days 30 and 60). This again was mainly due to residue-decomposing microorganisms. Remarkably, the percentage of SOM-derived C in MB in all residue treatments exceeded the control, only at day 120 indicating a relative increase of SOM-C incorporation in MB. A stronger positive correlation between ^{13}C labeled MB (Res_C_{MB}) than SOM was found with total CO_2 and residue-originated CO_2 effluxes, at all sampling periods (Table S2-S1, Supplementary Material). Similarly to specific PE, the PE calculated per unit of Res_C_{MB} was greater at low than at high residue addition (Fig. S2-4b). At both addition levels, the PE per unit Res_C_{MB} from roots was significantly higher than that of leaves and stems.

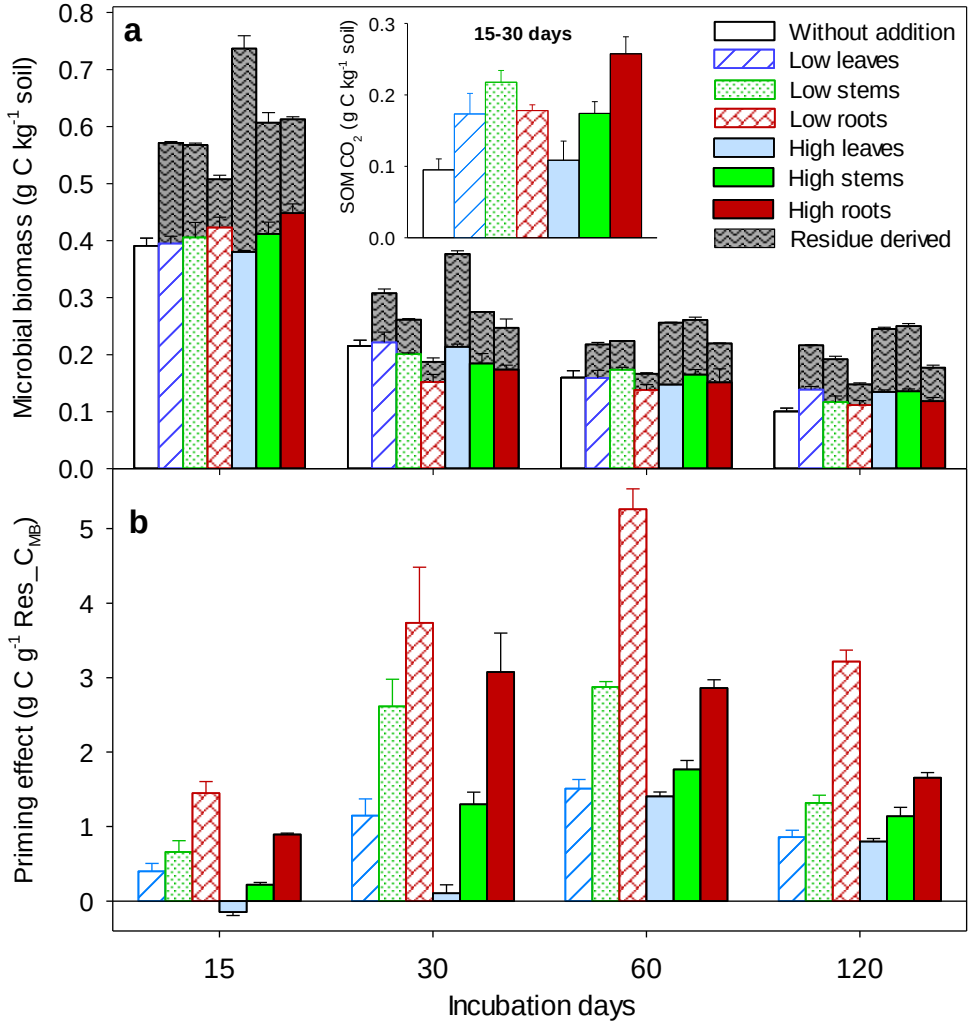

Figure S2-4. The contribution of soil organic matter (SOM) and crop residues originated C (Res_C_{MB}) to total microbial biomass (a); and the amount of priming effect per unit of Res_C_{MB},(b), depending on the residue type, addition level and time of sampling. The inset (a) shows amount of total SOM derived CO_2 during the period of 15-30 days. Means with standard errors (n = 3). The p-values from the ANOVA showing the factors effect on SOM originated microbial biomass (type = 0.043, level = 0.174, time < 0.001, type × level = 0.042, type × time <0.001, level × time = 0.661, and level × type × time = 0.546); and on Res_C_{MB} (for all factors i.e. type, level, time and their interactions, $p < 0.001$). Similarly p-values for Priming effect per unit Res_C_{MB} (b) are as, for all factors and interactions ≤ 0.001 (except the interaction of type × time, $p = 0.307$).

3.2.4.5 Enzyme activities

The activities of all tested enzymes increased significantly after residue addition. Enzyme activity highly depended on residue type and the amount of addition (generally increasing with addition level) and sampling time (Fig. S2-S2, Supplementary Material). The correlation between enzyme activity and MB-C was relatively weak, but it was highly strengthened when

it correlated with ^{13}C MB-C (Res_C_{MB}) (Table S2-2, Supplementary Material). The correlation between enzyme activity and SOM-derived MB was mainly negative or insignificant. Therefore, enzyme activities are presented as specific activity per unit of Res_C_{MB} (Fig. S2-5).

Overall, the specific enzyme activities (nmol μg^{-1} Res_C_{MB} h^{-1}) were lowest on incubation day 15 (Fig. S2-5). At both addition levels, the activities involved in the C-cycle (ß-glucosidase, ß-cellobiohydrolase, chitinase, xylanase) remained stable. The acid phosphomonoesterase and leucine aminopeptidase activity increased until day 60 and then decreased again (Fig. S2-5). Enzyme specific activity was significantly higher under root than under both leaf and stem addition at all sampling periods. With the increase of residue addition level, however, the specific activity significantly decreased compared with low residue addition (Fig. S2-5). No significant differences in enzyme activities between leaf and stem additions were observed at both addition levels. The positive correlation between the specific PE and enzyme activities consistently increased with the increased specific PE and was the strongest for ß-glucosidase, acid phosphomonoesterase and leucine aminopeptidase activity (Table S2-2).

Table S2-2. The Pearson correlations (r) between specific priming effect and values of specific enzyme activities at days 15, 30, 60 and 120 of incubation in soils amended with residues

	ß-Glucosidase	Acid Phosphatase	Chitinase	Xylanase	Cellubiosidase	LAP*
15 days	0.74	0.61	-0.19	0.43	0.27	0.71
30 days	0.69	0.78	0.66	0.66	0.40	0.67
60 days	0.82	0.80	0.64	0.69	0.68	0.82
120 days	0.90	0.82	0.77	0.72	0.74	0.81

*leucine aminopeptidase

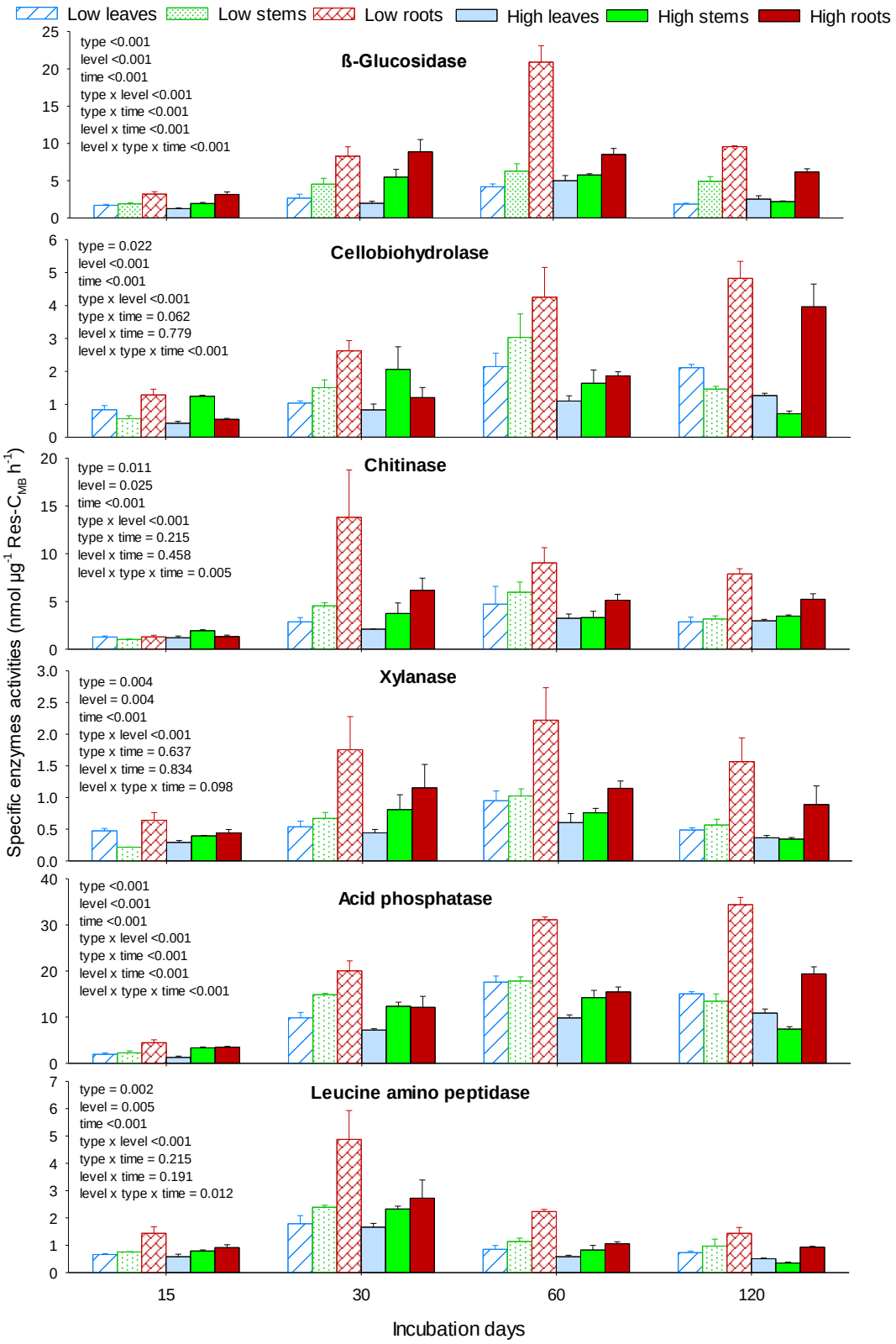

Figure S2-5. Specific enzyme activities (enzyme activities per unit of residue originated microbial biomass (Res_C_{MB})), depending on the residue type, addition level and time of incubation. Mean values with standard errors (n = 3). The presented *p*-values are from the ANOVA of the data (residue type, addition level, time of sampling and their interactions)

3.2.5 Discussion

3.2.5.1 Residue and soil organic matter decomposition

3.2.5.1.1 Residue quality effects

Our first hypothesis (i.e. SOM decomposition dependence on residue type) was confirmed by the simultaneous but inverse intensity of CO_2 efflux originating from SOM and from crop residues. SOM decomposition was very low during the intensive phase of residue mineralization (up to 3 weeks) and thereafter increased when residue mineralization rate declined. Roots had a lower and shorter intensive mineralization phase than leaves and stems, but relatively intensive SOM decomposition indicated high SOM vulnerability (Chen et al. 2015; Shahbaz et al. 2016a). Note, however, a 2-fold increase of residue addition triggered an up to 2.3 and 2.6 times higher mineralization of leaves and stems, whereas SOM decomposition remained unaffected. This demonstrated that high rates of residue mineralization reflect great substrate C availability, which did not cause an increase in SOM decomposition. Microorganisms preferably utilize substrates if their availability is high and therefore SOM decomposition is not necessarily to be increased (Nottingham et al. 2009; Wang et al. 2015). In contrast to this, SOM decomposition was substantially increased (up to 15%) at the doubled amount of root additions. This proved the high susceptibility of SOM to decomposition in the presence of decaying roots (Shahbaz et al. 2016a). Despite roots were characterized by the C/N ratio close to leaves, the roots were least decomposed. This can be attributed to the biochemical composition: root contains relatively less readily decomposable compounds and high amount of recalcitrant substances such as lignin, suberin, phenols and tannin (Aber and Melillo 1982; Bertrand et al. 2006; Lian et al. 2016; Rasse et al. 2005). Soil microorganisms feeding on such slowly decomposable substances produce enzymes able to degrade similar compounds in SOM via co-metabolism (Horvath 1972; Kuzyakov et al. 2000). Here we extend the meaning of co-metabolism assuming that microorganisms producing such enzymes can also utilize SOM decomposition products.

3.2.5.1.2 Priming effect as a function of residue mineralization threshold levels

The occurrence of PE after residue incorporation suggests that substrate-addition (C availability) changed microbial stoichiometry that accelerated SOM decomposition for balanced microbial growth (Chen et al. 2014). This explains, in accordance with the priming conceptual models, the development of PE due to microbial C limitation (Blagodatskaya and

Kuzyakov 2008). Here, we further developed a concept on the PE dynamics as depending on the quality and quantity of crop residues.

In accordance with our second hypothesis, specific PE (per unit of residue-C) was lower at high than at low residue additions. Such decrease in PE mostly occurs when the abundant amount of easily decomposable substrate is available (Guenet et al. 2010). This is often explained by the microbial substrate C saturation and their preferential substrate utilization over SOM (Blagodatskaya and Kuzyakov 2008; Xiao et al. 2015). The decrease of PE at high C additions highlights the relation of PE with residue decomposition (Wang et al. 2015). The PE was lowest during the intensive phase of residue mineralization and thereafter increased strongly. According to a unifying model (logistic and power functions) a threshold of ca. 20, 29-44 and 39-51% mineralization of roots, stems and leaves, respectively, exhibited the strong increase of PE at the slow residue decomposition phase (Fig. S2-2). This showed that the onset of strong priming growth was up to 2.5 times faster (earlier) under root additions compared with leaf and stem additions. Accordingly, at high crop residue addition, the start of strong increase of PE was delayed by a factor of up to 1.5 for both leaves and stems (exhibiting quantity effect) versus not for roots. This residue decomposition and PE phenomena (depending on quality and quantity of input) indicated a changing microbial substrate utilization pattern (Nguyen and Marschner 2016; Wang et al. 2015), which may result is a variable amount of apparent and real PE (Garcia-Pausas and Paterson 2011; Blagodatskaya et al. 2014).

3.2.5.2 Mechanisms of priming effect

3.2.5.2.1 Apparent and real priming effect in relation to residue mineralization

The mechanisms of PE relate the extra-CO_2 emission with the sources of the primed organic matter. During the intensive residue mineralization phase: the low or even negative PE suggested a preferential substrate utilization (or pool substitution) mechanism mainly for leaves (ca. 2-3 weeks) and stems (Blagodatskaya and Kuzyakov 2008; Fontaine et al. 2003). Importantly, this phase lasted much longer for leaf and stem additions than for the roots, showing apparent PE (Figs S2-2 and S2-6) (Paterson and Sim 2013). Afterward (15-60 days), the increase in primed CO_2 was accompanied by up to 60% decrease in MB, and by increased specific enzyme activities (compared with at day 15). Surprisingly, no incorporation of SOM-derived C into MB was detected during this period. This suggested an occurrence of a new mechanism of real PE primarily from re-utilization of microbial-necromass (produced after a

strong decrease in MB), indicating that necromass served as SOM primer (Fig. S2-6) (Miltner et al. 2009; 2012). At a later stage, the PE strongly exceeded residue decomposition and it was accompanied by incorporation of SOM-derived C into MB. This indicated an occurrence of real PE, possibly due to microbial shifts i.e. from fast- to slow-growing (e.g. fungi) SOM-feeding populations (Blagodatsky et al. 2010; Nannipieri et al. 1978). This phase of PE was much faster (started earlier) and stronger under root versus leaf and stem addition (Figs S2-2 and S2-6). Therefore, the sequence of PE mechanisms was more complex during decomposition of aboveground crop residues compared with roots.

3.2.5.2.2 Priming effect mechanisms in relation to microbial biomass and enzyme activities

The lower amount of root- than leaf- or stem-originated C in MB confirms that root-C was relatively less labile and its decomposition was slower (Cotrufo et al. 2013; Stewart et al. 2015). The MB peaked due to residue additions at day 15, without an increase in SOM-originated C compared to the control. The correlation between the PE and enzyme activity was relatively weak in that period. We therefore, interpret the PE occurring during the intensive decomposition phase as apparent, mainly due to the pool substitution (or preferential use) mechanism (Blagodatskaya and Kuzyakov 2008; Garcia-Pausas and Paterson 2011). The fast decline of MB demonstrates the exhaustion of the labile portion of residue and SOM-originated C, which is often observed in other studies (Blagodatskaya et al. 2011b; Wang et al. 2016). The decline of MB during 15-30 days e.g. by ca. 0.2 g C kg^{-1} in the without addition control was confirmed by at least 0.1 g C kg^{-1} of SOM derived CO_2-C emissions during that period (Fig. S2-4a). Similar pattern observed in the residue treated soils where SOM-derived CO_2-C emission corresponded well to the decline in SOM-originated microbial C. Such PE can already be considered as real assuming extra CO_2 originated from the labile SOM-fraction (i.e. microbial-necromass) and that the PE was accompanied by increased enzyme activity (Blagodatskaya and Kuzyakov 2008; Miltner et al. 2009; Paterson and Sim 2013). The specific PE and enzyme activities mainly correlated with residue-metabolizing microbial biomass, indicating the link of the residue-feeding microbial fraction to PE. Remarkably, this PE mechanism has never been before experimentally demonstrated. The significant increase of SOM derived C in MB after 120 days corresponded to the strong correlation between the PE and enzyme activity (Fig. S2-4a and Table S2-2). This suggests that the possible mechanism of real PE was due to the co-metabolism of recalcitrant SOM during the decomposition of less labile crop residues.

Residue addition enhanced microbial activity, boosting enzyme production (Fig. S2-S2, Supplementary Material) (Blagodatskaya and Kuzyakov 2013; Fontaine et al. 2003). Due to stronger positive correlations, the residue-originated (Res_C_{MB}) rather than the SOM-originated soil microflora appeared to be mainly responsible for decompositions and enzyme activities (Table S2-S1, Supplementary Material), further supporting our third hypothesis.

Residue addition stimulated specific activities (per unit of Res_C_{MB}) of all six tested enzymes (Fig. S2-5). The decrease of specific enzyme activities at high residue additions can be due to the decreasing rate of enzyme production because of lower energy demands (microbial saturation by substrate) (Xiao et al. 2015). The increase of these activities after the intensive phase of residue decomposition confirms that microorganisms were at a nutrient limitation - or starving stage, causing (real) PE (Blagodatskaya et al. 2014). Accordingly, a strong interaction between specific enzyme activities and PE further supported microbial-necromass re-utilization mechanism, i.e. a real PE.

At both addition levels, higher root-induced specific PE and enzyme activities than leaves and stems may be due to the complex root structure (less decomposability) and low C availability. Thus, microorganisms synthesized more enzymes able to hydrolyse root components as well as similar compounds from SOM. This caused real PE based on co-metabolism (Kuzyakov et al. 2000; Paterson and Sim 2013). Although residue-originated C in MB under root additions was lowered compared with leaves and stems, microorganisms involved in root degradation produced enzymes (specific activity): probably this community was more efficient due to substrate quality (Fig. S2-5). Our study emphasizes the role of crop residue-feeding microorganisms as active players explaining the mechanisms and thresholds of PE, which are induced by contrasting crop residue quality and quantity.

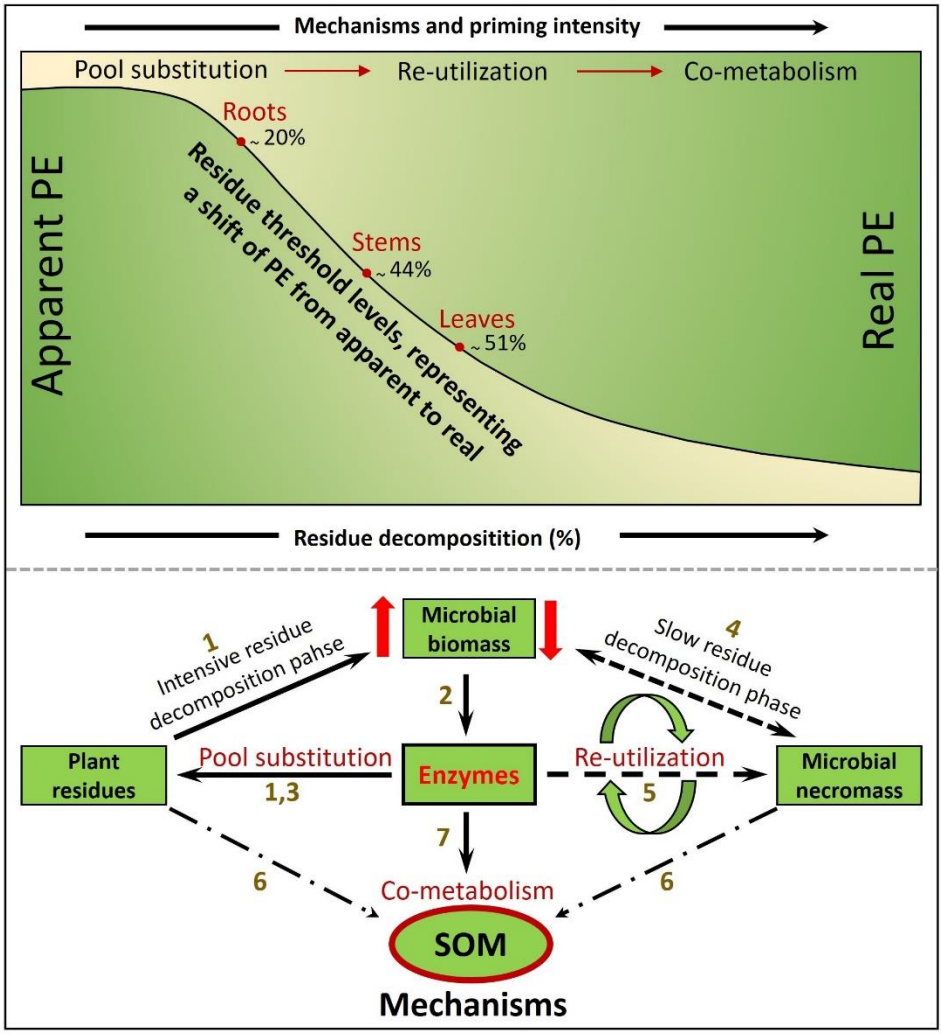

Fig. S2-6. Conceptual scheme of apparent and real priming effect (PE) in soil after residue addition, explained by three main mechanisms: pool substitution, re-utilization and co-metabolism. Residue mineralization threshold levels, as estimated by a unifying model (logistic plus power functions), represent the shifts of PE from apparent to real when ca. 20% (roots), 44% (stems) and 51% (leaves) of added residues (i.e. at high additions) were mineralized.

Mechanisms: 1– Microbial uptake of plant residues degraded by existing enzymes lead to initial increase of microbial (active) biomass (MB), which is mainly due to the residue-feeding population; 2– Acceleration in enzyme production after residue-induced increase in MB (depending upon residue type); 3– Preferential substrate utilization (under high substrate C availability) leading to pool substitution mechanism (1,2,3); 4 – Decrease of MB during slow residue decomposition phase (after exhaustion of labile substrate) which results in the accumulation of microbial-necromass i.e. after microbial starvation and cell death; 5– Re-utilization (2,4,5) of microbial-necromass under low substrate availability; 6– Direct contribution of microbial-necromass and plant compounds in soil organic matter (SOM) formation; 7– Increase of enzymatic SOM decomposition by co-metabolism under low availability of labile crop residue compounds (depending on residue quality).

Overall, we summarize that the partitioning of C sources (residue and primed) during residue decomposition is combined with residue-originated MB and specific enzyme activity (Figs S2-4 and S2-5). The results demonstrated that instead of the total MB, the crop residue-feeding microorganisms, served as the main player regulating PE mechanisms, which depended on residue mineralization stage (threshold levels). Residue quality and amount strongly influenced the MB and microbial activity (high MB under leaf, low under root additions) involved in crop residue and SOM decomposition (Fig. S2-6) (Blagodatskaya et al. 2014). The increase in MB after residue addition was mainly due to crop residue feeding microbial fraction. During intensive phase, crop residues preferably decomposed due to accelerated enzyme production (specific), which mainly correlated with the residue-feeding microbial population. This caused apparent PE by a pool substitution (roots and stems) and negative PE by preferential residue decomposition mechanisms (Fig. S2-1d, stages 1, 2 and 3 in Fig. S2-6). Later, a strong decrease in MB (resulted in an increase of microbial-necromass) and a high correlation of enzyme activity with PE occurred. This indicated real PE induced primarily by the re-utilization of microbial-necromass (stage 2, 4 and 5 in Fig S2-6, Miltner et al. 2012). Subsequently, co-metabolism of recalcitrant SOM was seen when an increase in SOM-originated C in MB was accompanied by an increase in specific enzyme activities. The specific enzyme activity strongly depends on crop residue C availability: unless the decomposability or C availability is high, microorganisms will produce less enzymes capable to co-metabolize SOM and there will be a less need for re-utilization of microbial-necromass (resulting in low or negative PE). Under low residue decomposability, i.e. less C availability (e.g. roots), microbial dynamics yield only a brief pool substitution stage. Nonetheless, re-utilization and co-metabolism will be the dominating processes, creating real PE (Fig. S2-6).

3.2.6 Conclusions

Root residues induce faster and stronger PE than aboveground plant parts. For all residue types, specific PE (per unit of C addition) decreased with added residue amounts. The slow root decomposition leads to stronger PE. The leaf and stem residues were intensively mineralized and yielded negative or apparent PE for extended periods, due to preferential utilization and pool substitution mechanisms. This resulted in a shorter real PE compared to root addition. During the 15-60 days, the MB declined strongly but specific enzyme activities increased. Remarkably, no incorporation of SOM-derived C into MB was detected during up to 60 days. Therefore, this suggest that the PE was primarily caused by re-utilization of microbial-

necromass i.e. necromass served as SOM primer. At the end of incubation, the incorporation of SOM-originated C into microbial biomass and a corresponding increase in enzyme activities indicated the co-metabolism of SOM. The amount of primed SOM correlated with the residue-feeding microorganisms and depended on residue decomposition phases, residue quality and the added amounts. This underlines the role of residue–feeding microbial community as an active player for PE that is responsible for the contrasting PE mechanisms. We recorded threshold levels for the onset of strong PE increase versus the fraction of mineralized residues at ca. 20, 29-44 and 39-51% mineralization of low and high input of root, stem and leaves, respectively. We conclude that for microbially-mediated SOM decomposition the residue mineralization stage is crucial, which depends not only on the quality but also the quantity of added residues. Further research efforts should focus on evaluating the role of microbial-necromass in stable SOM formation and PE under contrasting substrate quality, and on utilizing enzyme assays (e.g. for oxidative enzymes) to assess the recalcitrance of newly-formed SOM compounds.

3.2.7 Acknowledgements

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3.3 Study 3

Decadal nitrogen fertilization decreases mineral-associated and subsoil carbon: a 32 year study

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3.3.1 Abstract

Crop residues and manure are important sources of carbon (C) for soil organic matter (SOM) formation. Crop residue return increases by nitrogen (N) fertilization because of higher plant productivity, but this often results only in minor increases of SOM. In our study, we show how N fertilization and organic C additions affected SOM and its fractions within a 32-year long field-experiment at Puch, Germany. Five organic additions: no-addition (control), manure, slurry, straw and straw+slurry, were combined with three mineral N fertilization rates (no-, medium- and high-fertilization), which resulted in 1.17-4.86 Mg C-input ha^{-1} yr^{-1}. Topsoil (0-25 cm) SOM content increased with N fertilization, mainly due to the C in free light fraction (f-LF). In contrast, subsoil (25-60 cm) SOM decreased with N fertilization, probably because of roots' relocation in Ap horizon with N fertilization at the surface. Despite high inputs, straw contributed little to f-LF but prevented C losses from the mineral-associated SOM fraction (ρ >1.6 g cm^{-3}) with N fertilization, which was observed without straw addition. Above- (straw) and belowground (roots) residues had opposite effects on SOM fractions. Root C retained longer in the light-fractions and was responsible for SOM increase with N fertilization. Straw decomposed rapidly (from f-LF), and fueled the mineral-associated SOM fraction. We conclude that SOM content and composition depended not only on residue quantity, which can be managed by the additions and N fertilization, but also on the quality of organics. This should be considered for maintaining the SOM level, C sequestration and soil fertility.

Keywords: Soil organic matter; Density fractionation; Nitrogen fertilizer; Manure & straw slurry; Cropland soil.

3.3.2 Introduction

Improving and maintaining soil organic matter (SOM) levels is necessary for the functioning of physicochemical and biological properties of soils (Keesstra *et al*., 2016; Laudicina *et al*., 2015). Poor soil physicochemical functioning can lead to land and nutrients degradations (such as due to erosions) in cropland soils (Auerswald *et al.,* 2009; Novara *et al.,* 2013; Rodrigo Comino *et al.,* 2016a). Crop residue return is important for soil conservation practices because it is a major carbon (C) source for improving SOM levels (Cerdà *et al.,* 2016). The SOM is vital for nutrients conservation and soil structural development, therefore protect the soil against degradation (Brevik *et al.,* 2015; Withers *et al.,* 2007).

The pool size of SOM depends on its formation from plant residues and its mineralization to CO_2 (Cotrufo *et al*., 2015). Generally, it is assumed that increasing amounts of C inputs to soil, improves SOM levels. Several field studies, however, show that increasing C inputs did not always increase SOM levels (Heitkamp *et al*., 2012a; Novara *et al*., 2016; Stewart *et al*., 2008). Such phenomena of SOM change is often linked with C storage capacity of SOM fractions, which are important for nutrients conservation and soil physicochemical functioning (Prosdocimi *et al.,* 2016; Six *et al*., 2002).

The SOM fractions are mainly distinguished according to their protection mechanism and decomposition stage (Schrumpf *et al*., 2013). Mostly, SOM fractions with various protection mechanisms are separated based on density and their association with soil silt and clay particles (Gunina & Kuzyakov, 2014). The physically unprotected fraction of SOM represented by the free light fraction (f-LF) which is strongly affected by recent C inputs. Within soil aggregates, SOM is physically protected by spatial separation from decomposing microorganisms (i.e. their extracellular enzymes) and by low oxygen diffusion into aggregates, which slows decomposition (Six *et al.,* 2002). The aggregates associated fraction often termed the occluded light fraction (o-LF). Decomposition of light fractions and also microbial turnover leads to the formation of microbial-residues, which mostly are sorbed to fine mineral-particles and form the heavy fraction (HF) of SOM (Schrumpf *et al*., 2013). This physicochemical stabilization (after microbial substrate degradation) substantially reduces the turnover of SOM in HF. The SOM increase due to large C additions (such as crop residue) mostly explained by C accumulation in HF. However, due to the limited physical or physicochemical protection capacity, large C additions may cause only minor increase of bulk SOM, especially in high-C soils (Six *et al*.,

2002; Shahbaz *et al*., 2016). This indicates that C-input driven by e.g. high crop residues return, therefore, would not be directly beneficial for SOM.

Crop residue return frequently increased by nitrogen (N) fertilization, however, the effect of the N-fertilization-triggered increase of C addition is not always certain (Dou *et al*., 2016; Zhang *et al*., 2016). This is because, the stable SOM build-up is not mainly input driven but also depends on residue decomposability and its protection from microbial degradation (Barbera *et al*., 2010; García-Orenes *et al*., 2016). Recent views suggest that stable SOM formation is mainly related to the conversion of residue C input into microbial-residues that make up most of the C associated with HF (Cotrufo *et al*., 2013; Gleixner, 2013; Lehmann & Kleber, 2015). N-fertilization improves aboveground residue quality and decreases C/N ratio. Residues with lower C/N ratios support high microbial-residues formation compared to low-quality (such as roots; high C/N and recalcitrant compounds), which decomposes slowly (Cotrufo *et al*., 2013). However, in contrast to low quality, accelerated decomposition of high-quality residues (e.g. under high N availability) can promote C losses (as CO_2 emissions or leaching of dissolved C) more than stabilization within SOM (De Almeida *et al*., 2016; Pabst *et al*., 2016). The soil N availability and residue decomposability (with contrasting quality) can, therefore, affect the partitioning of C within SOM fractions and its distribution along soil depths.

The importance of management effects on SOM levels has mostly been examined for topsoil (0-25 cm, plough layer); information for subsoil is scarce (Gregory *et al*., 2014; Ogle *et al*., 2005). Subsoil contains a large fraction of total organic C and is sensitive, for instance, to land use changes (Rumpel and Kogel-Knabner, 2011). The subsoil SOM stabilization is primarily affected by root growth (its exudations) and dissolved C leaching from topsoil (Don *et al*., 2009; Rumpel & Kögel-Knabner, 2011). In general, due to relatively less exposure to environmental extreme events and high degree of mineral-associations, subsoil SOM is assumed to be more stable than topsoil (Cerdà *et al*., 2010; Rumpel & Kögel-Knabner, 2011). However, subsoil SOM can be destabilized due to priming effects, specifically under high N fertilization (Kuzyakov *et al*., 2000; Khan *et al*., 2007). Subsoil mostly had a mineral-associated C, thus its SOM stabilization can be affected by the factors affecting C accumulation in topsoil HF (Stewart *et al*., 2008; Hobley & Wilson, 2016). Nonetheless, no clear information is available on the long-term management effects, on total SOM change that can be explained by C stabilization in its fractions, and we know little about the ultimate effects on subsoil C.

The present study was therefore designed to explain and compare the integrated long-term impacts of C inputs (varies in quality and quantity) and N fertilization rates on topsoil (0-25 cm) SOM and its fractions, and to estimate the effects on subsoil (25-60 cm) SOM contents. Density fractionation approach was used to separate C in topsoil SOM fractions. We assumed that C storage in SOM fractions will reflect the total SOM change, but their response rate can differ. In particular, the specific goals of this study were: i) to estimate and compare the changes in topsoil SOM levels due to C inputs (variable organics) and N fertilization over the study period, i.e. 32 years, ii) to analyze the effects of topsoil managements on SOM accumulation in subsoil, iii) to quantify and compare the effects of C inputs and N fertilization on partitioning of C among topsoil SOM fractions (f-LF, o-LF and HF), and overall impact of these fractions on SOM formation.

3.3.3 Materials and methods

3.3.3.1 Site description

The long-term (well designed and documented) field experiment (48°11′37.85″ N, 9°13′04.55″ E) is located at Puch, a village close to Munich, Germany. The study site represents a common soil type in Central Europe and covers a wide range of management options in a widespread, cereal-based crop rotation. The soil was classified as Luvisol (Parabraunerden) (IUSS-WRB, 2015), derived from loess sediments with silt-loam texture (sand: 9%, silt: 73%, clay: 18%) overlying moraine deposits of the Riss glaciation. The mean annual precipitation and temperature since 1983 were 868 mm y^{-1} and 8.4°C, respectively (Heitkamp *et al*., 2012a). Prior to the experiment, the site was used as cropland for decades or even centuries, and we, therefore, assume no major disequilibrium of C contents due to land use changes. In the plough layer (0-25 cm, maintained since 1983) the pH decreased from 6.4 to 6.1 in the studied period (1983-2015). The pH and a test with 10% hydrochloric acid (HCl) indicated the absence of carbonates. To estimate the changes in SOM contents during the study period, starting conditions of SOM (in 1983) were analyzed using topsoil samples (0-25 cm). Soil samples were taken for plots receiving various organic additions but bulked across replicates and N fertilization rates (see below, Fig. S3-1). Therefore, in 1983, different amounts of SOM contents (g C kg^{-1}) were calculated for different organic additions that ranged 11.2 (no-addition control); 10.6 (Slurry as well as for Straw) and 10.8 (Manure and Straw+Slurry) (Fig. S3-4a), see further details in Heitkamp *et al*. (2012a).

3.3.3.2 Experimental design

The crop rotation is silage maize (*Zea mays* L.) – winter wheat (*Triticum aestivum* L.) – winter barley (*Hordeum vulgare* L.). The experiment was laid out as a full factorial strip design with two factors (n = 3) (Fig. S3-1). Organic additions were considered as the first factor and N fertilization rate (three levels) as the second factor (Fig. S3-2, Table S3-1). In all organic additions, application of phosphorus (P) and potassium (K) fertilization was equal but varied between years according to crop needs (Hege & Offenberger, 2006). Five organic addition levels were selected for factor one: i) Control (no addition, straw removed); ii) Manure (straw removed, cattle farmyard manure applied every third year); iii) Slurry (cattle slurry application, straw removed); iv) Straw (alone straw incorporated); v) Straw+Slurry (straw incorporated combined with slurry application).

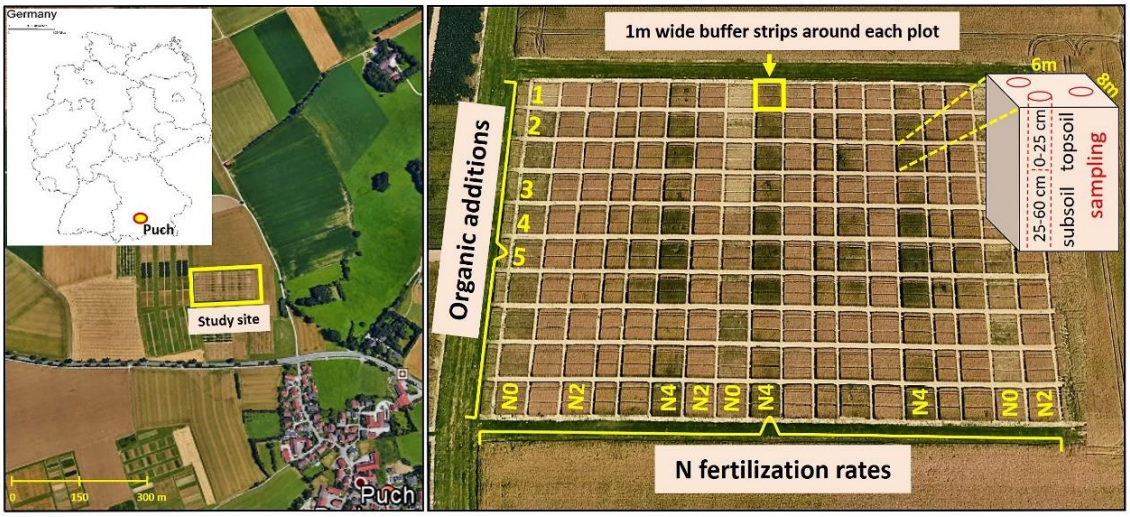

Figure S3-1. Aerial view of the study site (48°11′37.85″ N, 9°13′04.55″ E), located at Puch close to Munich (Germany), showing the field experimental design which consists of two factors: organic additions (1 – control, 2 – manure, 3 – straw, 4 – slurry, 5 – straw+slurry) and N fertilization rates (N0, N2 and N4 represents; no, medium and high N fertilization, respectively). The expanded box shows random soil sampling points (3 sample which were bulked) for both top- and subsoil of individual plot.

In August and April, before the maize crop, slurry (on average 7.6% dry matter, 5.8% OM, 4.4 kg N m^{-3}, 2.8 kg NH_4 N m^{-3}) was applied at rates of 30 m^3 ha^{-1} (corresponds to the regional practices). Since 1999, the slurry application was changed to account for more recent management of the region (Table S3-1): to maize, two applications of 25 m^3 (each at sowing time) and additionally (in spring) to winter wheat or winter barley (before sowing), 25 m^3 ha^{-1}

was applied each time. The manure was spread every third year in August before the maize crop. From 1983 to 1998, manure was applied on the fresh mass basis of 30 Mg ha^{-1} (on average 23% dry matter, 17% OM, 5.5 kg N Mg^{-1}) and, from 1998 on, the application rate was increased to 40 Mg ha^{-1}.

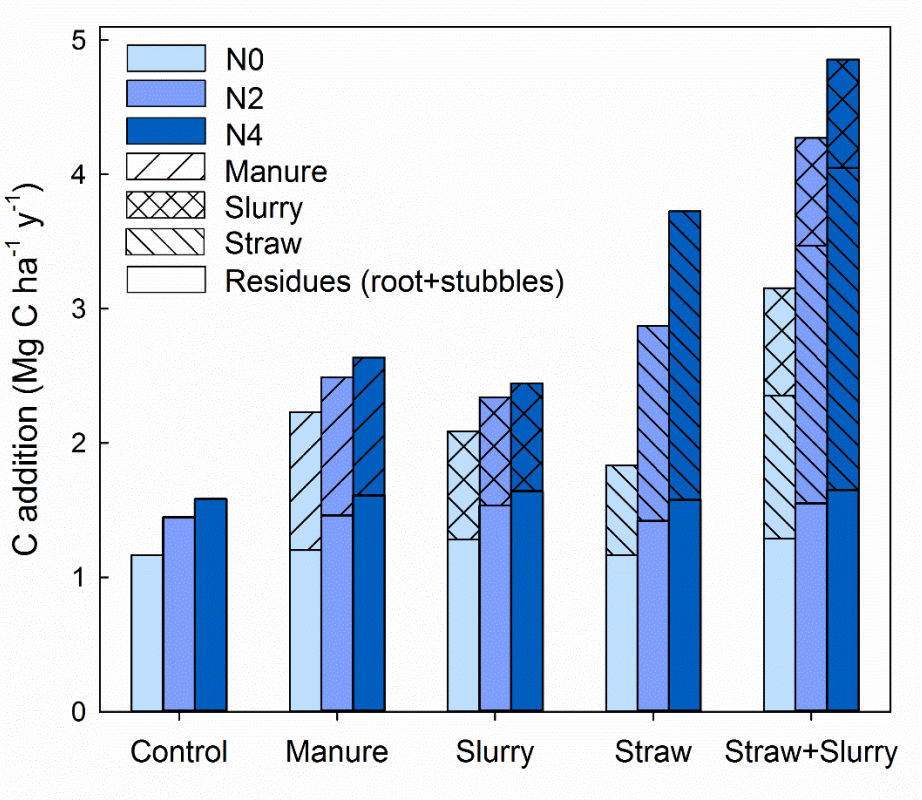

Figure S3-2. The contribution of organic carbon (C) sources to total annual C additions (Mg C ha^{-1}) starting from 1984. The C input by manure, slurry, straw and crop roots (stubbles) was measured and calculated (see detail Heitkamp *et al*., 2012a). N0, N2 and N4 represents; no, medium and high N fertilization, respectively. Control: without organic additions; Manure: straw removed, farmyard manure applied every third year; Slurry: cattle slurry application, straw removed; Straw: straw incorporated; Straw+Slurry: straw incorporated combined with slurry application.

The second factor, N fertilization (three levels, no (N0), medium (N2) and high (N4) fertilization), varied between crops. The amount of N fertilization rates given to winter wheat and barley was different, because of the specific nutrient demands of the crops (Table S3-1).

Table S3-1. Mineral N fertilization rates

N-fertilization (kg N ha^{-1} y^{-1})	N0	N2	N4
1984-1998			
barley	0	60	80/40
wheat	0	50/30*	70/50/40
maize	0	100	120/80
since 1999			
barley	0	50/30	80/40/40
wheat	0	50/20/30	80/60/60
maize	0	100	120/80

* N-amounts divided by slash indicate split applications.

Until 1993, straw yield was measured in all organic addition plots, and thereafter only for organic additions with straw removal. A C content of 45% was considered for straw, and the straw yield was estimated based on the mean harvest index (grain to aboveground biomass ratio), which was remarkably stable (0.49 ± 0.01) through time and among treatments. The straw was incorporated directly into the plot of its origin. This provided realistic on-farm conditions since the amount and chemistry of straw may be directly influenced by the respective treatments. Consequently, the amount of incorporated straw increased with N fertilizer rate (Fig. S3-2). The amount of manure and slurry was fixed and measured before addition, and their C contents were calculated by dividing organic matter by 1.92 (Larney *et al.*, 2005). The fraction of crop residues in soil added by roots and crop-stubbles was estimated as described by Heitkamp *et al*. (2012a). The used regression model of yields with crop residues does not separate between roots and stubbles. Nevertheless, it is reasonable to assume that a major part of the estimated C input of residues stems from roots. According to estimates of Bolinder *et al.* (2007), we calculated that ca. 75% of our estimates may be contributed by roots. Therefore, we argue that the C input by crop residues in treatments without straw incorporation is clearly dominated by roots. Overall, mean annual C input for all organic additions ranged from 1.17 to 4.86 Mg C ha^{-1} y^{-1} and increased with N fertilization rate (Fig. S3-2). For detailed information about the experiment (i.e., C input estimation and calculations, crop yields, N balances) see Hege & Offenberger (2006) and Heitkamp *et al*. (2012a).

3.3.3.3 Soil sampling

Following the wheat harvest in August 2015, soil samples were taken from a depth of 0-25 cm (topsoil) and 25-60 cm (subsoil) with the help of a soil auger. The sampled topsoil (0-25 cm) represents the plough horizon (Ap), which is annually mixed by tillage since 1983. The subsoil (25-60 cm) was sampled to estimate the SOM accumulation which is affected due to topsoil

managements. For each organic addition, the soil was sampled in three field replicates of each selected N fertilizer rate. Within each selected N field replicate plot, three random sampling (for both top- and sub-soil) was done and thereafter soil was bulked and represented one composite field replicate of each selected N fertilization level (Fig. S3-1). Three levels of N fertilization were selected: N0, N2 and N4 (Table S3-1). The soil samples were air dried at room temperature, sieved (< 2mm), and visible parts of large crop residue (e.g. mixed during sampling from recent crop harvest) removed. Additionally, we sampled soil with 100 cm^3 cylinders (three replicates per plot, 10-15 cm depths) to determine bulk density. The samples were oven dried (105°C), left for cooling in a desiccator and weighed.

3.3.3.4 Density fractionation

The SOM density fractionation approach was applied to both top- (0-25 cm) and subsoil (25-60 cm), but the yield of f-LF and o-LF from subsoil was too small to carry out precise measurements. Therefore, the density fractionation was only presented for topsoil and we assumed the subsoil SOM was mainly HF-C.

To isolate the density fractions of topsoil SOM, 20 g of air-dried soil portioned into two replicates (i.e. 10 g each) was placed into a centrifugation tube. A 30 mL of sodium polytungstate solution with a density of 1.6 g cm^{-3} was added to each soil portion in the tube (Cerli *et al*., 2012). The tube was then gently turned several times by hand, the solution was centrifuged at 4,000 rpm for 40 min, and the supernatant with floating material (ρ >1.6 g cm^{-3}) was filtered (cellulose acetate filter, 0.45 µm; Sartorius, Germany) and washed with ca. 1 L distilled water to obtain a salt-free f-LF. To isolate o-LF, a similar amount of sodium polytungstate solution (ρ = 1.6 g cm^{-3}) was added to the remaining sample after removing of f-LF. The sample was mixed with sodium polytungstate and then the soil-aggregates were dispersed by ultrasound (Retesch, Germany) with a calibrated input energy at 440 J ml^{-1}. After dispersion, the suspension was left to settle overnight and centrifuged for 40 min at 4,000 rpm. The supernatant (consist of o-LF) was filtered and washed as described above. To separate sand particles (> 53 µm) from the remaining sample after the separation of f-LF and o-LF, wet sieving was done with 53 µm mesh size. The measured organic C contents of sand fraction were very low (<0.01%) and therefore fraction <53 µm (silt plus clay) was considered as the HF (Breulmann *et al*., 2016). The HF was washed with distilled water, and suspended particles were precipitated by adding few drops of 0.5 M $AlCl_3$. The clear supernatant was removed and

the precipitated HF was collected. All the density fractions were dried (at 40°C, to constant weight) and weighed.

3.3.3.5 Analysis of total carbon contents

Before analysis, all density fractions, topsoil and subsoil samples were dried (40°C). The soil samples were ball milled (MM2, Fa Retsch, Germany), while density fractions of SOM were homogenously manually ground by using mortar and pestle. The C contents were measured using an elemental analyzer (Vario EL II, Germany). The soil was carbonate-free, therefore we consider total soil C as organic C. Further, we reported C measurements only as contents instead of calculating stocks because there were no significant effects of the tested factors on topsoil bulk density (organic addition p= 0.310, N fertilization p=0.788 and their interactions, organic addition x N fertilization p= 0.209).

The ΔSOM contents (%C) of topsoil, in relation to its initial and final C values were calculated (Johnson *et al*., 2014) as;

$$\Delta SOM = [(SOM_n - SOM_i)/SOM_i] * 100$$

Where, SOM_n is the SOM contents (g C kg^{-1}) at our sampling time (2015), and SOMi is the initial SOM contents in 1983.

3.3.3.6 Statistics

Statistical analyses were performed using R (version 2.11.1). A linear mixed model (LMM) was used to test the effect of organic additions (factor one, five levels) and N fertilization (factor 2, three rates) on top- and subsoil C and topsoil density fractions. The main two factors were used as fixed effects, while the spatial structure (strip design) was introduced as a random factor. Results are presented as means (n=3) ± standard error.

To quantify changes of C contents since the beginning of the experiment, replicate values of ΔC were related to the annual C inputs (Mg C ha^{-1} y^{-1}) (Halvorson & Schlegel, 2012; Johnson *et al*., 2014). An exponential relationship showed the best fit according to the highest adjusted R^2 values. The effect of C additions on density fractions was additionally quantified. Linear regressions between density fractions and C inputs were applied to the whole dataset (n = 45) and within each organic addition among N fertilizer rates (n = 9). Results different at $p < 0.01$ level are considered as significant.

3.3.4 Results

3.3.4.1 Soil organic matter contents depending on C inputs and N fertilization

After 32 years of C input (1.17 to 4.86 Mg C ha^{-1} y^{-1}), the relation of changes in topsoil C contents (% change compared to initial C values) against total C inputs was best described by an exponential model (Fig. S3-3). The total SOM contents in topsoil were significantly affected by both organic addition and N fertilizer rates (Fig. S3-4a). The topsoil SOM contents ranged from 9.6 to 12.6 g C kg^{-1} and generally increased with N fertilization rate. Compared to initial values in 1983, the highest positive change (up to 17%) of C along N fertilization rate over 32 years was recorded under slurry addition, either with straw removal or incorporation (Fig. S3-3). C contents remained stable under manure additions, which represent the traditional practice in the region. Highest losses of C (12-14%) were recorded when N fertilizer was applied alone (control) and or with straw (2-8%) incorporation.

The C content in subsoil SOM was also affected by N fertilization and organic additions (Fig. S3-4a). Contrary to topsoil, however, the subsoil SOM contents decreased with increasing N fertilization rate. The total SOM content in the subsoil ranged from 4.1 to 5.5 g C kg^{-1} soil. Compared to other organic additions, the control had the highest amount of subsoil SOM at each N fertilization rate (Fig. S3-4). Note that starting conditions in 1983 were not measured for subsoil. The measured C contents in topsoil SOM were highest in the control and, therefore, we assume that the high C contents in this treatment reflect the heritage of the starting conditions. That this restriction also applies for the N fertilization is highly unlikely: while organic additions were arranged in strips, plots receiving N fertilizer rates were randomized. Thus, the highly significant effect of N fertilization ($p \leq 0.001$) strongly indicates a real treatment effect and cannot be explained by spatial heterogeneity.

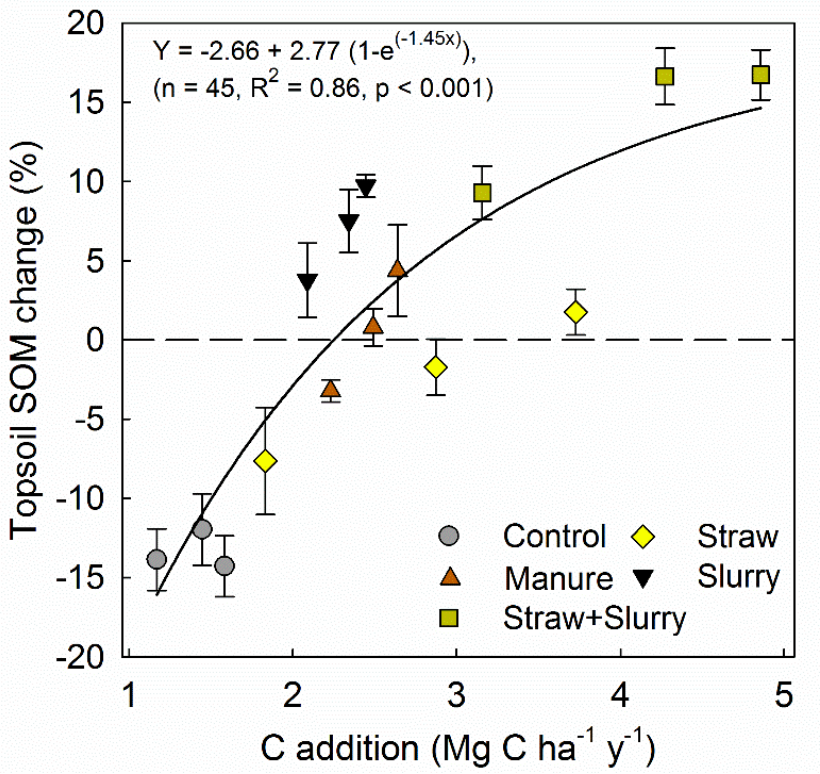

Figure S3-3. The curve represents the exponential relationship between the mean annual C additions and changes of topsoil soil organic matter (SOM) contents (%, between 1983 and 2015) over 32 years. 0-line corresponds to C content in soil at the start of the experiment (32 years ago). Control: without organic additions; Manure: straw removed, farmyard manure applied every third year; Slurry: cattle slurry application, straw removed; Straw: straw incorporated; Straw+Slurry: straw incorporated combined with slurry application. Bars represent the ± standard error of the mean (n=3). The probability levels of the linear mixed model describing the effects (C addition, N fertilization, interaction) for accepting the null hypothesis that the factors have no effect for the change of total SOM (%) are as follows, C addition ≤ 0.001; N fertilization ≤ 0.001 and interactions: C addition × N fertilization = 0.409.

3.3.4.2 Effect of C inputs and N fertilization on SOM fractions

The total sample recovery after soil density fractionation was ca. 97%. Total C contents in f-LF ($p < 1.6$) ranged from 0.26 to 0.74 g C kg^{-1} and in o-LF from 0.35 to 0.54 g C kg^{-1} of soil (Fig. S3-5a, c). Therefore, both fractions together comprised up to 20% of total SOM. The C content of the f-LF was affected by both N fertilization and organic addition. C contents of the o-LF depended mainly on organic additions. The highest occlusion was found when slurry (alone or in combination with straw) or manure was applied. The effect of N fertilization on o-LF varied depending on the organic addition (Fig. S3-5c, d), indicated no response (control, slurry), increased (manure, straw) and decreased (slurry+straw).

The C contents of the HF ranged from 6.5 to 8.7 g C kg^{-1}, thus constituting up to 80% of total SOM contents. The HF was affected by organic addition, with the highest C content under slurry+straw and the lowest C content in the control (Fig. S3-5e, f). Despite the increasing C additions with increasing N fertilization, the C contents in HF decreased across N fertilizer rates, when straw was removed (Figures 2 and 5e, f). Nonetheless, upon straw incorporation, non-significant slopes (across N rates) indicated that the C associated with HF at least did not decrease with increasing N fertilization rate (Fig. S3-5f).

3.3.5 Discussion

3.3.5.1 Effect of C inputs and N fertilization on top- and subsoil SOM

Compared to the initial C values, slurry application (alone or with straw) increased topsoil C contents over 32 years (Fig. S3-3 and S3-4a). This indicated that slurry contained either relatively stable C and thus was retained in soil (Shahbaz *et al*., 2014; Weyman-Kaczmarkowa & Politycka 2002), or that slurry improved root growth (Kandeler *et al*., 1994). The total SOM changes remained unaffected under manure addition, presumably because this management is closest to the traditional practice and SOM contents were therefore in dynamic equilibrium. The more labile nature of straw explains why its addition did not maintain the C contents at a similar level as manure or slurry. Other researchers have reported that substantial amounts of straw incorporation did not have marked effects on total SOM contents (Powlson *et al*., 2011; Poeplau *et al*., 2015; Novara *et al*., 2016). C contents increasing with N fertilizer rates in soils without straw incorporation can be explained by the increasing crop residue return by stubbles and roots. Since straw was removed, roots with their slower decomposition were mainly responsible for the SOM increases (Heitkamp *et al*., 2012b; Rasse *et al*., 2005).

The exponential relationship between change in topsoil C and C inputs was especially evident with straw incorporation (alone or combined with slurry). This shows a decreasing overall efficiency of C accumulation with increasing amounts of aboveground biomass. One reason is a closer C/N ratio of straw with increasing N fertilizer rates because litter with closer C/N ratios decomposes faster (Ogle *et al*., 2005). Faster decomposition, however, should also increase the amount of microbial-residues, which form a major part of stable SOM (Cotrufo *et al*., 2013; Gleixner, 2013). Recently, Castellano *et al*. (2015) linked the stabilization efficiency of the labile litter with the concept of "C saturation". That concept proposes that effective stabilization of microbial-residues occurs only when mineral surfaces have free capacity for sorption. This

explanation does not fit to our dataset. Although the shape of the relationship between ΔC and C additions (Fig. S3-3) does fit the "C saturation" concept, the data from the subsoil and density fractions contradicts this hypothesis. The subsoil is characterized by low C contents, and the C stabilization capacity should be high, resulting in linear relationships with C input. Instead, we show that C decreased in subsoils receiving more C through increased biomass by N fertilization (Fig. S3-4).

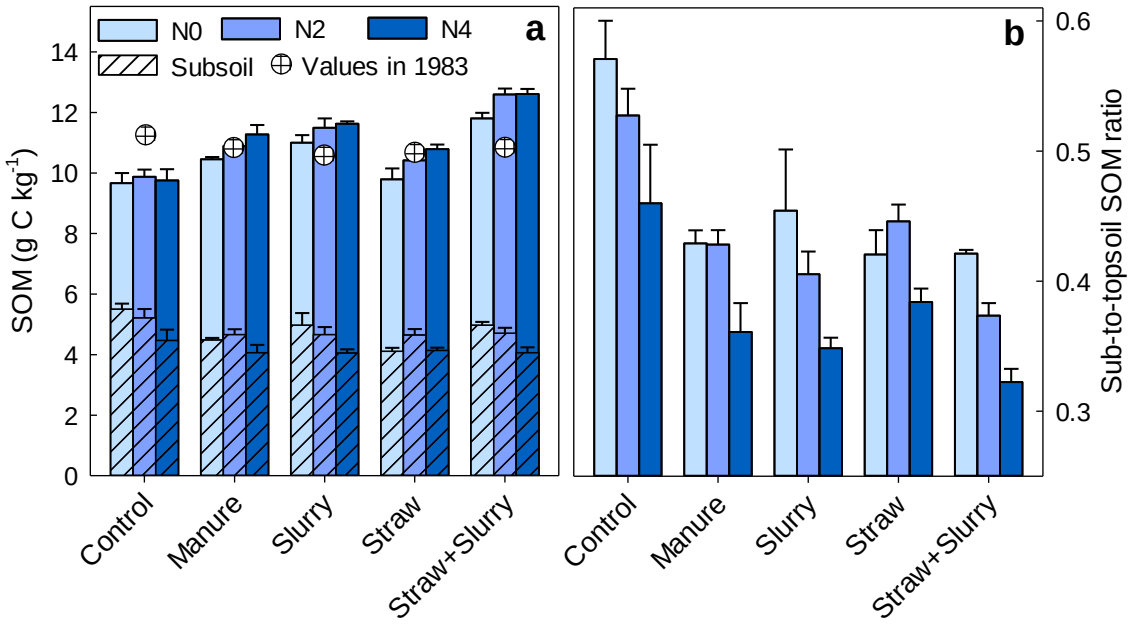

Figure S3-4. Contents of soil organic matter (SOM) in topsoil (0-25 cm), subsoil (25-60 cm) and initial topsoil (32 years ago, crossed circle) **(a)**; and the ratio of sub- to topsoil SOM contents **(b)**. N0, N2 and N4 represents; no, medium and high N fertilization, respectively. Control: without organic additions; Manure: straw removed, farmyard manure applied every third year; Slurry: cattle slurry application, straw removed; Straw: straw incorporated; Straw+Slurry: straw incorporated combined with slurry application. Bars represent the ± standard error of the mean (n=3). The probability levels of the linear mixed model for accepting the null hypothesis that the factors have no effect are as follows: topsoil SOM (organic addition ≤ 0.001; N fertilization ≤ 0.001; and interactions: organic addition × N fertilization = 0.750), subsoil SOM (organic addition = 0.002; N fertilization ≤ 0.001; and interactions: organic addition × N fertilization = 0.323), sub-to- topsoil SOM ratio (organic addition ≤ 0.001; N fertilization ≤ 0.001; and interactions: organic addition × N-fertilization = 0.691).

The decrease of subsoil SOM contents with N fertilization, probably indicates roots' relocation in Ap horizon with N fertilization at the surface. However, other studies have shown that the supply of fresh C and high N fertilization can destabilize subsoil C due to priming effects (Fontaine *et al.*, 2007; Khan *et al.*, 2007; da Silva Oliveira *et al.*, 2016). Recent findings also indicate that microbial community composition can strongly change with N fertilization

(Kuslien *et al*., 2014; Fanin *et al*., 2015) and that microbial activity governs the integration of new C into the soil (Lange *et al*., 2015). Regardless of the specific mechanism involved, the results show that subsoil C drops as N fertilizer rates increase (Fig. S3-4). A similar finding was recently reported by Steinmann *et al*. (2016) on a large sample set (n = 268, for the Cologne-Bonn region, Germany), where despite increasing topsoil SOM contents, the subsoil C contents declined over 8 years. This was in contrast to the general notion that subsoil SOM is highly stabilized and insensitive to management. Moreover, fractions that are supposed to be stabilized – such as the organo-mineral HF – can also clearly be affected by management (Hobley *et al*., 2016).

3.3.5.2 Distribution of organic matter in density fractions

The C contents of f-LF increased with N fertilizer rates and therefore with C additions (Fig. S3-5). However, this increase of C in f-LF was much stronger when straw was removed (in control, manure and slurry), indicating the importance of root-derived C (Fig. S3-5a). The amount of manure and slurry application in our experiments was fixed, and thus any changes in f-LF must be due to residue C originating mainly from roots (and to a lower degree from crop stubbles). Strongly increasing C contents in the f-LF under straw removal (Fig. S3-5) reflect an enrichment of root C (Schrumpf *et al*., 2013) due to its slower mineralization rates compared to straw (Rasse *et al*., 2005; Shahbaz *et al*., 2016). f-LF is known to be most responsive to C input, especially derived by cattle manure additions (Gregorich *et al*., 2006; Yagüe *et al*., 2016). Nonetheless, under straw incorporation (dominant aboveground biomass) the low response of f-LF contents to C input indicated that straw was rapidly decomposed. The retention of roots and the fast decomposition of straw in the f-LF may explain the behavior of the HF.

The HF-C decreased with increasing C additions and N fertilization when straw was removed. This means that root-derived C was unable to increase, or even sustain, C contents in the HF under high N fertilization (Fig. S3-5 and 6). In a meadow ecosystem, N fertilization increased plant biomass production without changing bulk SOM or its fractions (Neff *et al*., 2002). This was explained by a substantially increased C turnover in plant material and f-LF, followed by replacement of C in the HF by microbial-residues derived from labile substances (Cotrufo *et al*., 2013; Gleixner, 2013; Gunina & Kuzyakov, 2014). However, roots or low-quality residue inputs (high C/N) can also contribute markedly to SOM storage (Rasse *et al*., 2005; Barbera *et al*., 2010). Therefore, we propose that roots and straw fulfilled different functions with regards to SOM storage. On the one hand, root-derived C was retained in the f-LF and thus directly

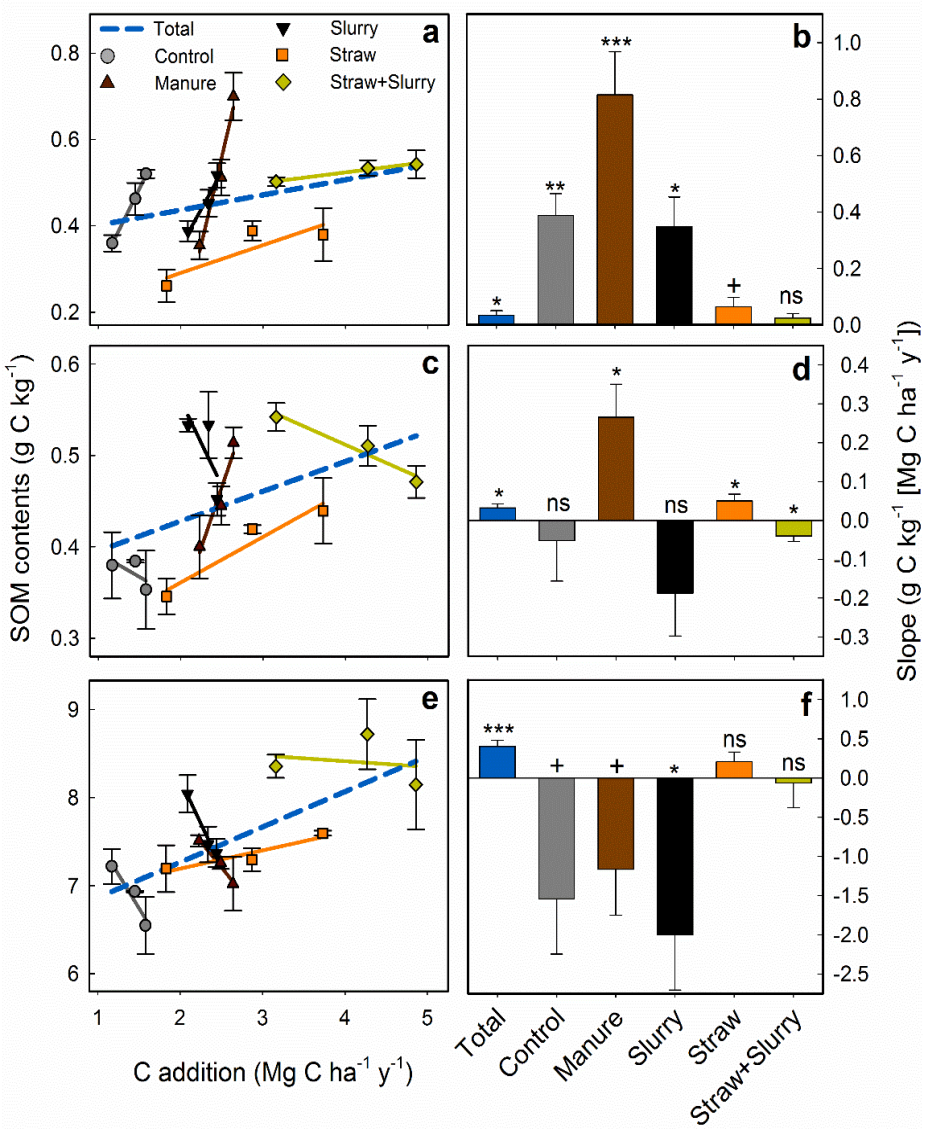

Figure S3-5. Soil organic matter (SOM) contents and their relationships with mean annual C addition over 32 years. The slopes of the linear regressions (± standard errors) either of the total dataset (n= 45) or within individual treatment (organic additions n = 9) along N fertilization rates are given. Free light fractions (f-LF) **(a)** and their slopes **(b)**, occluded light fractions (o-LF) **(c)** and their slopes **(d),** heavy fractions (HF) **(e)** and their slopes **(f)**. Control: without organic additions; Manure: straw removed, farmyard manure applied every third year; Slurry: cattle slurry application, straw removed; Straw: straw incorporated; Straw+Slurry: straw incorporated combined with slurry application. ***: $p \leq 0.001$; **: $p \leq 0.01$;*: $p \leq 0.05$; +: $p \leq 0.1$; ns: $p > 0.1$. The probability levels of the linear mixed model for accepting the null hypothesis that the factors have no effect on C contents are as follows: f-LF (C addition ≤ 0.001; N fertilization ≤ 0.001; and interaction: C addition × N fertilization = 0.010), o-LF (C addition ≤ 0.001; N fertilization = 0.428; and interaction: C addition × N fertilization = 0.005), HF (C addition ≤ 0.001; N fertilization = 0.0109; and interaction: C addition × N fertilization = 0.326).

reflected the amount of C input and explained the often observed minor increase of SOM with N fertilization in cropland soils (Lu *et al*., 2011). On the other hand, straw, exhibiting (relative to roots) faster decomposition, fueled mineral-associated SOM with microbial-residues. The decreasing C contents in the HF without straw incorporation indicate that the stimulating effect of N on SOM turnover (Neff *et al*., 2002; Qiao *et al*., 2016) could not be counteracted by remaining C inputs. Importantly, this discussion centers around the effect of N fertilization solely within one type of organic addition. Accordingly, our finding is *sensu strictu* valid only for C inputs, which are stimulated by N fertilization.

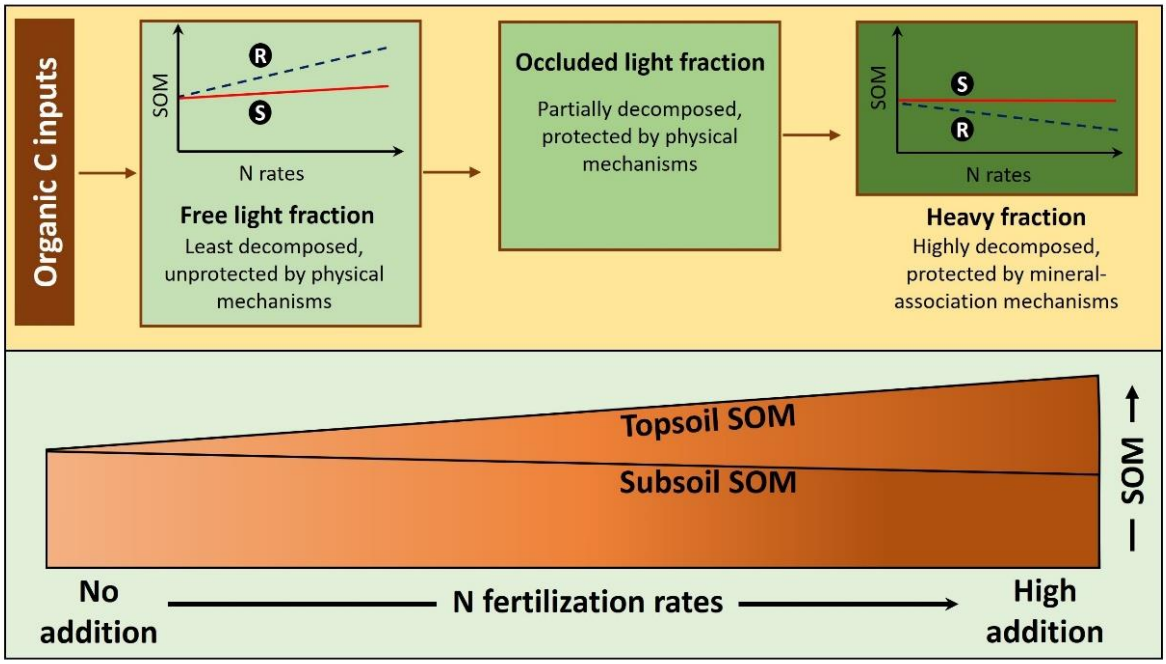

Figure S3-6. The stabilization of top- and subsoil soil organic matter (SOM) under long-term organic C inputs and N fertilization rates (lower part). The upper part represents the partitioning and stabilization (by different mechanisms) of added organic C into topsoil SOM fractions. The inset on free light fraction and heavy fraction shows the contribution of root dominated (R in circle) or straw dominated (S in circle) C inputs in C storage within SOM fraction along N fertilization rates.

The C contents in o-LF showed inconsistent results and were mainly affected by organic additions (Fig. S3-5c, d). The increase of C contents in o-LF with manure additions is consistent with other findings, where high aggregates formation to animal manure additions attributed to the increase of occlusion. The addition of manure along N fertilization can increase favorably

root biomass (Hati *et al*., 2006) which are important for aggregate formation during growth (Denef *et al*., 2002) and decomposition (Majumder & Kuzyakov, 2010; Shahbaz *et al*., 2016). The results, however, indicated that straw incorporation resists, while slurry addition favored the decreases of occluded C under increasing N fertilizer rates.

Overall, the SOM fractions (f-LF, o-LF and HF) are a sensitive indicator for evaluating changes in the soil quality, due to their vital role in nutrient cycling, SOM formation and soil structural development (Brevik *et al.,* 2015; Schrumpf *et al.,* 2013; Six *et al*., 2002). In our study, the C content of f-LF increased and of HF decreased with N fertilization, particularly when straw was absent (Fig. S3-5 and S3-6). In contrast, straw incorporation with N fertilization did not improve C contents of f-LF but prevented the loss of C in HF, which is less responsive to environmental changes. HF is considered the most important principal component for long-term SOM stabilization and it plays a pivotal role in soil structural development due to its strong bindings effect (Six *et al.,* 2002). It has been shown that soil mineral particles (HF) with low SOM content exhibit faster dispersal in soil water compared with SOM-rich mineral fractions (Dexter and Czyz, 2000; Schjønning *et al.,* 2009). Under low HF-associated SOM contents (as without straw additions, Fig. S3-5e), therefore, the prevalence of erosion-induced land degradation may increase possibly due to decrease of soil stability (Jiménez *et al.,* 2016; Keesstra *et al.,* 2016; Schjønning *et al.*, 2009). In addition to the positive impact on mineral-associated SOM fraction, straw incorporation also provides soil physical protection against raindrop impact, resulting in reduced sediment detachment (Auerswald *et al.,* 2009; Cerdà *et al.,* 2016; Prosdocimi *et al.,* 2016). Although, the overall effect of straw removal on bulk SOM (at our low-erosion study site) was minor, our findings however, support the potential benefits of straw incorporation (to protect decadal mineral-associated SOM from loss, and so may improve soil quality e.g. by mulching effect and improving soil structure) at exposed site suffering from high rates of erosion (Auerswald *et al.,* 2009; Rodrigo Comino *et al.,* 2016a, 2016b; Prosdocimi *et al.,* 2016).

3.3.6 Conclusions

Nitrogen fertilization substantially increased C input by roots and straw into the soil because of higher plant productivity. Therefore, total SOM increased with N fertilizer rates during the 32 years in the Ap horizon (0-25 cm). Subsoil (25-60 cm) SOM, however, decreased with increasing C additions and N fertilization, probably because roots are relocated to the topsoil as the N supply increased. The increase of total SOM in the topsoil was driven mainly by the

light fraction. Mineral-associated C, however, decreased with increasing C input induced by N fertilization. Straw contributed little to the f-LF but prevented C losses from the mineral-associated HF. We ascribe this finding to different functions of roots (dominating crop residue input when straw was removed) and straw: Whereas under root-dominated residue input, light SOM fraction increased linearly with N fertilization, more easily decomposable straw was transformed (from f-LF) by microorganisms and stabilized on minerals thereafter. Accordingly, the often described minor increase of SOM with N fertilization reflects the opposite response of functionally variable SOM fractions to root and aboveground residues. This calls for caution when recommending removal of aboveground crop residues, such as straw, e.g. for bioenergy or other purposes. Although the overall effect of straw removals on bulk SOM can be minor, the SOM stabilized on mineral fractions, which are less responsive to environmental changes, could be lost over decades. In conclusion, organic residues increase the SOM level, but their effects strongly depend not only on their quantity (e.g. regulated by management and N fertilization) but also on the quality and functions of plant residues remaining and added on and in soil.

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4 Additional Study

This chapter presents a study that is completed but the writing is still in progress. The goal is to present the short description of the work that has been done and that still needs to be done before submitting a manuscript. The presented results are not exhaustive but are selected because they enhance the understanding of SOM stabilization when crop residues are partially decomposed and labile C (such as root exudates) is available. The results present the potential of the future manuscript.

Study 4 (in preparation)

Interactive effect of glucose and partially decomposed plant residues priming: A three source partitioning study

4.1 Background

Crop residue return to the soil is aimed to improve SOM levels and soil physical properties. The pool size of SOM depends on the balance between SOM formation from added plant residue and roots decomposition and its mineralization to inorganic C. Much is known about factors controlling residue and SOM decomposition rates, information related to how labile C inputs (e.g. exudates released by roots) can alter residues as well as then SOM stabilization is scare. Under natural conditions, labile C is usually released into the soil through root exudation. Glucose is an organic component that resembles the root exudates and it represents simple monosaccharides which are produced during cellulose decomposition. The availability of such labile substrate influence the microbial growth and activities (due to rapid C assimilation by microbes) which can affect decomposition of already degrading crop residues (present is soil) and SOM. The impact of contrasting substrates quality, separately i.e. less labile plant residues or labile C (such as glucose) additions on SOM is mostly studied. It has been reported that glucose and plant residues can prime the decomposition of SOM. However, we lack the knowledge about the simultaneous effects of glucose on SOM versus plant residue mineralization. Further, information related to combined effect of these substrates: labile C and especially partially decomposed residues (as under field conditions), on SOM is need to be investigated.

4.2 Objectives and hypotheses

The aim of this study is to explore the responses of SOM versus residue mineralization in response to labile C (glucose) addition in a soil having partially decomposed wheat residues (leaves, root), over an incubation period of 3 months. The combined effect of residues plus glucose on SOM stabilization is also studied. We followed a three-source partitioning approach using dual isotopic labeling (^{13}C and ^{14}C) to partition the decomposition of glucose, residues and SOM. Specific objectives of this study were: (1) to compare the priming effects of glucose versus residues on SOM decomposition when they were added separately, (2) to differentiate the effects of glucose addition on SOM versus residue in soil-residue mixtures, (3) to provide insights into the possible consequences of glucose amendment on different C pools.

Two type of wheat residues are used representing contrasting quality i.e. easily decomposable leaves and recalcitrant to decomposition roots. To meet the study objectives, we hypothesized that: regardless of the type of plant residues glucose will be mineralized at the same rate, and therefore the magnitude of priming will increase at the same rate between glucose+leaves and glucose+roots treated soils. The base of this hypothesis is that priming of SOM mineralization is facilitated by increased availability of labile C for microbial activity. That means the rate of priming is constrained by the maximum potential activity of microorganisms and that microbial biomass composition/size and residues/SOM recalcitrance are soil-specific limitations on SOM mineralization.

4.3 Methods

We used dual $^{13}C/^{14}C$ isotopic labeling approach to partition soil CO_2 efflux and C pools into three sources: glucose (^{14}C), plant residues (^{13}C) and SOM. To obtain partially decomposed plant residues, ^{13}C labeled wheat residue (leaves, stems separately) were preincubated in the soil for 30 days, to reach a constant residue mineralization rate (Fig. 4-1). This was done because under natural conditions during residues decomposition (partial degradation) labile substrate (such as root exudates) are released to the soil. After partial degradation of residue, soil alone or with residues was amended with or not with ^{14}C-labeled glucose solution (160 µg C g^{-1}) over a period of 3 months at 22 °C (Fig. 4-1). Accordingly, six treatments were established (Soil alone, Soil+glucose, Soil+leaves, Soil+leaves+glucose, Soil+roots, Soil+roots+glucose) with three replicates. To see the changes in microbial biomass over the

incubation period, the microbial biomass C contents were estimated before the glucose additions (at day 30 of pre-incubation) and at the end of incubation.

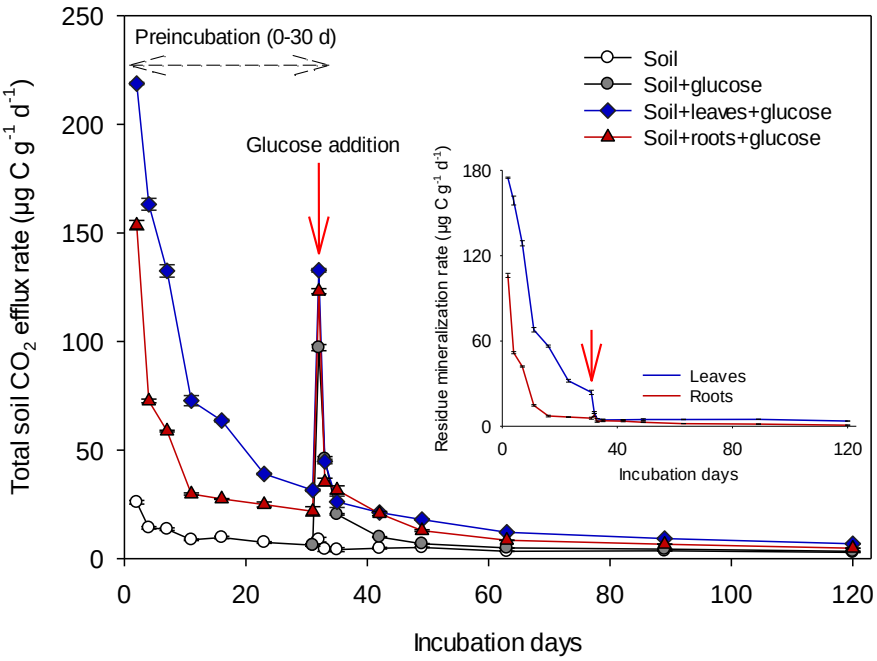

Figure 4-1: Mineralization rate of total soil CO_2 efflux, before (during 30 day pre-incubation period) and after glucose additions. The inset represent the mineralization rate of wheat residues (leaves and stems). Error bar represents standard error (n=3)

Results and discussion

4.2.1 Glucose and residue mineralization

Glucose decomposition was calculated based on the $^{14}CO_2$ efflux. The results show that glucose mineralization rate was dependent on the residue quality. Glucose mineralization rate was highest during the initial days (up to 11 days) and remained similar between with roots and alone soil additions. Adding with leaves, glucose mineralization increased by 17% than with roots or alone soil (Fig. 4-2).

After glucose addition, mineralization rate of leaves declined up to 65% and of roots remained unaffected (Fig. 4-1). However, at the end of incubation, the cumulative leaves mineralization under glucose addition also reached to the similar level as without glucose additions. A fast

decline in leaves residues but high glucose mineralization indicated a strong shift of residue decomposing microorganism toward added glucose C. Root mineralization rate remained unaffected after glucose additions. This indicates that roots decomposition was already reached to a stable conditions (i.e. slow-growing) and the glucose mineralization was not mainly due to the root decomposing microorganisms.

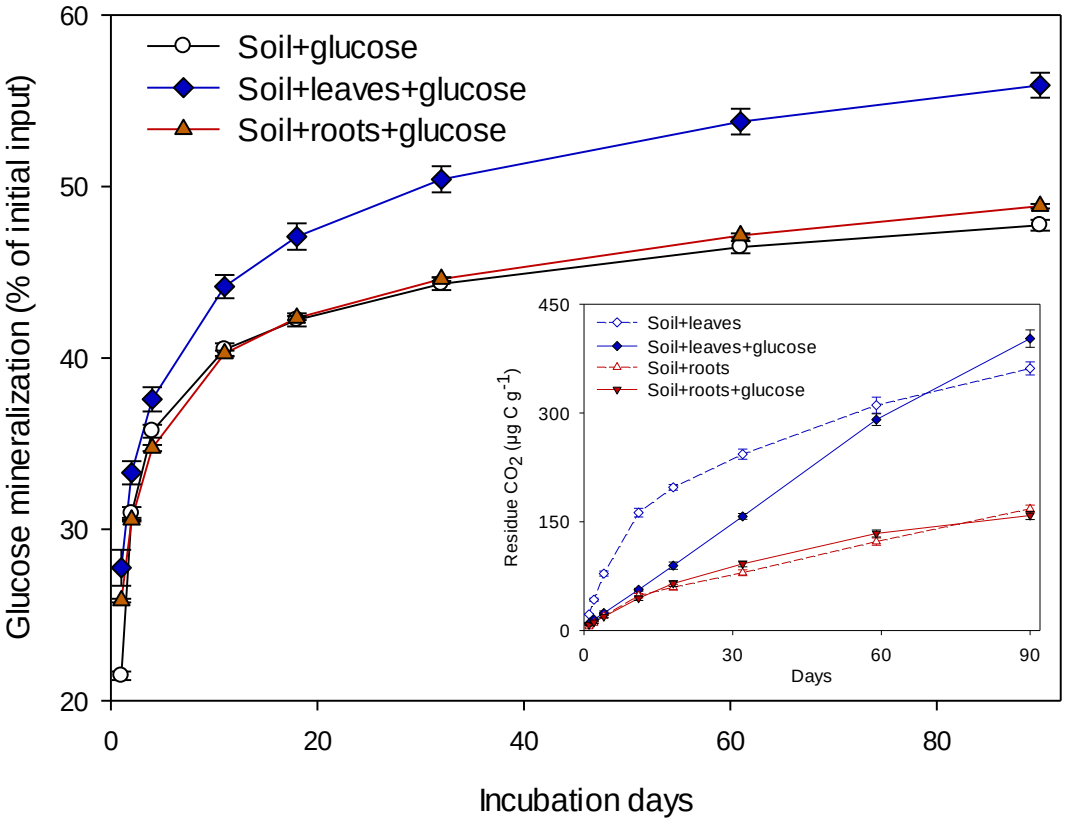

Figure 4-2: Cumulative glucose mineralization over the incubation period depending on plant residue type. The inset shows cumulative residue derived CO_2 with or without glucose additions. Error bar represents standard error of mean (n=3)

4.2.2 Priming effect

The addition of glucose to the alone soil induced a cumulative positive priming effect (0.19 mg C g^{-1}) on SOM over 90 days, a much higher (42%) effect than residue-induced priming in the presence of glucose. Compared to soils with only residue amended, glucose and residue added in combination increased SOM mineralization by 44%. This increase in priming effect was solely due to the contribution of primed SOM during the initial (up to 11 days) period of glucose

mineralization (Fig. 4-3). Meaning that microbial activation after glucose addition caused mainly SOM mineralization.

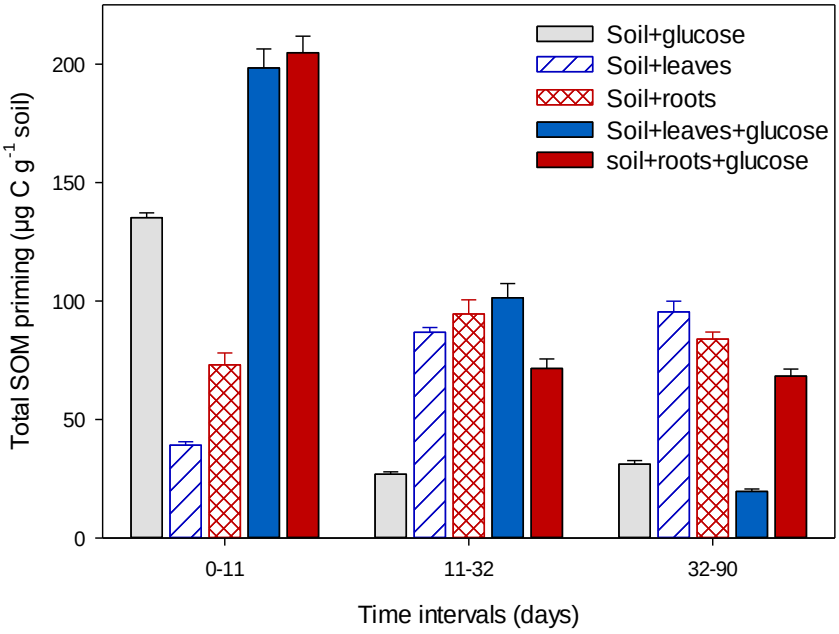

Figure 4-3: Cumulative soil organic matter (SOM) priming effect at different time intervals, depending on: time, glucose and residue addition, type of residue and combination of glucose and residues. Error bar represents standard error of mean (n=3)

To assess the priming effects of glucose on residue (i.e. leaves and roots) versus SOM in the soil-residue mixture, the residue- and SOM-derived CO_2 effluxes from the Soil+residue+glucose amendments were compared to those with soil+residue treatments. The results showed that glucose had opposite priming effect on residue and SOM decomposition. During the initial period (up to 11 days of addition), glucose caused a strong priming effect on SOM (higher when added with leaves than roots), but thereafter cumulative priming effect was decreased (Fig. 4-4). The total SOM priming effect due to glucose addition in residue treatments was up to 120 µg C g^{-1} soil. In contrast, the priming effect of glucose on residue mineralization was negative (slightly for roots and strongly for leaves) in the initial 2 weeks but then became positive. Accordingly, residue mineralization with glucose gradually reached to the level without glucose additions after 90 days (Inset Fig. 4-2, Fig. 4-4).

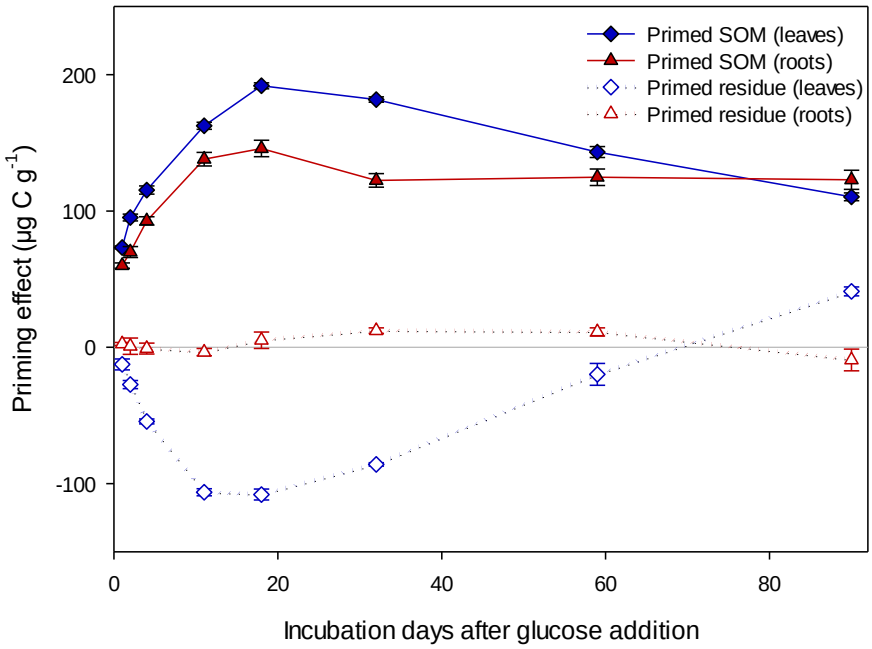

Figure 4-4: Priming effects of glucose on the mineralization of residues (leaves and roots) versus soil organic matter (SOM) in soil-residue mixtures. The priming effects are differences in SOM mineralization between the Soil+residue+glucose and soil+residue amendments. Error bars represent standard error of mean (n=3).

4.2.3 Microbial biomass

To assess the changes in residue and SOM feeding microorganisms, the microbial biomass C were estimated at the time of glucose additions and at the end of incubation. At the end of incubation, a higher amount of microbial biomass was recorded under leaves addition followed by root additions than alone soil control. Partitioning of microbial biomass C into various sources revealed that a remarkable amount of SOM originated C caused an increase in microbial biomass C either with alone residues, or the combination of glucose and residues. Glucose increased microbial biomass C, which was mainly due to SOM decomposing microorganisms. However, a significant portion of residue decomposing microorganism was lost after glucose addition (Fig. 4-5).

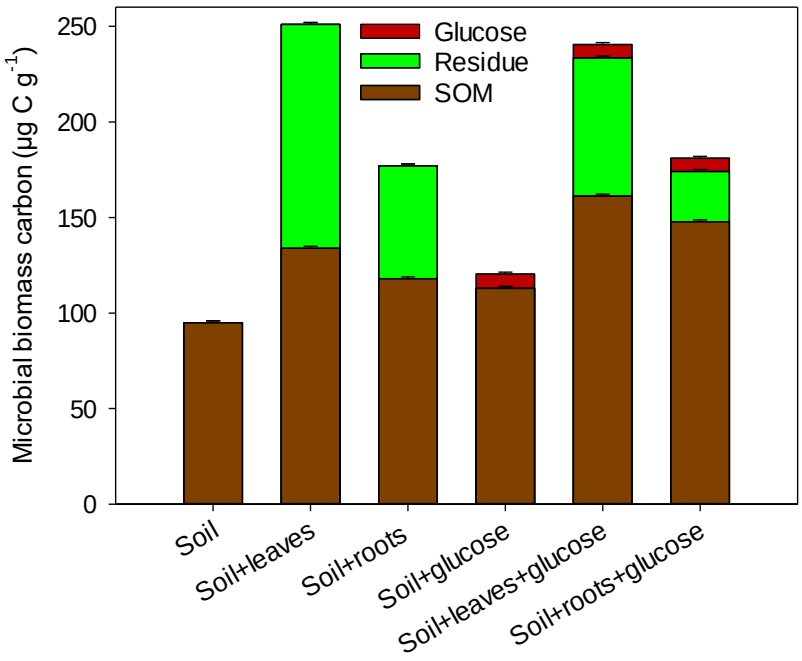

Figure 4-5: Microbial biomass carbon originating from three sources: soil organic matter (SOM), residue and glucose following incubation. Error bars represent standard error of mean (n=3).

4.2.4 Conclusions

Increased SOM- but not residue-derived C in microbial biomass suggested that glucose caused a preferential microbial utilization of SOM over crop residues. This was evident by high SOM priming under glucose addition. Glucose additions caused contrasting priming effect on residues and SOM. Residue mineralization rates decreased (strongly of leaves) but SOM decomposition strongly increased after glucose addition. Thereafter (after ca. 2 weeks), residue mineralization gradually reached to the residue mineralization levels without glucose addition. Overall, addition of glucose after residues preincubation (30 days of decomposition) enhanced the SOM priming significantly, either glucose was added alone or in combination with residues. Glucose induced priming effect was mainly evident by SOM decomposing microorganisms. Further, the priming effects of residue on SOM are changed by the presence of glucose.

5 General conclusions

A combined approach of controlled laboratory experiments and a less controlled long-term (32 year) field experiment enhanced the understanding of SOM formation and stabilization. SOM stabilization is related to soil physical properties, source and amount of crop residue inputs (root or straw dominated), priming effects and residue C partitioning between SOM fractions. Addition of large amounts of crop residues improved soil physical structure, but efficiency of residue C stabilization in SOM was lower than for smaller additions. Increased C input was associated with a low percentage of residues physically protected within aggregates and a high rate of SOM mineralization. This explained the reduced long-term C stabilization in SOM with increasing residue additions.

A more precise effect of crop residue quality and quantity on SOM stabilization was investigated, especially in terms of SOM priming. An increased level of addition led to a disproportionate increase in the residue mineralization rate for aboveground residues, but not for roots (belowground). SOM priming decreased with greater residue additions for all residue types. However, we demonstrated that root residues induced faster and stronger SOM priming than aboveground residues. This was attributed to the recalcitrance of roots to decomposition, which causes microorganisms to decompose SOM for nutritional needs by increasing their enzyme activities. Remarkably, the amount of primed C and enzyme activities were mainly correlated with the residue-feeding microorganisms, indicating a possible link between the residue-feeding microbial fraction and priming. To describe this link, we suggested a unifying logistic model for all residue types describing SOM priming as a function of residue mineralization. We recorded threshold levels for the onset of strong priming in terms of the fraction of mineralized residues at high additions: ca. 20, 44 and 51% mineralization of roots, stems and leaves, respectively. Therefore, we concluded that the quality of added substrate is crucial for microbe-mediated SOM decomposition.

The density fractionation approach revealed the importance of crop residue quality in C partitioning between SOM fractions and role of these fractions in determining SOM contents in the field experiment. The C input in the field experiment was mainly ascribed from two main sources: root- or straw, which were both increased by N fertilization. The topsoil SOM contents increased with higher input (N induced) to only a limited extent, and was mainly accounted for by the free light fraction of SOM. Mineral-associated C, however, decreased with the increasing

C input induced by N fertilization. The aboveground residue (straw) contributed little to the free light fraction, but prevented C losses from the mineral-associated SOM fraction. We ascribed this finding to the different behaviors of roots (the dominant crop residue input when straw is removed) and straw: Under root-dominated residue input, the light SOM fraction increased linearly with N fertilization, whereas the more easily decomposable straw was transformed (from the light SOM fraction) by microorganisms and stabilized in the mineral-associated fraction thereafter. Although the overall effect of straw addition on bulk SOM was minor, it prevented the loss of mineral-associated SOM fractions (observed when straw removed), which are stable over decades. In contrast to topsoil, the subsoil (25-60 cm) SOM contents decreased with increasing C additions (along with N fertilization), probably because roots were more localized in the topsoil as N supply increased. This was contrary to our hypothesis, i.e. high C inputs at the surface may cause high subsoil SOM accumulation.

In conclusion, organic residues improve soil structure and SOM levels, but their effects strongly depend on not only their quantity (which can be managed through additions) but also on the quality and behavior of plant residues added to the soil. Roots and aboveground residues exhibit variable effects and functions for SOM stabilization. Root-dominated C inputs contribute greatly to the unprotected or less decomposed SOM fractions (free light fraction) for a longer period than aboveground plant residues. Although roots are more recalcitrant to decomposition than aboveground residues, they have a lower mineralization threshold at which SOM priming increases. Therefore, in addition to SOM destabilization through priming, root additions may result in lower levels of microbially-mediated stable SOM formation following residue decomposition, which is necessary for mineral-associated stable SOM formation. Although larger aboveground residue additions (leaves, stems) improve soil aggregation and protect the mineral-associated SOM fraction, low physical protection and high mineralization decreases the efficiency of their stabilization in SOM. The often-described minor increase of SOM with high crop residue inputs emerges from the opposing responses of functionally variable SOM fractions to root and aboveground residues.

Overall, our findings connect the quantity and quality of crop residues for better prediction and understanding of the mineralization and stabilization of SOM. In order to sustain sufficient SOM levels, efficient crop residue management under specific field conditions is required. This is important when recommending removal of aboveground crop residues, such as straw, e.g. for bioenergy purposes.

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7 Appendix

7.1 Supplementary material Study 3.2

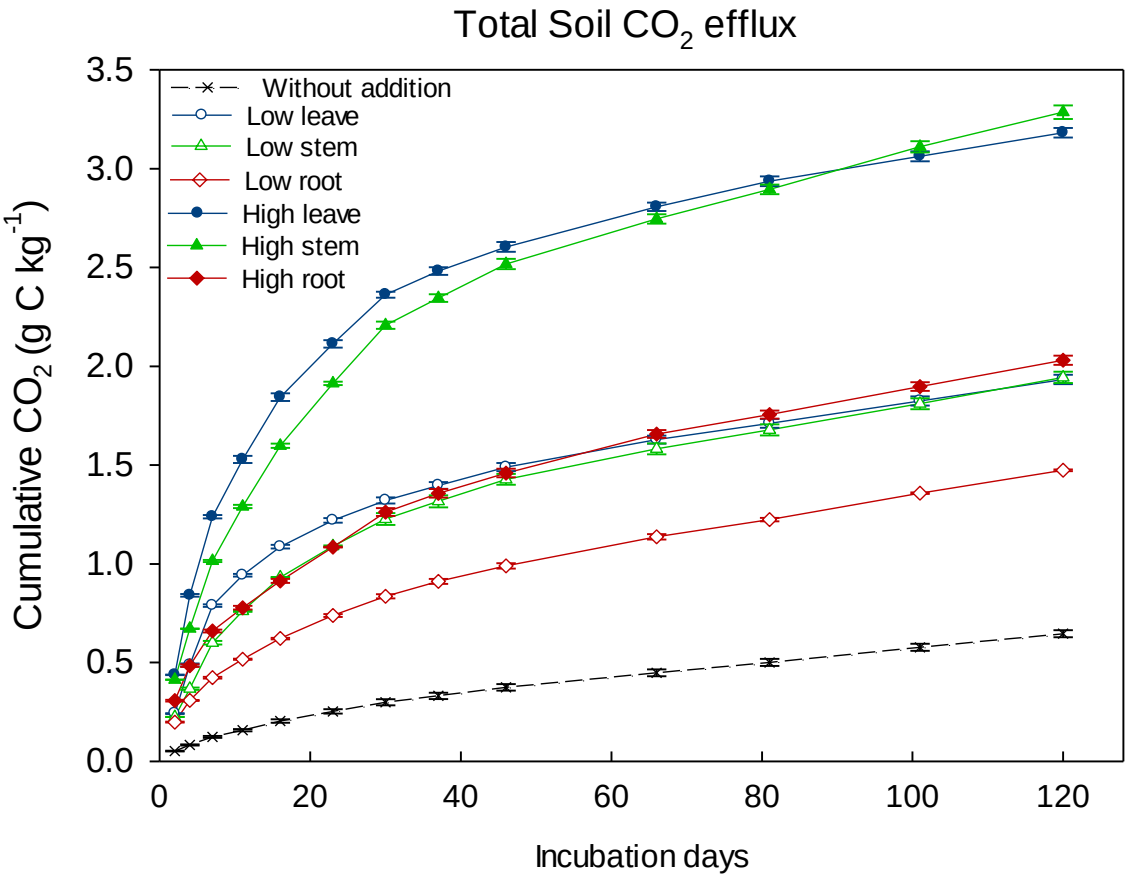

Figure S2-S1. Cumulative total soil CO_2 efflux over 120 days of incubation, depending on type and level of crop residue additions. Mean values with standard errors (n = 3).

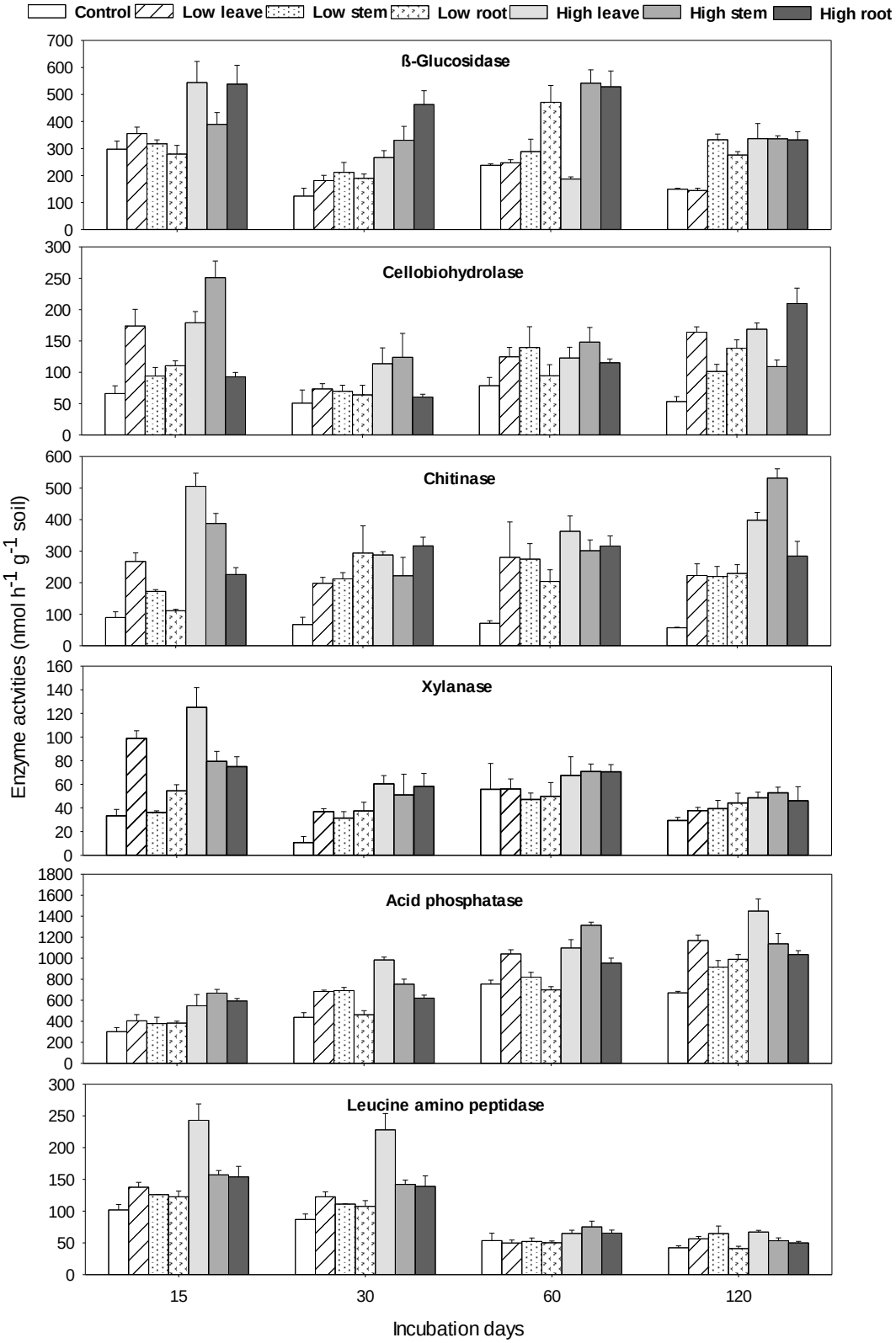

Figure S2-S2. The effect of residue type and level of addition on potential soil enzymes activities over the incubation period of 120 days. Mean values with standard errors (n = 3).

Table S2-S1: The Pearson correlations (r) of residue and soil organic matter (SOM) derived microbial biomass carbon (MBC) with total and residue originated CO_2 efflux, and with the values of the potential activities of hydrolytic enzymes in soils amended with residues at incubation day 15, 30, 60 and 120.

	Total CO_2	Residue CO_2	ß-Glucosidase	Acid Phosphatase	Chitinase	Xylanase	Cellubiohydrolase	LAP*
Residue MBC								
15 day	0.88	0.89	0.66	0.33	0.85	0.76	0.39	0.82
30 day	0.54	0.64	0.08	0.90	0.02	0.40	0.40	0.85
60 day	0.85	0.86	-0.09	0.81	0.39	0.47	0.21	0.55
120 day	0.76	0.78	0.27	0.53	0.86	0.29	-0.25	0.33
SOM MBC								
15 day	-0.43	-0.46	-0.02	0.08	-0.40	-0.30	-0.40	-0.26
30 day	0.04	0.13	-0.23	0.62	-0.05	0.00	0.09	0.38
60 day	0.39	0.40	0.07	0.35	0.02	0.13	0.11	0.17
120 day	0.48	0.53	-0.09	0.50	0.65	0.11	-0.05	0.14

*Leucine aminopeptidase

7.2 Contribution to the studies

Names order corresponds to the estimated importance of the each contribution

Study 1 (chapter 3.1): Decrease of soil organic matter stabilization with increasing inputs: mechanisms and controls

Study design: Felix Heitkamp, **Muhammad Shahbaz,** Yakov Kuzyakov

Sample collection: **Muhammad Shahbaz**

Sample preparation, incubation and analysis: **Muhammad Shahbaz**

Data interpretation: **Muhammad Shahbaz**, Felix Heitkamp

Manuscript writing: **Muhammad Shahbaz**

Comments on manuscript: Felix Heitkamp, Yakov Kuzyakov

Study 2 (chapter 3.2): Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds

Study design: **Muhammad Shahbaz,** Felix Heitkamp, Yakov Kuzyakov

Sample collection: **Muhammad Shahbaz,** Muhammad Sanaullah

Sample preparation, incubation and analysis: **Muhammad Shahbaz,** Amit Kumar, Muhammad Sanaullah

Data interpretation: **Muhammad Shahbaz**, Evgenia Blagodatskaya

Logistic model development: Vladimir Zelenev, Evgenia Blagodatskaya, **Muhammad Shahbaz**, Felix Heitkamp

Manuscript writing: **Muhammad Shahbaz**

Comments on manuscript: Evgenia Blagodatskaya, Yakov Kuzyakov, Muhammad Sanaullah, Felix Heitkamp

Study 3 (chapter 3.3): Decadal nitrogen fertilization decreases mineral-associated and subsoil carbon: a 32 year study

Field experiment maintenance: Matthias Wendland

Sample collection: **Muhammad Shahbaz,** Shafique Maqsood, Felix Heitkamp

Sample preparation and analysis: **Muhammad Shahbaz,** Shafique Maqsood

Data interpretation: **Muhammad Shahbaz**, Felix Heitkamp, Yakov Kuzyakov

Manuscript writing: **Muhammad Shahbaz**

Comments on manuscript: Felix Heitkamp, Yakov Kuzyakov

Study 4 (chapter 4, in preparation): Interactive effect of glucose and partially decomposed plant residues priming: A three source partitioning study

Study design: **Muhammad Shahbaz,** Yakov Kuzyakov, Felix Heitkamp

Sample collection: **Muhammad Shahbaz**

Sample preparation, incubation and analysis: **Muhammad Shahbaz**

Data interpretation: **Muhammad Shahbaz**, Evgenia Blagodatskaya

Preliminarily results writing: **Muhammad Shahbaz**

7.3 Declarations

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form.
   Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

   Göttingen,

   ……
   (Signature)

   MUHAMMAD SHAHBAZ
   (Name in block capitals)

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorized aid.

   Göttingen,

   .....
   (Signature)

   MUHAMMAD SHAHBAZ
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7.4 Curriculum vitae

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Education

July 2014 – Dec 2016	**PhD, Agricultural Soil Science** Georg-August-Universität Göttingen, Göttingen, Germany
Sep 2009 – Jun 2011	**M.Sc. (Hons) Agricultural Soil Sciences** University of Agriculture Faisalabad, Faisalabad, Pakistan
Aug 2005 – Sep 2009	**B.Sc.(Hons), Agricultural Soil Science** University of Agriculture Faisalabad, Faisalabad, Pakistan

Research Work Experience

Oct 2012 – April 2014	Justus-Liebig University of Gießen, Institute of plant nutrition, Gießen, Hessen, Germany	**Research project:** Role of abscisic acid and auxin in acid invertase activity and kernel setting of various maize hybrids under salt stress
May 2009 – Oct 2011	University of Agriculture Faisalabad, Institute of Soil and Environmental Sciences, Faisalabad, Punjab, Pakistan	**Research project:** Investigating the role of foliar feeding of micronutrients for crop yield maximization in Punjab

Training/Workshops

2014 - 2016	Georg-August-Universität Göttingen, Germany: Modules:	• Linear statistical models with R • Systematic review and meta-analysis in ecology • Isotopes in ecosystems sciences and environment • Scientific writing and publishing in crop sciences • Soil biogeochemistry discussions
2013 (7 days)	DAAD-Berlin (Wandlitz), Germany	Making sustainability internationally
2010 (4 weeks)	University of Agriculture Faisalabad, Pakistan	Remote sensing and digital image processing
2010 (8 weeks)	Fauji Fertilizer Company (Pvt. Ltd) Pakistan	Assessment of farmer field problems and provision of fertilizer recommendations

Awards & Grants

Oct 2012	PhD Scholarship: German Academic Exchange Service (**DAAD**), Germany
Oct 2011	Award: master thesis research funding from International Center for Development and Decent work (**ICDD**), University of Kassel, Germany
Sep 2011	Award: **Highest CGPA (4.00/4.00)** award in M.Sc. (Hons); institute of soil and environmental Sciences, University of Agriculture, Faisalabad, Faisalabad Pakistan
Aug 2009	Award: **Highest CGPA (3.98/4.00)** award in B.Sc. (Hons); institute of soil and environmental Sciences, University of Agriculture, Faisalabad, Faisalabad Pakistan

Research Interests and Skills

Research interests	Soil organic matter, Soil structure, Carbon sequestration, Priming effects, Partitioning of CO_2 fluxes, Stable and radioactive isotopic applications (^{13}C, ^{14}C and ^{15}N), Soil organic matter dynamics, Crop residue quality effects, Soil fertility, Sustainable agriculture
Languages	English (fluent), German (medium), Urdu (mother tongue)
Scientific Memberships	European Geosciences Union (EGU), Vienna, Austria
Softwares	MS-office, SigmaPlot, Statistix 8.1, Linear statistical model R (basic), Mendeley

Journal Publications

Shahbaz M, Kuzyakov Y, Maqsood S, Weendlad M, Heitkamp F: Decadal nitrogen fertilization decreases mineral-associated and subsoil carbon. *Land Degradation & Development* 11/2016. DOI: 10.1002/ldr.2667

Shahbaz M, Kuzyakov Y, Heitkamp F: Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls. *Geoderma* 06/2016. DOI:10.1016/j.geoderma.2016.05.019

Shahbaz M, Kuzyakov Y, Sanaullah M, Heitkamp F, Zelenev V, Kumar A, Blagodatskaya E: Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. *Biology and Fertility of soils* 12/2016. DOI: 10.1007/s00374-016-1174-9

Shahbaz M, Akhtar MJ, Ahmed W, Wakeel A: Integrated effect of different N-fertilizer rates and bioslurry application on growth and N-use efficiency of okra *(Hibiscus esculentus* L.*)*. *Turkish Journal of Agriculture and Forestry* 03/2014. DOI:10.3906/tar-1303-65

Yaseen M, Ahmed W, Shahbaz M: Role of foliar feeding of micronutrients in yield maximization of cotton in Punjab. *Turkish Journal of Agriculture and Forestry* 07/2013. DOI:10.3906/tar-1206-56

Conference Proceedings

Shahbaz M, Sanaullah M, Kuzyakov Y, Heitkamp F, Zelenev V, Blagodatskaya E: Microbial residues accelerate decomposition of Soil Organic Matter: new mechanism, actors and thresholds. Workshop "SOMmic – Microbial Contribution and Impact on Soil Organic Matter, Structure and Genesis". Leipzig, Germany, November 9-11, 2016 (Oral presentation)

Stelmach, W, Shahbaz M, Bieganowski A, Kuzyakov Y: Sludge C stabilization and mineralization in soil as assessed by ^{13}C natural abundance. 11th International Conference on Agrophysics, September 26-28 2016, Lublin (Poland)

Stelmach W, Shahbaz M, Bieganowski A, kuzyakov Y: Sequestration of sludge C in soil as assessed by ^{13}C abundance and ^{14}C labeling. 15th International Workshop for Young Scientists, BioPhys Spring 2016, 05/2016, Prague (Czech Republic)

Shahbaz M, Kuzyakov Y, Heitkamp F: *Mineral-associated and aggregate-occluded soil carbon decreased with increasing nitrogen and residue input for three decades*. EGU General Assembly 2016, Vienna (Austria); 04/2016 (Oral presentation)

Shahbaz M, Kuzyakov Y, Heitkamp F: *High rate of residue addition decreases stabilization efficiency due to priming and low physical protection*. 5th International Symposium on Soil Organic Matter; Göttingen (Germany); 09/2015 (Poster presentation)

Shahbaz M, Kuzyakov Y, Heitkamp F: *Dynamics of soil organic matter and residue addition.* Colloquium of the Regulation of soil organic matter and nutrient turnover in organic agriculture, Witzenhausen-university of Kassel (Germany) 11/2015 (Oral + Poster presentation)