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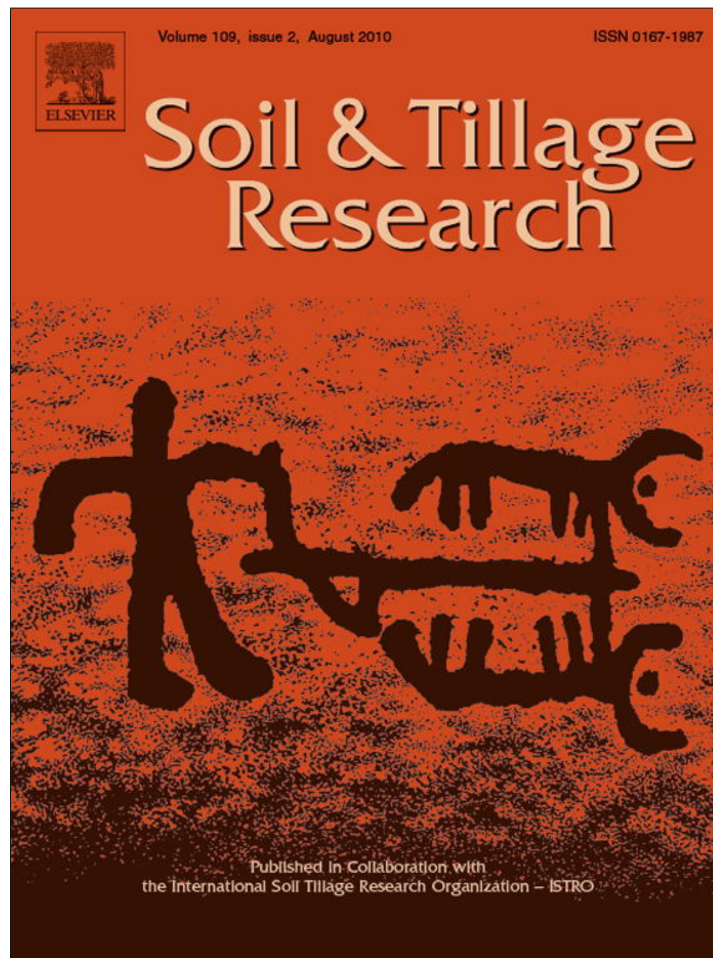

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Soil & Tillage Research 109 (2010) 94–102

Contents lists available at ScienceDirect

Soil & Tillage Research

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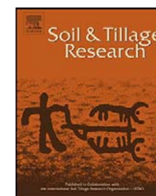

Effect of fertilization on decomposition of ^{14}C labelled plant residues and their incorporation into soil aggregates

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ARTICLE INFO

Article history:
Received 8 January 2010
Received in revised form 4 May 2010
Accepted 10 May 2010

Keywords:
Aggregate size fraction
^{14}C-labelled plant residue
Soil organic matter
Decomposition
CO_2 efflux
C sequestration
Microbial biomass

ABSTRACT

Returning crop residues to soil helps to maintain soil C stocks. Organic C stocks and microbial biomass are important factors controlling the decomposition or retention of crop residues in soil and the formation of aggregates. Little is known about the specific contribution of crop residues to soil aggregate size fractions in the framework of long-term fertilization. This study investigated the effects of long-term fertilization on the decomposition of ^{14}C-labelled plant residues and their incorporation into soil organic matter (SOM) of different aggregate size fractions. Soils were collected from 0–10 cm in the Ap horizon of a long-term (since 1988) field experiment at Grossbeeren (Germany). The following four fertilization treatments were used: 1) without fertilization or manuring (Control), 2) nitrogen applied by mineral fertilizer (N), and 3) manure with low (M) and 4) high (2 M) application doses. Soils were incubated for 100 days at 20 °C, with or without ^{14}C-labelled plant residue. The incorporation of ^{14}C into three aggregate size fractions–large macroaggregates (2–1 mm), small macroaggregates (1–0.25) and microaggregates (<0.25 mm)–was analyzed.

After 15 days of incubation, 44–57% of plant residue was mineralized in the order: M > N > control soil > 2 M. Adding plant residues increased soil β-glucosidase activity and microbial biomass C. On day 16 of incubation, more residue ^{14}C was retained in small and large macroaggregates than in microaggregates in the control soil. In contrast, in fertilized soils the reverse was measured. Additionally, N, M and 2 M soils showed significant differences by incorporation of ^{14}C in microbial biomass and β-glucosidase activity in different aggregate size fractions. The results imply that long-term fertilization significantly increased the residue ^{14}C retention in microaggregate size fractions and its decomposition in soils.

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1. Introduction

Land-use is the most important factor affecting the soil organic carbon (C_{org}) stock. The crop residue inputs in soil influence aggregate structure by changing C_{org} (Tisdall and Oades, 1982; Haynes and Swift, 1990; Hulugalle and Cooper, 1994). At the same time, aggregation affects C_{org} storage by occluding organic materials, making them inaccessible to degrading organisms and their enzymes (Sollins et al., 1996). Tillage disruptions of soil aggregates, however, expose such physically protected organic material (Paustian et al., 2000; Six et al., 2000) and enhance C mineralization and/or CO_2 fluxes (Elliott, 1986; Reicosky et al., 1995). Therefore, understanding the key factors and processes controlling aggregation and C_{org} storage and turnover is critical because any changes in C_{org} could significantly affect the CO_2 levels in the atmosphere.

Various fertilizer inputs such as mineral fertilizers and manure are used mainly to increase crop yield and thus the amount of crop residue, much of which is returned to the soil. Therefore, intensive crop cultivation with high inputs of organic fertilizers results in high organic matter input, which acts as a binding agent for aggregate formation. Aggregates are composed of mineral particles and binding agents (Tisdall and Oades, 1982; Haynes and Swift, 1990) and the initial unit is termed microaggregate. According to Tisdall and Oades (1982), soil microaggregates (<250 μm) are bound together by organic compounds of different origin to form stable macroaggregates (>250 μm). Fresh organic materials such as plant residues have abundant readily decomposable C. They therefore increase aggregate stability (De Gryze et al., 2005; Abiven et al., 2007) by enriching young organic matter in macro- versus microaggregates (e.g. Puget et al., 1995; Jastrow et al., 1996; Six et al., 2000; John et al., 2005; Yamashita et al., 2006; Helfrich et al., 2008). Little information, however, is available on how and to what extent plant residues influences aggregation and on the partitioning of recently added C within aggregate size fractions during decomposition. One of our hypotheses is that the high manure doses accelerate C stabilization in soil more than the average dose.

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doi:10.1016/j.still.2010.05.003

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The first aim of the present study was therefore to gain quantitative knowledge about the stabilization of added plant residues in soil aggregates. This information is necessary to understand, assess and predict the effects of plant residue on C storage in aggregate size fractions.

Soil microbes decompose added plant residues. These microbes derive their energy by oxidizing organic C. The plant residue C passes through the microbial biomass at least once as it is transferred from one C pool to another and finally mineralized to CO_2 (Ryan and Aravena, 1994). The remaining microbially undecomposed part of the plant residues contribute to the soil C stock (Lorenz and Lal, 2005). Land-use management with fertilization or manuring affects the residue decomposition rate and thus C_{org} turnover (Paustian et al., 1997). This requires knowledge about the decomposition mechanism of the added plant residue as influenced by long-term fertilizer management strategies. This, in turn, is necessary to identify the pathways of C sequestration in soils. In order to differentiate and quantify C decomposed from added plant residue, ^{14}C-labelled plant residues were used in an incubation experiment with soils from a long-term cultivated experiment with different land-use practices. We investigated aggregate formation during litter decomposition and the role of newly formed aggregates in storing plant-derived C. This study therefore (i) evaluates the long-term management effect on plant residue decomposition, (ii) assesses the plant residue C stability in soil by analyzing soil microbial biomass and enzyme activity, (iii) assesses the amount of stabilized plant residue C added to aggregate size classes, and (iv) quantifies the relationship between plant residue C input and C sequestration in soil.

2. Materials and methods

2.1. Soil and long-term field experiment

Soil samples were taken in January 2008 from the 0–10 cm layer of a sandy Cambisol at the experimental plots of the long-term field trial "Trasse 2" of the Institute of Vegetable and Ornamental Crops (IGZ), Grossbeeren, Germany (52°20′56.70″ N, 13°19′07.90″ E). The climate conditions (1973–2002) of the site are characterized by a mean annual temperature of 8.8 °C and an average rainfall of 520 mm y^{-1} plus 150 mm y^{-1} irrigation water. The soil pH ($CaCl_2$) was 6.6, and sand and clay contents of the soil were 81 and 5%, respectively. The soil had a bulk density of 1.59 g cm^{-3}.

Soil samples were selected from four treatments having a different fertilization history during 20 years: 1) control soil (without mineral and manure fertilization), 2) with mineral nitrogen application 270 kg N ha^{-1} a^{-1} (N), 3) farmyard manure (FYM) application 30 t ha^{-1} a^{-1} (M) and 4) FYM application 60 t ha^{-1} a^{-1} (2 M). Before ploughing, the respective amounts (30 and 60 t ha^{-1}) of well-decomposed FYM was applied to the specified plots (size: 4.5 m x 5.0 m) of the experiment. During the experimental period, the mean dry matter concentration of fresh FYM was 0.22 g g^{-1} and the mean C content was 0.31 g g^{-1} (oven-dry basis). Mineral N fertilizer was applied as calcium ammonium nitrate at a high N level corresponding to 270 kg N ha^{-1} a^{-1}. The crops grown annually were white cabbage (*Brassica oleracea* L. var. capitata f. alba), carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), leek (*Allium porrum* L.), and celery (*Apium graveolens* L. var. rapaceum Mill.). At harvest, all the above-ground plant material (yield plus crop residue) was removed from the plots. After sampling, the soil was air-dried (30 °C), thoroughly mixed and sieved (2 mm), after which all visible roots were carefully removed both with the electrostatic method (Kuzyakov et al., 2001) and manually by tweezers.

2.2. Incubation

The laboratory incubation was conducted in closed vessels at 20 °C for 100 days. Forty grams of air-dried soils were weighed into 250 ml glass vessels (Schott Duran, Mainz, Germany). The experiment was set up with eight treatments in triplicates including two factors. The first factor was four land-use managements: C, N, M and 2 M (described above). The second factor was ^{14}C-labelled plant residue additions: No residue (- residue) or 20 mg ^{14}C-labelled plant residue (+ residue) (36.5% C and 2.9% N) ground with a ball mill (Retsch) were added and thoroughly mixed with the soil of the four land-use treatments. The ^{14}C labeled residues (22 Bq mg^{-1}) were produced by labelling *Lolium perenne* by 7 pulses in $^{14}CO_2$ atmosphere (for details see Kuzyakov et al., 2002). The soil moisture was kept at 70% of its water holding capacity (24.4%) with deionized water. Small vials with 3 ml of 1.0 M NaOH were placed in the vessels to trap CO_2. The traps were changed daily in the initial days and then 3 times per week throughout the incubation period. Additional triplicate blank vessels containing only the vials with NaOH served as controls to account for the CO_2 trapped from the air inside the vessels.

2.3. Aggregate-size fractionation at optimal soil moisture

Before incubation and sixteen days after the start of incubation, the soil samples were prepared for aggregate fractionation. Aggregates were isolated according to Kristiansen et al. (2006): 40 g were transferred to a nest of sieves (1 and 0.25 mm) and shaken for 90 s and the 1–2 mm aggregates were collected (large macroaggregates). The same procedure was done for the material retained on the 0.25 mm sieve, isolating the 1–0.25 mm aggregate-size class (small macroaggregates). The remaining material passed through the 0.25 mm sieve was identified as the <0.25 mm aggregate class (microaggregates). The recovery after sieving was about 98% of soil weight (Table 1). Preliminary tests showed that the sieving duration was sufficient to quantitatively separate the various aggregate-size classes while minimizing aggregate abrasion during sieving (Dorodnikov et al., 2009).

Table 1
Aggregate size fractions of the soil as depending on 20 years of fertilization (0–10 cm) at the long-term field trial Trasse-2 in Grossbeeren.

Aggregate size class	Control[a]	N	M	2 M
	% of soil mass			
Large macroaggregates: 1–2 mm	16.4 ± 2.0	12.8 ± 1.6	16.1 ± 1.0	16.5 ± 3.9
Small macroaggregates: 1–0.25 mm	35.1 ± 1.5	44.8 ± 0.1	42.1 ± 3.0	51.1 ± 1.4
Microaggregates: <0.25 mm	47.6 ± 1.3	41.1 ± 1.6	40.4 ± 2.1	31.7 ± 5.3
Sum of three fractions	99.0	98.7	98.5	99.2

N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage.
[a] mean ± standard deviation.

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2.4. C_{org} and ^{14}C analyses

The total C content (C_{org}) was determined by dry combustion with a LECO CN2000 analyzer. As the soil is carbonate free, total C was equivalent to soil organic carbon.

The ^{14}C content of plant residues and of the soil at the end of the experiment was determined after combustion of 1 g of sample within an oxidizer unit (multi N/C 2100; Analytik Jena, Germany) by absorbing ^{14}C in NaOH. Thereafter, the ^{14}C activity in NaOH solution was measured by adding 2 ml scintillation cocktail Rothiscint-22x (Roth Company, Germany) to 1 ml aliquot of NaOH after the decay of chemiluminescence.

2.5. Microbial biomass carbon and CO_2 efflux

Soil microbial biomass of bulk soil and isolated soil aggregates was determined by the chloroform fumigation-extraction method (modified from Vance et al., 1987). Each replicate was divided into two equivalent portions; one was fumigated for 24 h with ethanol-free chloroform and the other was the unfumigated control. Both fumigated and unfumigated soils were shaken for 30 min with 0.05 M K_2SO_4 (1:4 soil: extraction ratio), centrifuged at 3000 rpm for 10 min and filtered. Extracts were analyzed for total organic carbon and total N using the 'multi N/C 2100′ analyzer. Microbial biomass C was calculated using a K_C value of 0.45 for C and ^{14}C (Wu et al., 1990) and was presented as a percentage of 1 g dry soil. The soil water content was determined in another 1 g of soil dried at 105 °C.

To estimate total CO_2 efflux, the CO_2 trapped in 1.0 M NaOH solution was precipitated with 0.5 M barium chloride ($BaCl_2$) solution and then the NaOH was titrated with 0.1 M hydrochloric acid (HCl) against phenolphthalein indicator (Zibilske, 1994). The ^{14}C activity in CO_2 collected in NaOH solution was measured by adding 2 ml scintillation cocktail Rothiscint-22x (Roth Company, Germany) to 1 ml aliquot of NaOH after the decay of chemiluminescence. The ^{14}C counting efficiency was about 87% and the ^{14}C activity measurement error did not exceed 2%. The CO_2 efflux was measured daily during the first 4 days, every 2–3 days for the next 10 days, and weekly thereafter.

2.6. β-Glucosidase activity

Enzyme activities in bulk soil and isolated aggregates were measured using fluorogenically labelled substrates according to a modified technique described in Dorodnikov et al. (2009). Briefly, fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used: MUF-β-D-glucopyranoside (MUF-G; EC 3.2.1.21) to detect β-glucosidase activity. To dissolve the MUF-substrates, 2 mL of 2-methoxyethanol (Hoppe, 1983) were used. Predissolved MUF-substrates were further diluted with sterile distilled water to give the desired concentrations. All chemicals were purchased from Fluka (Germany).

The soil samples (1 g) were suspended in water (20 mL) and shaken on an overhead shaker for 15 min at room temperature and at maximum speed to ensure thorough mixing. A subsample of the soil suspension (1.0 mL) was added to the 3 mL MUF-substrate solution (containing either 400 μmol MUF-G), already pipetted in deep-well plates (24-wells x 10 mL, HJ-Bioanalytik GmbH, Germany), and incubated at 20 °C for 1 h. The incubation temperature was based on the average annual soil temperature of the experimental site (S. Marhan, personal communication). The calibration solutions were prepared using soil suspension (1 mL) and MUF of different concentrations (0–100 μmol, 3 mL). Deep-well plates with the soil-MUF-substrates and soil-calibration-MUF concentrations were centrifuged (20 g, 5 min). Thereafter, 0.5 mL of supernatant was pipetted to the 24-well microplates (Becton Dickinson, USA). These microplates contained 1.25 mL sterile distilled H_2O and 0.25 mL of 20 mmol glycine-NaOH buffer solution (pH 11) to stop enzyme reactions. Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width 25 nm, with a Victor[3] 1420–050 Multilabel Counter (PerkinElmer, USA). Enzyme activities were expressed as MUF release in micromoles per gram bulk soil/aggregate dry weight and hour ($\mu mol\ g^{-1}\ h^{-1}$).

2.7. Estimation of labile and refractory (stabile) pools

The decomposition of the ^{14}C-labelled plant residue was also estimated by the double exponential model.

$$^{14}CO_2 = C_{lab} * (1 - e^{-k1t}) + C_{re} * (1 - e^{-k2t}) \tag{1}$$

where $^{14}CO_2$ is the cumulative ^{14}C-CO_2 released (% of input ^{14}C) by time t; C_{lab} and C_{re} are the sizes of the labile and refractory C pools (% of plant residue ^{14}C; $C_a + C_b = 100\%$), respectively; k1 and k2 are the mineralization rate constants for each pool; and t is the incubation period.

To determine the fertilization effect on plant residue mineralization rates k1 and k2, C_{lab} had been taken as an average of the calculated values derived from the double exponential model, i.e., 50% of residue C.

2.8. Statistical analysis

A non-linear least-squares regression analysis was used to calculate parameters from cumulative C mineralization data (Eq. (1)) (Software STATISTICA 6.0, StatSoft Inc.). The comparison of model fits was evaluated by the coefficient of determination, R^2. The decomposition parameters were compared for the tested land-use types.

The cumulative $^{14}CO_2$ efflux and C mineralization parameters from the double exponential model (Eq. (1)) were subjected to one-way analysis of variance to test for significant differences between the treatments. Differences were considered significant at $p < 0.05$.

The effects of fertilization on C_{org}, ^{14}C, MBC content of bulk soil and the three aggregate size classes were subjected to one-way ANOVA to test for significance of differences between the treatments. Differences were considered significant at $p < 0.05$. The results presented are arithmetic means and standard error (SE) of three replicates.

3. Results

3.1. Decomposition of added plant residue in differently fertilized soils

Adding plant residues significantly ($p < 0.05$) increased the cumulative CO_2 emission over the 104 d of incubation for all fertilized and control soils (Fig. 1). The CO_2 efflux for plant residue addition varied slightly within the treatments and followed the order: M > N > control >2 M.

Decomposition of added plant residue started immediately after adding water at the beginning of incubation. The applied plant residue-^{14}C was mineralized by 32.1 to 41.9% and 44 to 57% after 3 and 15 days of incubation, respectively (Fig. 2). The subsequent decomposition was slow. The mineralization rate decreased ten times from day 15 to day 100 of incubation.

Soil fertilization significantly influenced the $^{14}CO_2$ efflux from decomposition of the plant residues ($p < 0.05$, Fig. 2). After 100 days, the cumulative $^{14}CO_2$ amounted to 52.3 to 66.0% of the ^{14}C input and was in the order: M > N > control soil > 2 M (Fig. 2). However, the $^{14}CO_2$ release decreased sharply from 0–15 days and,

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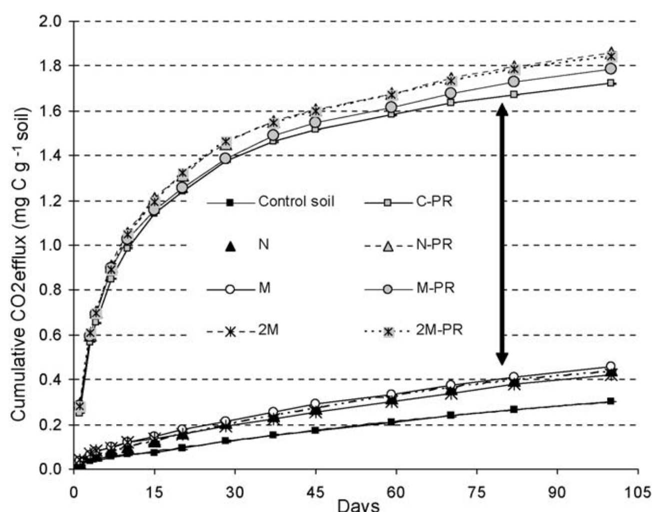

Fig. 1. Cumulative CO_2 emission from soil of different management practices (C-PR: control soil with plant residue; N and N-PR: mineral (N) fertilized soil before and after plant residue addition; M and M-PR: manure amended soil before and after plant residue addition, 2 M and 2M-PR: manure amended in double manure dosage before and after plant residue addition) (Standard errors are less than symbol size (n = 4).

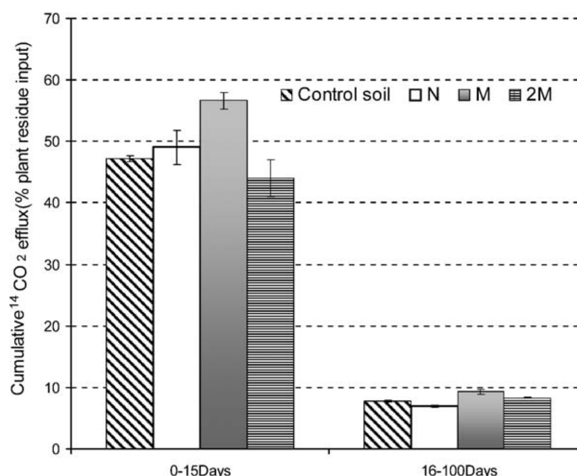

Fig. 3. Cumulative $^{14}CO_2$ emission during two decomposition phases depending on soil fertilization. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage). (*error bars* represent the standard error of mean).

thereafter, became nearly constant for all fertilized and control soils (Fig. 3).

The labile or easily decomposable part of the plant residues (C_{lab}) mineralized strongly at the initial phase of incubation. The C_{lab} content of the added residues as estimated by the double exponential model was almost same for all the soils. This is because residue lability does not depend on soil properties. The average constant value was taken (50% of plant residue) to calculate decompositions rates. The decomposition rates of C_{lab} (k1) as estimated by the double exponential model was the lowest in 2 M soil and increased in the order, 2 M > control soil > N > M. The corresponding half-lives were 2.6, 2.1, 1.7 and 1.3 days (Tab. 3). Moreover, decomposition rates of C_{re} (k2) differed significantly among the soils fertilized with mineral N and organics ($p > 0.05$, Tab. 3); the rate varied from 0.0010 to 0.0054 d^{-1}, with an average half-life of 571 days.

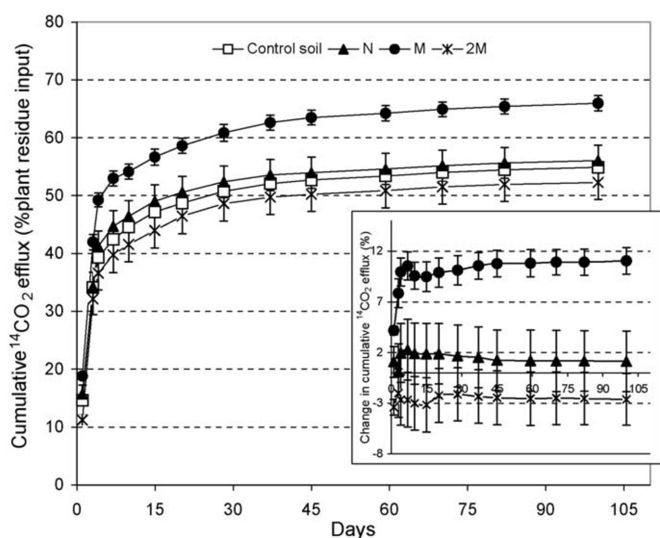

Fig. 2. Cumulative $^{14}CO_2$ emission from differently fertilized soils. The inset shows effect of fertilization (Fertilized – control soil) on ^{14}C labelled added plant residue decomposition. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage). (*error bars* represent the standard error of mean).

3.2. Fertilization and plant residue effect on microbial biomass (MB) and β-glucosidase activity

The lowest MB was observed in the control soil (21.7 mg C kg^{-1}) and it increased with the mineral fertilizer (36 mg C kg^{-1} for N) and manure amendments (48 mg C kg^{-1} for M and 54 mg C kg^{-1} for 2 M) (Fig. 4). The contribution of the MB carbon (MBC) towards C_{org} ranged from 0.4–0.6% within the treatments.

Adding plant residues significantly ($p < 0.05$) increased the MBC content of soils. After 15 days of incubation with residues, the MBC content was highest in M (194 mg C kg^{-1} soil) followed by control soil > 2 M > N (Fig. 4). The total MBC is partly derived from SOC and partly from the added ^{14}C labelled residue (MB-^{14}C). 1.0 and 2.4% of the residue-^{14}C was incorporated into MB in N fertilized and control soils respectively and followed by 1.7 and 2.4% for M and 2 M soils respectively(Fig. 4). This fertilization effect on MB-^{14}C was also distinct amongst the aggregate size fractions (Fig. 5). MB-^{14}C was significantly ($p < 0.05$) higher in microaggregates than in large and small macroaggregates in all soils. The exception was the high manure amended soil (2 M), where MB-^{14}C was best conserved in small macroaggregates.

Soil β-glucosidase activity increased significantly ($p < 0.05$, Fig. 6) in the order of M > N > 2 M > control soil after addition of

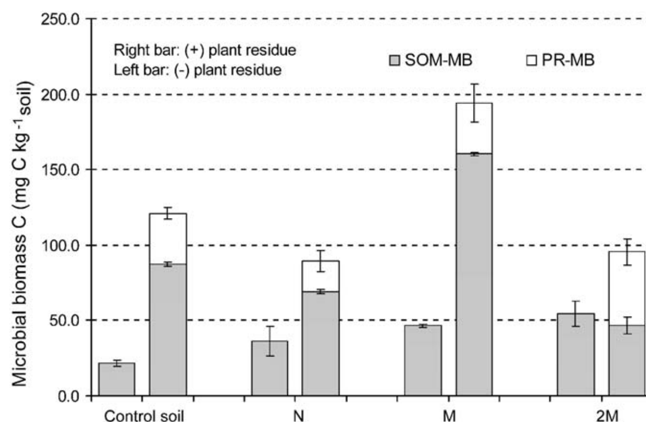

Fig. 4. Plant residue effect on MBC of differently fertilized and unfertilized soils. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage). (*error bars* represent the standard error of mean).

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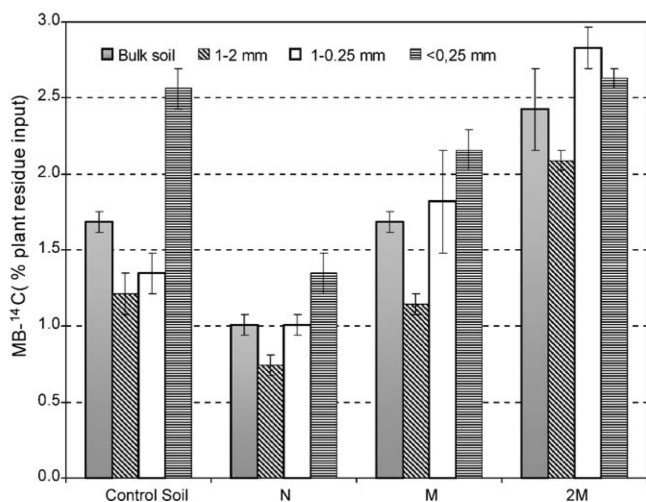

Fig. 5. Microbial biomass- ^{14}C (MB-^{14}C) content in different aggregate size fractions of fertilized and unfertilized soils. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage). (*error bars* represent the standard error of mean).

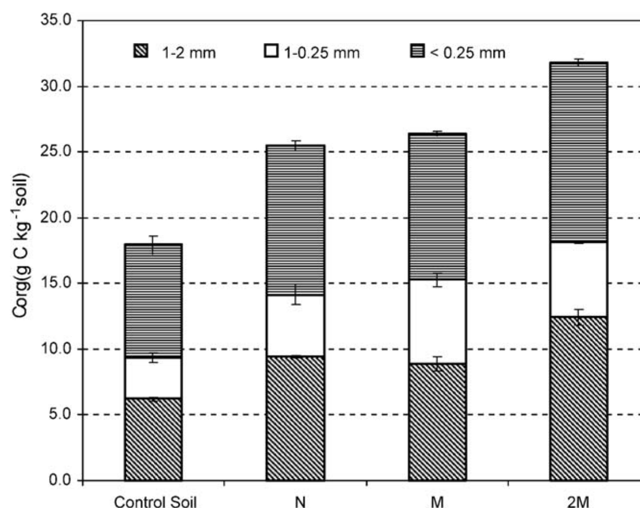

Fig. 7. Total soil organic C (C_{org}) distribution in different aggregate size fractions of differently fertilized soils. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage).

residues. Plant-residue-induced soil glucosidase activity was lower in the N soil, followed by manure-amended (M and 2 M) soils. Accordingly, the plant residue effect on microbial biomass and soil glucosidase activity was more pronounced in microaggregates than in small and large macroaggregates of soils.

3.3. Effect of fertilization on plant residue ^{14}C incorporation in different aggregate size fractions

The studied sandy soil without fertilization had a low C_{org} content (0.58%). The mineral and organic fertilization since 1988 led to significant differences in C_{org}. Thus, the C_{org} content in N, M and 2 M fertilized treatments increased by 42.1, 53.2 and 72.6%, respectively, over the control (Fig. 7). This fertilization effect on the C_{org} content significantly affected the retention of the newly added plant residue C. After 15 days of incubation, 37.2–50.2% of added plant residue-^{14}C remained in the soils, the order being control soil > 2 M > N >M (Fig. 8). In control soil, ^{14}C was incorporated almost evenly in large and small macroaggregates and microaggregates. Mineral (N) and manure (M and 2 M) fertilized soils had more plant residue C (^{14}C) retained in microaggregates than in large and small macroaggregates.

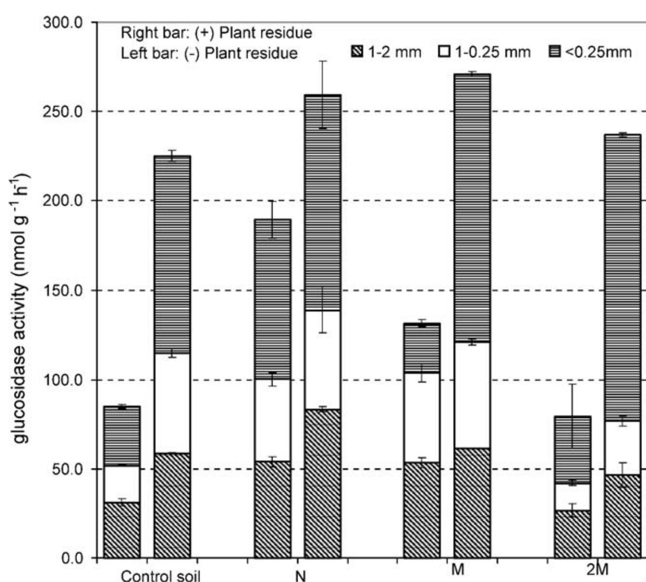

Fig. 6. Plant residue effect on glucosidase activity in differently fertilized and unfertilized soils. (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage). (*error bars* represent the standard error of mean).

3.4. ^{14}C budget

After 15 days of incubation, 44–57% of plant residue-^{14}C was decomposed by microorganisms and released as CO_2. Some of the ^{14}C (37–50%) was retained in soil, and only a small portion (1.0–2.4%) was incorporated in microbial biomass in all the fertilized and control soils. Total plant residue ^{14}C recovered from soil aggregates, microbial biomass and CO_2 efflux ranged from 88–97%.

4. Discussion

4.1. Mineralization of C_{org} and added plant residues in differently fertilized soils

Throughout the incubation period the cumulative CO_2 emission was 5.0–5.3% of the $C_{org,}$ and adding the plant residues increased the emission by 8.0 to 12.2 times (Fig. 1). Low clay content (5%) of

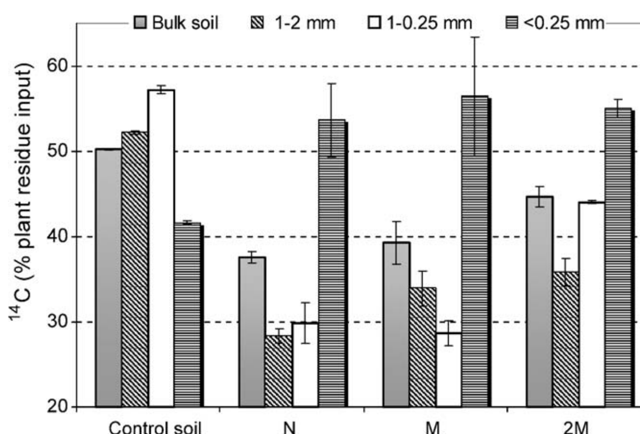

Fig. 8. Percentage of plant residue ^{14}C retained in aggregate size fractions of fertilized and unfertilized soils (N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage).

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the experimental soil may promote the decomposability of C_{org} because clay-rich soil shows low respiratory losses of C per unit of MBC (Kaiser et al., 1992). The residue effect on the total CO_2 efflux was only slightly affected by soil fertilization and was sustained up until the exhaustion of the labile residue fractions. Thereafter, the constant CO_2 emission rate in all soils may be attributable to the resistant and stable fraction of both soil organic matter (SOM) and the residues (Ellert and Bettany, 1988; Wander et al., 1994).

The decomposition of added residues, measured as $^{14}CO_2$ efflux from the soil amended with plant residue-^{14}C, showed two distinctive phases (Fig. 3). In the first phase (0–15 days), non-cellulosic polysaccharides, proteins and hemicellulose–readily available to microorganisms–were decomposed (Alvarez et al., 1995, Lorenz and Lal, 2005). During the second phase (16–100 days), more stable forms of plant C such as lignin and cellulose were degraded (Lorenz and Lal, 2005) (Fig. 3). Swift et al. (1979) reported that residues contain up to 25% soluble, readily decomposable compounds and accordingly the mineralization rate of such soluble substrate-derived ^{14}C was highest during the first 3 days of incubation (Chotte et al., 1998). In our experiment, after 3 days of incubation, 32 to 42% of the ^{14}C input from the plant residues was mineralized in the soil; the values were 44–56% by day 15. Thereafter, slow decomposition in the second phase (16–100 days) indicated an altered substrate quality. The decomposition (7.0–9.3% of ^{14}C input) in the second phase reflects a progressive loss of the less biologically labile components and a subsequent increase in the proportion of more resistant components in the remaining plant material (Wang et al., 2004). Finally, the un-decomposed plant residues and the plant C that passed through microbial biomass contributed to the stable C_{org} compounds in each soil (Bottner et al., 1998). At the end of the 100 day incubation, 52 to 66% of ^{14}C input was evolved as $^{14}CO_2$ (Fig. 2).

The only differentiating factor for plant residue decomposition and $^{14}CO_2$ efflux was the long-term fertilization and manure effect on soils. These followed the trend of M > N > control soil > 2 M throughout the incubation. Manure contains numerous forms of labile C (e.g. carbohydrates, fatty acids, amino acids and peptides) that can stimulate further growth of the soil microbial biomass (Paul and Beauchamp, 1989; Sørensen, 1998). After cessation of manure application, its cumulative effect can persist for several years (Ginting et al., 2003). Nonetheless, we found a different result for 2 M versus M soil. The quantity and quality of organic inputs are crucial factors affecting not only microbial biomass and community structure (Peacock et al., 2001) but also enzyme activities (Acosta-Martínez et al., 2007). The higher manure dose throughout 20 years of cropping (2 M soil) altered the microbial population and thus lowered the decomposability of plant residues by microorganisms. Several studies (Waldrop et al., 2004; Knorr et al., 2005, Chen et al., 2009) showed that increased N availability in soil accelerates the decomposition of rapidly decomposing plant substrates but retards that of slowly decomposing materials (e.g. lignin). As a result, 49% of the added plant residue-^{14}C in N soil decomposed within the first 15 days of incubation but showed a lower $^{14}CO_2$ efflux value in the second phase of decomposition (Fig. 3).

4.2. Amounts of labile and stable components in plant residues and their decomposition rates

The double exponential model (Eq. (1)), widely applied to predict C mineralization of soil organic matter or plant materials (Bottner et al., 1998; Wang et al., 2004, Chen et al., 2009), describes the decomposition of two pools of plant residue-C with different degradation rates. One is the labile pool (C_{lab}) with a high degradation rate (k1) and the other is the refractory pool (C_{rec}),

Table 2
^{14}C-labelled plant residue mineralization parameters using a double exponential model ($C_{cum} = C_{lab}(1-e^{-k1t}) + C_{re}(1-e^{-k2t})$, $C_{lab} + C_{re} = 100\%$).

Land use	K1 (d^{-1})	$T_{1/2}$ of C_{lab} (day)	K2 (d^{-1})	$T_{1/2}$ of C_{re} (day)
Control soil	0.33 ± 0.02	2.09 ± 0.08	0.0010 ± 0.0003	695.2 ± 110.0
N	0.42 ± 0.02	1.65 ± 0.04	0.0023 ± 0.0002	305.6 ± 14.3
M	0.55 ± 0.07	1.26 ± 0.09	0.0054 ± 0.0005	128.2 ± 7.4
2M	0.27 ± 0.02	2.55 ± 0.14	0.0006 ± 0.0003	1155.7 ± 621.0

N: mineral (N) fertilized soil, M: manure amended soil, 2 M: manure amended in double manure dosage; C_a and k1 and C_b and k2 = amount and decay rate of labile and resistant C pool; t = time (d); $T_{1/2}$ = half life of C_a or C_b

which is more resistant to microbial attack and has a low decomposition rate (k2). The rates (k1 and k2) are important in estimating the half-lives of mineralization listed in Tab. 3.

The labile carbon pool is the fraction of SOC which has the most rapid turnover rates. Its oxidation drives the CO_2 flux from soils to the atmosphere. This pool is important as it fuels the soil food web and therefore greatly influences nutrient cycling; this helps maintain soil quality and productivity (Chan et al., 2001). However, the decomposition of the C_{lab} and C_{re} pools of plant-residue C was different in the differently fertilized soils. Several studies have already established that the soil microbial population and activities change with the long-term application of N or manure fertilizers. Consequently, this microbial diversity contributed to plant residue decomposition by changing its susceptibility toward the labile pool of the residues. As a result, the long-term manure effect was the highest for the decomposition rate of the C_{lab} pool (k1) of plant residue C in M soil, followed by N, control soil and 2 M (Table 2). Moreover, a significant difference was observed in the decomposition rates (k2) of the refractory pool (C_{re}) and in the corresponding half-lives of the mineral (N) and manure (M and 2 M) amended soils. This indicates that the decomposition of the C_{re} fraction was essentially controlled by the long-term fertilization. The lower decomposition rates (k2) of the refractory C pool may be associated with a more efficient C storage because soil microorganisms may decompose less of the plant substrate (Smith, 1994).

4.3. Soil microbial biomass and glucosidase activity

Manure amended (2 M and M) soils showed higher MBC than N or control soils (Fig. 4). Organic amendments increase MB (Dhillion, 1997; Hu et al., 1999, García-Gil et al., 2000; O'Donnell et al., 2001) by supplying labile C substrate, thus increasing the C_{org} of soils. In the studied soil, mineral fertilizer slightly increased MB compared to the control. Majumder et al. (2008) also found some positive effect of long-term fertilization (N-P-K) on MBC, but several studies (Fließbach and Mäder, 2000; Černý et al., 2008) have observed the reverse.

The most labile part of plant residue C (e.g., hemicelluloses, pectin and non-cellulosic polysaccharides), readily available to microorganisms (Alvarez et al., 1995, Lorenz and Lal, 2005), is an important C and energy source for microorganisms. This can stimulate the growth of the soil microbial biomass (Paul and Beauchamp, 1989; Sørensen, 1998) by assimilating part of the plant C into soil MB (MB-^{14}C). In the present experiment, 1.0 to 2.4% of the input plant residue ^{14}C was conserved into microbial biomass after 15 days of incubation (Fig. 5). Moreover, adding plant residues to soil increased MBC from 1.7 to 5.4 times than that of the unamended soils (Fig. 4). Plant residue C passes through the MB at least once as it is transferred from one C pool to another (Ryan and Aravena, 1994). Our results thus indicated that the plant residue C utilization efficiency of soil organisms was enhanced by increased manuring (M to 2 M) (Fig. 5). The long-term mineral N fertilization

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decreased microbial biomass, microbial activity and diversity (Compton et al., 2004; Chen et al., 2009) in soil, which may decrease the incorporation of plant residue C into MB.

Previous research (Santrucková et al., 1993; Schutter and Dick, 2002; Chotte et al., 1998; Guggenberger et al., 1999) has demonstrated a heterogeneous distribution of MB among aggregate-size classes. This heterogeneity might reflect differences in aggregate structural properties that selectively or generally restrict nutrient transfer (Mendes et al., 1999). This would lead to different MB-^{14}C values (Fig. 5). The different aggregate separation procedures, e.g., wet and dry sieving, may help to explain the different MB results (Dorodnikov et al., 2009). According to Ashman et al. (2003), fractionation is clearly a destructive procedure, and some microorganisms are transferred from the surfaces of larger to smaller aggregates (dry sieving) or adhere to aggregates during slaking in water (wet sieving), persisting in macroaggregates. In this context, the approach we used (optimal moist sieving approach) was less destructive (Kristiansen et al., 2006) by enabling a high recovery of microbial biomass (Dorodnikov et al., 2009). Accordingly, we observed that MB-^{14}C was high in microaggregates due to decreased decomposition of plant residue-^{14}C in microaggregates as also reported by Kong et al. (2005). Similarly, less MB-^{14}C in large macroaggregates than in small macroaggregates and microaggregates (Fig. 5) points to faster decomposition of plant residue in macroaggregates. Considering the fertilization effect on soil, higher MB-^{14}C in both macro- and microaggregates in 2 M and control soil versus M and N soils supported the findings of lower cumulative $^{14}CO_2$ emission from 2 M and control soils for 0–15 days of incubation (Fig. 3).

Residue decomposition can also be explained by enzyme activity, which is used as an index of microbial functional diversity (Nannipieri et al., 2002). Amongst the enzymes, β-glucosidase is the most abundant and easily detectable enzyme, involved in plant component (cellulose) degradation (Turner et al., 2002). It also provides an early indication of changes in organic matter status and turnover (Bandick and Dick, 1999; Debosz et al., 1999; Monreal and Bergstrom, 2000). Thus, plant substrate significantly ($p < 0.05$) influenced glucosidase activity after 15 days of incubation, depending on the aggregate size fractions in the soil (Fig. 6). Adding residues promoted glucosidage activity more in microaggregates than in large and small macroaggregates. This supported the findings of quantitative and qualitative C_{org} differences between the two aggregate sizes classes (Jastrow et al., 2007) due to residue incorporation. In this context, high MB-^{14}C in microaggregates supports the high glucosidase activity, which accelerates further residue decomposition in microaggregates compared to other fractions after 15 days of incubation. This heightened glucosidase activity may be depleted when the microbially available part of the residue is exhausted (Stemmer et al., 1998). Thus, residues in macroaggregates were decomposed by 15 days, whereas residues in microaggregates were still susceptible for further decomposition.

The specific activity of the microbial biomass, an indicator of the microbial decomposability of added plant residues, is expressed by the soil enzyme activity per unit MBC. Applying this concept, the lower 'glucosidase activity per unit MBC' in microaggregates and small macroaggregates of control soil indirectly confirms the low microbial decomposability of plant residues after 15 days. In contrast, higher specific activity was observed in microaggregates in M and 2 M soils, where MB-^{14}C was significantly more abundant in micro- than in macroaggregates. Mineral fertilizer accelerates MB (Majumder et al., 2008). This potentially enhances the glucosidase activity, producing a higher specific activity in macroaggregates.

4.4. Effect of fertilization on plant residue-^{14}C retention

For different soils having the same silt and clay composition, the C_{org} content is the determining factor for input C retention (Stewart et al., 2008). Thus, as in the case of control soil, long-term cultivation without fertilization caused a net depletion of the C_{org} content of soil (Majumder et al., 2007). As a result, after 15 days of incubation, more plant residue-^{14}C was retained in control versus fertilized soils. On the other hand, less (37.5 and 39.3%) ^{14}C retention in N and M soils supported a higher $^{14}CO_2$ mineralization rate due to higher microbial activity than that of control and 2 M soils. The reason behind the high ^{14}C retention in 2 M soil (having lower $^{14}CO_2$ emission) is still unexplained.

The distribution of plant residue-^{14}C in different aggregate sizes was significantly affected by soil fertilization (Fig. 8). Soils having more C_{org} due to long-term fertilization conserved more plant residue C in micro- than in macroaggregates. Our observation that newly added ^{14}C was retained in microaggregates was similar to other findings (Kristiansen et al., 2006; Bossuyt et al., 2002). In contrast to fertilized soils, the plant-derived ^{14}C in unfertilized control soil was higher in the small and large macroaggregates than microaggregates. Added residues in control soil might act as a binding agent by forming macroaggregates (Elliott, 1986; Golchin et al., 1995) and might be more resistant to microbial decomposition. The soil glucosidase activity and MB-^{14}C content also support this interpretation. In contrast, ^{14}C present in microaggregates in fertilized soils was microbially susceptible to decomposition. Guggenberger et al. (1999) reported that this sequestered ^{14}C in microaggregates was not only subject to mineralization but also to distribution processes amongst the aggregates.

5. Conclusions

We studied the effect of long-term fertilization on plant residue mineralization and retention in soil and its aggregate fractions. The mineralization of residues followed the order M > N > control soil >2 M. The double exponential model (Eq. (1)) described the C_{lab} pool of plant residue-^{14}C as having the highest degradation rates (k1) in M soils, followed by N, control and 2 M soils. Thus,

i) Plant residue-^{14}C was retained more in control soil because of its lower C_{org} value compared to fertilized soils. In this context, plant residue-^{14}C was allocated to a higher degree in small and large macroaggregates than in the microaggregates of control soil. The low 'glucosidase activity per unit MBC' value in control soil corresponded to a lower decomposability of sequestered plant residues.
ii) The decomposition of plant residue during 15 days of incubation was high in mineral N fertilized soil. After 15 days the remaining ^{14}C was higher in microaggregates than in macroaggregates. However, the plant residue effect on glucosidase activity and MB-^{14}C was less pronounced. This implies a relative stability of plant residue C in mineral N fertilized soil.
iii) Amongst the treatments, long-term manuring with average doses caused the highest decomposition of plant residues. The presence of more ^{14}C in microaggregates corresponded to a sharp increase in glucosidase activity there. The high 'glucosidase activity per unit MBC' value implied further decomposability in microaggregates. The manure application at the higher dose (2 M) may have caused a different environment and a different response to newly added plant residues.

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Acknowledgements

We are grateful to the Alexander-von-Humboldt Foundation for funding the work. We are thankful to Dr. Jörg Rühlmann for providing the soil samples of long-term experiment in Trasse-2, Grossbeeren. We also thank Dr. Prasanta Kumar Bandyopadhyay for kindly going through the draft manuscript and offering valuable suggestions for strengthening it.

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