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

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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 hypothesise that (1) root residues will be mineralised 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-labelled 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 δ^{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 mineralisation 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 mineralisation 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.

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1. 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) mineralisation (Houghton, 2012; Lal, 2004). The soil C losses can be mitigated by recarbonisation 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 silty 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 mineralisation (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

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within aggregates is another important mechanism to protect litter from mineralisation (Six et al., 2004; von Lützow et al., 2008).

Aggregates, which protect SOM by physical occlusion, are formed by biological and physico-chemical 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 mineralisation. 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).

Summarising, 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 labelled 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.

2. Materials and methods

2.1. 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 labelled with ^{13}C every week after emergence for at least 8 h 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. Labelled (98 At%) NaH^{13}CO$_3$ was injected into H$_2$SO$_4$ 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 2.6). 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).

2.2. Incubation and sampling

Maximum water holding capacity (WHC) of the soil was determined by soaking for 24 h, subsequent free drain for 1 h 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 labelled wheat leaves, stalks or roots with low or high amounts and one control was 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 1.40 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.

2.3. 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 δ^{13}C analysis.

2.4. 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 aluminium 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.

2.5. 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 CHCl₃. Both subsamples were shaken for 1 h at 175 rev. min⁻¹ with 20 ml of 0.05 M K₂SO₄. The obtained extracts were kept cold (<4 °C) and analyzed the next day for total C concentration (Multi N/C 2100, Analytik Jena, Germany). Microbial biomass C was calculated as EC/kEC, where EC = (organic C from fumigated soils)–(organic C from non-fumigated soils) and kEC = 0.45 (Wu et al., 1990).

2.6. 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 min 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, Italy). 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):

$$At\%^{13}C = \left[no.of\,^{13}C\,atoms/\left(no.of\left(^{12}C + ^{13}C\right)atoms\right)\right] * 100 \qquad (1)$$

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

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

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 ($C_{res-derived}$) in various pools was computed using Eq. (3) (Poirier et al., 2013).

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

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 K₂SO₄ extract (mg kg⁻¹).

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

$$PE = \underbrace{(CO_{2-total} - CO_{2-res-derived})}_{\text{Soil with residues addition}} - \underbrace{CO_{2-control}}_{\text{Soil without addition}} \qquad (4)$$

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.

2.7. 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 student's t-test was used to test whether the increase in mineralisation 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. Results

3.1. 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. 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. 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
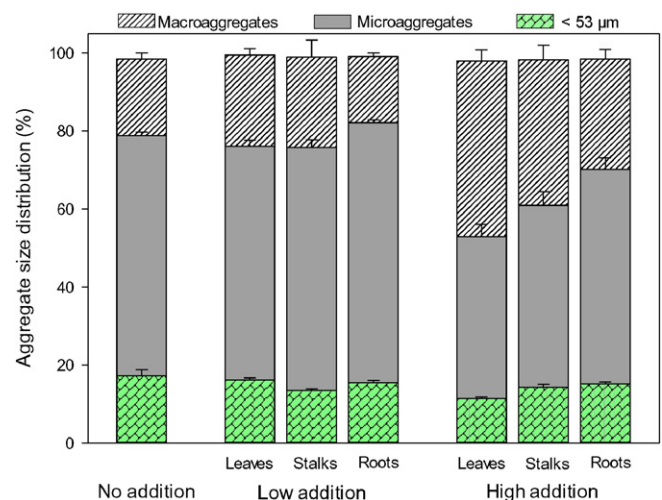

Fig. 1. The relative distribution of aggregate size classes (Macro > 250 μm, Micro 53–250 μm and silt plus clay < 53 μm) after 64 days of incubation depending on type and level of crop residue additions. Means and standard errors (n = 4). The probability levels of the ANOVA for accepting the null hypothesis 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).

aggregate fractions and about 37% was protected in macroaggregates (Table 1, Fig. 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. 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. 2). Moreover, the portion of root-derived C in microaggregates was significantly higher compared to stalk and leaves (Fig. 2).

3.2. 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. 3).

Microbial biomass C derived from SOM was affected by the interaction of residue type and level (p = 0.001, Fig. 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.

3.3. Mineralisation

Residue mineralisation (Fig. 4) after 64 days depended on the type and level of the addition (for both p < 0.001). For instance, residue mineralisation 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. 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, mineralisation of roots is less than 50% of leave and stalks. The increase of mineralisation 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. 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. 4).

4. 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 1, Fig. 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. 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. 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. 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 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 1, Fig. 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 mineralisation (as a proportion of total input) should be lower when aggregate occlusion is higher. Table 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

Table 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 mineralised 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).

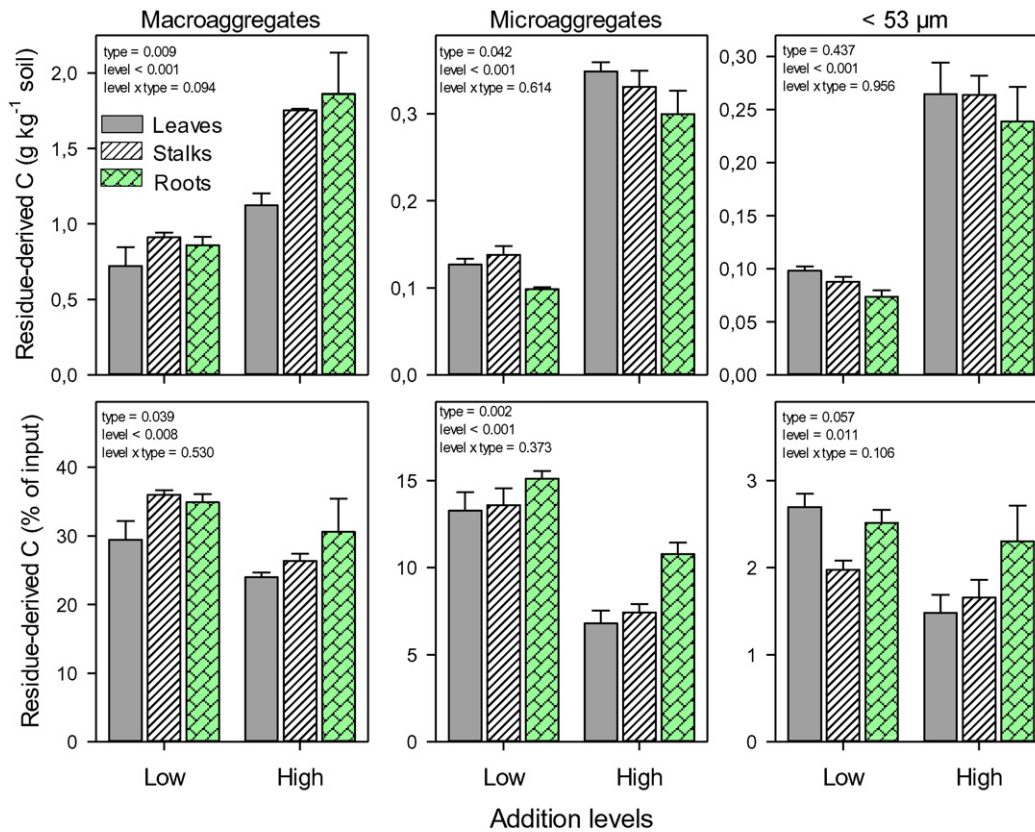| Treatment	Residue C (% of initial input)		Total recovery (%)
	Aggregate classes	CO_2	
Low addition level			
Leaf	47.3 (3.9)	42.6 (0.8)	89.9 (4.7)
Stalk	55.2 (1.5)	34.7 (2.2)	89.8 (3.5)
Root	58.6 (0.9)	37.1 (1.4)	95.7 (2.3)
High addition level			
Leaf	34.0 (0.3)	40.2 (0.9)	74.2 (0.9)
Stalk	38.2 (0.7)	39.0 (1.6)	77.2 (2.2)
Root	47.6 (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 × type	0.567	0.001	0.332

Fig. 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.

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 stabilizing 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 1) but also a higher association of root C with microaggregates and the <53 μm fraction (Fig. 2). Overall there was no evidence for physical short-term stabilization of aboveground plant parts and higher association of large amounts of roots may

indicate preferential long-term stabilization under field conditions (Baldock and Skjemstad, 2000; Six et al., 2002; von Lützow et al., 2008).

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 mineralisation of SOM which mainly depended upon the amount of addition. Regardless of residue type, mineralisation 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
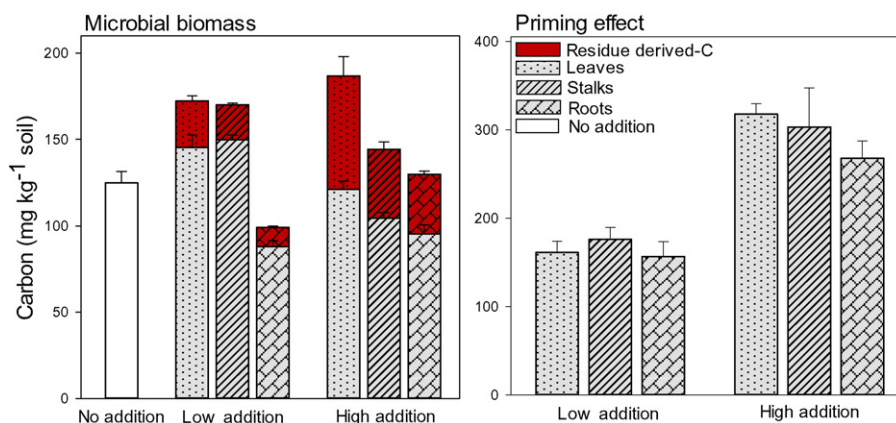

Fig. 3. The contribution of residue derived and soil organic matter (SOM) derived C to microbial biomass (left) and the amount of primed C due to low and high level of crop residue addition (right). Means with standard errors (n = 3). The probability levels of the ANOVA for accepting the null hypothesis 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).
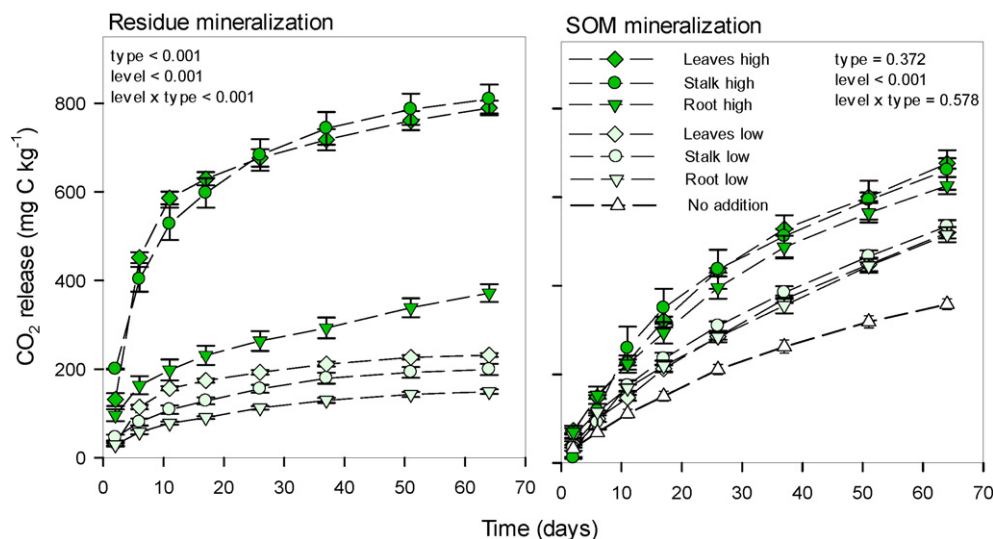
Fig. 4. Cumulative CO_2-C release during 64 days of incubation depending on type and level of crop residue additions. Left: release from crop residues; right: release from soil organic matter (SOM). Mean values with standard errors (n = 3). The p-values calculated by an ANOVA show probability levels for accepting the null hypothesis that the factors have no effect.

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. 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. 2, Table 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 mineralisation of residue types would be reflected in the intensity of priming. Roots at high level showed least mineralisation, but similar priming. Both findings can be linked to the higher incorporation of root residues into aggregates.

5. Conclusions

Our initial hypotheses were not all fully confirmed. Firstly, we hypothesised that mineralisation 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 required. Our findings highlight the necessity to connect the quantity and quality of crop residues for better predicting mineralisation and stabilization of SOM. Specifically, this may also help to resolve the global implications to characterize and identify key soil and residue parameters for modelling of greenhouse gas emissions from soil.

References

Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil aggregate stability – a literature analysis. Soil Biol. Biochem. 41, 1–12.

Andruschkewitsch, R., Geisseler, D., Dultz, S., Joergensen, R.G., Ludwig, B., 2014. Rate of soil-aggregate formation under different organic matter amendments-a short-term incubation experiment. J. Plant Nutr. Soil Sci. 177, 297–306.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org. Geochem. 31, 697–710.

Bertrand, I., Chabbert, B., Kurek, B., Recous, S., 2006. Can the biochemical features and histology of wheat residues explain their decomposition in soil? Plant Soil 281, 291–307.

Blagodatskaya, E.V., Anderson, T.H., 1999. Adaptive responses of soil microbial communities under experimental acid stress in controlled laboratory studies. Appl. Soil Ecol. 11, 207–216.

Blagodatskaya, E.V., Yuyukina, T., Blagodatsky, S., Kuzyakov, Y., 2011. Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. Soil Biol. Biochem. 43, 778–786.

Bromand, B., Whalen, J.K., Janzen, H.H., Schjoerring, J.K., Ellert, B.H., 2001. A pulse-labelling method to generate ^{13}C-enriched plant materials. Plant Soil 235, 253–257.

Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H.C., 2013. Linking litter decomposition of above- and below-ground organs to plant–soil feedbacks worldwide. J. Ecol. 101, 943–952.

Guenet, B., Neill, C., Bardoux, G., Abbadie, L., 2010. Is there a linear relationship between priming effect intensity and the amount of organic matter input? Appl. Soil Ecol. 46, 436–442.

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100.

Gunina, A., Ryzhova, I., Dorodnikov, M., Kuzyakov, Y., 2015. Effect of plant communities on aggregate composition and organic matter stabilisation in young soils. Plant Soil 387, 265–275.

Heitkamp, F., Jacobs, A., Jungkunst, H.F., Heinze, S., Wendland, M., Kuzyakov, Y., 2012a. Processes of soil carbon dynamics and ecosystem carbon cycling in a changing world. In: Lal, R., Lorenz, K., Hüttl, R.F., Schneider, B.U., von Braun, J. (Eds.), Recarbonization of the Biosphere. Springer, Dordrecht, pp. 395–428.

Heitkamp, F., Wendland, M., Offenberger, K., Gerold, G., 2012b. Implications of input estimation, residue quality and carbon saturation on the predictive power of the Rothamsted Carbon Model. Geoderma 170, 168–175.

Helfrich, M., Ludwig, B., Potthoff, M., Flessa, H., 2008. Effect of litter quality and soil fungi on macroaggregate dynamics and associated partitioning of litter carbon and nitrogen. Soil Biol. Biochem. 40, 1823–1835.

Houghton, R., 2012. Historic changes in terrestrial carbon storage. In: Lal, R., Lorenz, K., Hüttl R, F., Schneid er, B.U., von Braun, J. (Eds.), Recarbonization of the Biosphere. Springer, pp. S. 59–S. 82.

Kramer, S., Marhana, S., Ruess, L., Armbruster, W., Butenschoend, O., Haslwimmer, H., Kuzyakov, Y., Pauschf, J., Scheunemannd, N., Schoene, J., Schmalwasser, A., Totsche, K.U., Walker, F., Stefan, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55, 111–119.

Kuzyakov, Y., Blagodatskaya, E., Blagodatsky, S., 2009. The Biology of the Regulatory Gate: comments on the paper by Kemmitt et al. (2008) 'mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective' [Soil Biol Biochem 40, 61–73]. Soil Biol. Biochem. 41, 435–439.

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

Lehmann, J., Kinyangi, J., Solomon, D., 2007. Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. Biogeochemistry 85, 45–57.

Loeppmann, S., Blagodatskaya, E., Pausch, J., Kuzyakov, Y., 2016. Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. Soil Biol. Biochem. 92, 111–118.

Lorenz, K., Lal, R., 2012. Cropland soil carbon dynamics. In: Lal, R., Lorenz, K., Hüttl, R.F., Schneider, B.U., von Braun, J. (Eds.), Recarbonization of the Biosphere. Springer, pp. S. 303–S. 346.

Lugato, E., Bampa, F., Panagos, P., Montanarella, L., Jones, A., 2014. Potential carbon sequestration of European arable soils estimated by modelling a comprehensive set of management practices. Glob. Chang. Biol. 20, 3557–3567.

Moreno-Cornejo, J., Zornoza, R., Doane, T., Faz, Á., Horwath, W., 2015. Influence of cropping system management and crop residue addition on soil carbon turnover through the microbial biomass. Biol. Fertil. Soils 51, 839–845.

Poeplau, C., Kätterer, T., Bolinder, M.A., Börjesson, G., Berti, A., Lugato, E., 2015. Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments. Geoderma 237–238, 246–255.

Poirier, V., Angers, D.A., Rochette, P., Whalen, J.K., 2013. Initial soil organic carbon concentration influences the short-term retention of crop-residue carbon in the fine fraction of a heavy clay soil. Biol. Fertil. Soils 49, 527–535.

Poirier, V., Angersa, D.A., Whalen, J.K., 2014. Formation of millimetric-scale aggregates and associated retention of ^{13}C-^{15}N-labelled residues are greater in subsoil than topsoil. Soil Biol. Biochem. 75, 45–53.

Powlson, D.S., Glendining, M.J., Coleman, K., Whitmore, A.P., 2011. Implications for soil properties of removing cereal straw: results from long-term studies. Agron. J. 103, 279–287.

Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101, 133–149.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269, 341–356.

Sauerbeck, D.R., 2001. CO_2 emissions and C sequestration by agriculture - perspectives and limitations. Nutr. Cycl. Agroecosyst. 60, 253–266.

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Sci. Soc. Am. J. 62, 1367–1377.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155–176.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31.

Stewart, C.E., Plante, A.F., Paustian, K., Conant, R.T., Six, J., 2008. Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci. Soc. Am. J. 72, 379–392.

Vance, E., Brookes, P., Jenkinson, D., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008. Stabilization mechanisms of organic matter in four temperate soils: development and application of a conceptual model. J. Plant Nutr. Soil Sci. 171, 111–124.

Wang, H., Boutton, T.W., Xu, W., Hu, G., Jiang, P., Bai, E., 2015. Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. Sci. Rep. 5, 10102.

Webster, R., 2007. Analysis of variance, inference, multiple comparisons and sampling effects in soil research. Eur. J. Soil Sci. 58, 74–82.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass, by fumigation-extraction — an automated procedure. Soil Biol. Biochem. 20, 1167–1169.

Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biol. Biochem. 25, 1435–1441.

Xiao, C., Guenet, B., Zhou, Y., Su, J., Janssens, I.A., 2015. Priming of soil organic matter decomposition scales linearly with microbial biomass response to litter input in steppe vegetation. Oikos 124, 649–657.