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ORIGINAL PAPER

Nitrogen fertilization increases rhizodeposit incorporation into microbial biomass and reduces soil organic matter losses

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Abstract Agricultural soils receive large amounts of anthropogenic nitrogen (N), which directly and indirectly affect soil organic matter (SOM) stocks and CO_2 fluxes. However, our current understanding of mechanisms on how N fertilization affects SOM pools of various ages and turnover remains poor. The $\delta^{13}C$ values of SOM after wheat (C_3)-maize (C_4) vegetation change were used to calculate the contribution of C_4-derived rhizodeposited C (rhizo-C) and C_3-derived SOM pools, i.e., rhizo-C and SOM. Soil (Ap from Haplic Luvisol) sampled from maize rhizosphere was incubated over 56 days with increasing N fertilization (four levels up to 300 kg N ha^{-1}), and CO_2 efflux and its $\delta^{13}C$ were measured. Nitrogen fertilization decreased CO_2 efflux by 27–42% as compared to unfertilized soil. This CO_2 decrease was mainly caused by the retardation of SOM (C_3) mineralization. Microbial availability of rhizo-C (released by maize roots within 4 weeks) was about 10 times higher than that of SOM (older than 4 weeks). Microbial biomass and dissolved organic C remained at the same level with increasing N. However, N fertilization increased the relative contribution of rhizo-C to microbial biomass by two to five times and to CO_2 for about two times. This increased contribution of rhizo-C reflects strongly accelerated microbial biomass turnover by N addition. The decomposition rate of rhizo-C was 3.7 times faster than that of SOM, and it increased additionally by 6.5 times under 300 kg N ha^{-1} N fertilization. This is the first report estimating the turnover and incorporation of very recent rhizo-C (4 weeks old) into soil C pools and shows that the turnover of rhizo-C was much faster than that of SOM. We conclude that the contribution of rhizo-C to CO_2 and to microbial biomass is highly dependent on N fertilization. Despite acceleration of rhizo-C turnover, the increased N fertilization facilitates C sequestration by decreasing SOM decomposition.

Keywords CO_2 partitioning · C_3-C_4 vegetation change · Microbial biomass · SOM decomposition · Nutrient availability

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Introduction

Soil organic matter (SOM) increases soil fertility, sustainability, and crop yield in agricultural ecosystems (Lal 2006). Because SOM contains a large amount of carbon (C), even small changes in SOM storage can affect crop yield, CO_2 release in the atmosphere, and C budget (Lal 2006; Fischlin et al. 2007). Nitrogen (N) fertilization has been widely used as a common agricultural management strategy to increase crop yield. It also directly and indirectly affects the C input, SOM stocks, and CO_2 emissions (Mulvaney et al. 2009). The annual input of anthropogenically derived N is about 30–50% greater

than that from natural sources and is tenfold greater than 100 years ago (Galloway et al. 2008; Schlesinger 2009). Understanding how these additional N inputs affect terrestrial ecosystems is becoming increasingly important within the context of the global C budget, especially in agricultural ecosystems (Liu and Greaver 2010).

Nitrogen fertilization has divergent effects on soil CO_2 emissions, including increased (Cleveland and Townsend 2006), decreased (Burton et al. 2004), or unchanged levels (González Polo et al. 2015). Nitrogen can influence C turnover through direct effects on soil properties (e.g., N availability, pH), enzyme activity, microbial community composition, and microbial biomass (Ramirez et al. 2012; Zang et al. 2016). Nitrogen fertilizer also can either stimulate plant growth, increasing C inputs into soil (Liang et al. 2012), or even decrease root biomass and C inputs (Grulke et al. 1998), thus indirectly influencing C cycling in soil. N fertilizer can either reduce the decline in SOM or even cause a small increase of SOM in long term (Ladha et al. 2011). In soils with very low N availability, N inputs may stimulate microbial activity to mine nutrients from SOM, thereby accelerating SOM decomposition—a positive priming effect (PE). Conversely, in high N availability soils, microbes will switch from decomposing SOM to utilizing the external N and newly rhizodeposited C (rhizo-C) (Dijkstra et al. 2013). Generally, the amount and frequency of N addition are strongly related to changes in microbial biomass and soil CO_2 emission (Treseder 2008; Ramirez et al. 2012).

The SOM stability depends on various pools with different physico-chemical properties and turnover times (von Lützow et al. 2007; Blagodatskaya et al. 2011). Young C pools consist mainly of rhizodeposition as well as breakdown plant residues of small size and fast turnover rates (Pausch and Kuzyakov 2012; Lin et al. 2015). The turnover of young C is about six to seven times faster than that of old C (Blagodatskaya et al. 2011; Pausch and Kuzyakov 2012), which is presumably due to its lower stabilization on clay minerals, low molecular weight, and high solubility in water (Zang et al. 2015). The age of recent C varies between years and decades in various studies (Pausch and Kuzyakov 2012; Lin et al. 2015; Dou et al. 2016). Such aged C is already microbially processed and turned over many times within soil pools. To our knowledge, no study has estimated the turnover of very recent C, which is deposited within a few days or a week. This knowledge gap calls for gaining experimental evidence of the N fertilization effect on such very recently deposited C.

The discrimination of ^{13}C occurs during CO_2 assimilation of C_3 and C_4 plants results in natural differences in the $\delta^{13}C$ signature and enables separating young from old C pools (Balesdent and Mariotti 1996; Werth and Kuzyakov 2008). Therefore, when C_3 vegetation changes to C_4, old (C_3-derived) and young (C_4-derivered) SOM can be partitioned based on their $\delta^{13}C$ signature. N fertilization is predicted to increase the decomposition rate of labile C pools and decreases the decomposition rate of more recalcitrant pools (Riggs et al. 2015). Remarkably, the size of recent C pools in this study was very small (less than 2% of SOC) because of only 4-week maize cropping. Therefore, we assume that the decreased decomposition rate of old C will be more pronounced than the increased decomposition of recent C after N fertilization.

The objective of this study was to determine the effects of N fertilization on the decomposition of rhizo-C (here derived from C_4 maize) and SOM pools (here derived from original C_3 wheat) in an agricultural soil using ^{13}C natural abundance after a C_3-C_4 vegetation change. Thus, we used the soil from wheat (C_3 soil) and from maize rhizosphere (C_3-C_4 soil) to examine the effects of increasing N fertilization on CO_2 emissions, microbial biomass, and dissolved organic matter (DOM) over a 56-day incubation. The C_3 to C_4 vegetation change approach was also used to quantify the contribution of rhizo-C (C_4-derived) and SOM (C_3-derived) sources to CO_2 emission, microbial biomass, and DOM. Our hypotheses were (1) rhizo-C is strongly affected by N fertilization due to its very fast turnover; (2) N fertilization will reduce CO_2 efflux from soil because of the higher N availability and less microbial demand for mining SOM for N; therefore, N fertilization will suppress the mineralization of SOM pools; and (3) the relative availability of rhizo-C will increase with N availability.

Materials and methods

Soil sampling and preparation

Soil samples were collected from the upper layer (0–10 cm) of the Ap horizon of a wheat field in northwest Göttingen, Germany (51° 33′ 36.8″ N, 9° 53′ 46.9″ E). The soil is a Haplic Luvisol whose organic carbon originates from the permanent C_3 vegetation. The basic soil characteristics were pH (H_2O) 6.6, organic C 11.7 g C kg^{-1}, total N 1.2 g N kg^{-1}, NO_3^-830 μg N g^{-1}, and available P 160 μg P g^{-1} (Zang et al. 2016). The soil was air-dried, homogenized, and sieved (<2 mm). Fine roots and other plant residues were carefully removed manually.

The soil was placed into pots and kept at a very thin soil layer (about 5 cm). The maize (C_4) and wheat (C_3) seeds were germinated on wet filter paper in Petri dishes for 3 days, and sufficient seedlings were transferred to each pot. The plants were grown in a greenhouse at room temperature. During plant growth, artificial lighting was used and maintained at 100 μmol m^{-2} s^{-1} for 14 h day^{-1}; soil moisture was kept at 50–60% of the available field capacity. After 4 weeks of growth, all plants and fine roots were carefully removed from the soil, and then, the soil was mixed thoroughly. Because the

maize or wheat roots completely occupied the entire pots, the two soils were regarded as the wheat (C_3) or maize (C_3-C_4) rhizosphere and used for the subsequent incubation. Soil (C_3) under wheat without C_3-C_4 vegetation change was used as a reference to estimate the $\delta^{13}C$ shifts between the pools caused by isotopic fractionation. We define rhizo-C and SOM as newly deposited C from C_4 maize and SOM pools derived from original C_3 wheat, respectively.

Experimental design and soil incubation

Thirty grams (oven-dried weight) of the rhizosphere soil (C_3 or C_3-C_4 rhizosphere) was weighed into a 100-mL jar. The soil was adjusted to 50% of the water-holding capacity (WHC) and pre-incubated for 3 days at 20 °C. After pre-incubation, the increasing levels of NH_4Cl solution (low N 52, medium N 104, high N 208 μg N g^{-1} soil) and distilled water (control) were applied using a syringe to reach a final soil moisture content of 60% of WHC. Medium N input to the soil was equivalent to 150 kg N ha^{-1}, which is the conventional amount of mineral N fertilizer application in northern Germany. Thus, the increasing N addition was 0, 75, 150, and 300 kg N ha^{-1} for the control, low, medium, and high N treatment, respectively. Then, the jars were incubated in the dark at 20 °C for 56 days. During the incubation, the CO_2 evolved from the soils was trapped by 3 mL 1.0 M NaOH solution in small tubes that were exchanged at 1, 3, 5, and 7 days and then weekly. In addition, three jars for each treatment were destructively sampled at 3, 7, 21, 40, and 56 days to measure microbial biomass, DOM, and for $\delta^{13}C$ analyses.

CO_2 emission, microbial biomass, and DOM

The concentration of CO_2 trapped in the NaOH solution was measured by titration, and 0.5 mL solution was titrated with 0.1 M HCl against phenolphthalein after addition of 0.5 M $BaCl_2$ solution. Microbial biomass was determined by the chloroform extraction method (Vance et al. 1987; Wu et al. 1990). After destructive sampling, the soil was carefully mixed and 5 g soil was directly extracted using 20 mL of 0.05 M K_2SO_4. Another 5 g soil was fumigated with chloroform for 24 h and extracted in the same manner. The extracts were analyzed for the total C concentration using a 2100 TOC/TIC analyzer (Analytik Jena, Germany). The extracts of the non-fumigated samples were used to measure DOM. The microbial biomass C (MBC) was calculated based on the difference of K_2SO_4-extractable C between fumigated and non-fumigated soil samples using the k_{ec} factor 0.45 (Joergensen 1996).

Isotopic analyses

CO_2 trapped as Na_2CO_3 in NaOH was precipitated with 3 mL 1.0 M $SrCl_2$ aqueous solution. The NaOH solution containing the $SrCO_3$ precipitate was centrifuged three times at 1450*g* for 10 min and washed in between with deionized and degassed water to remove NaOH and to reach a pH of 7. After washing, the $SrCO_3$ was dried at 105 °C (Blagodatskaya et al. 2011). The $\delta^{13}C$ of the $SrCO_3$ was analyzed at the Center for Stable Isotope Research and Analysis (KOSI) of the University of Gottingen with an Elemental Analyzer (Eurovector) coupled to an IRMS (Delta Plus XL IRMS, Thermo Finnigan MAT, Bremen, Germany). Two acetanilide standards were measured every 12 samples. For the DOM and microbial biomass pools, the K_2SO_4-extracted solution was freeze-dried (Beta 1–8 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Harz, Germany) and analyzed using IRMS.

Calculation and statistics

Priming effect

Priming effects were calculated according to the following equation:

$$PE = [CO_2]_{treatment} - [CO_2]_{control} \quad (1)$$

$$\text{Relative PE} = \left([CO_2]_{treatment} - [CO_2]_{control}\right)/[CO_2]_{control} \quad (2)$$

where $[CO_2]_{treatment}$ and $[CO_2]_{control}$ represent the cumulative CO_2 respiration in N amendment treatments and control, respectively.

Contribution of rhizo-C to total CO_2 emission, microbial biomass, and DOM

A mass balance equation (Balesdent and Mariotti 1996) was used to determine the $\delta^{13}C$ value of total microbial biomass ($\delta^{13}C_{MB}$):

$$\delta^{13}C_{MB} = \left(\delta^{13}C_f \times C_f - \delta^{13}C_{nf} \times C_{nf}\right)/\left(C_f - C_{nf}\right) \quad (3)$$

where $\delta^{13}C_f$ and C_f are the $\delta^{13}C$ values and amount of C in fumigated samples, respectively, and $\delta^{13}C_{nf}$ and C_{nf} are the $\delta^{13}C$ values and amount of C in non-fumigated samples, respectively.

The proportional contributions of the C_3 (f_{C3}) and the C_4 (f_{C4}) source to total microbial biomass or CO_2 were calculated as follows (Amelung et al. 2008):

$$f_{C4} = \left(\delta^{13}C_t - \delta^{13}C_3\right)/\left(\delta^{13}C_4 - \delta^{13}C_3\right) \quad (4)$$

$$f_{C3} = 1 - f_{C4} \quad (5)$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the C pool under C_3-C_4 mixed sources and $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding C pool in reference soil (C_3 soil). The $\delta^{13}C_4$ was calculated based on the $\delta^{13}C$ value of maize (mean of root, shoot, and leaves) and corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}C_3$ of C_3 vegetation and $\delta^{13}C_3$ of SOM of the C_3 soil. This approach assumes equal isotopic fractionation by humification of C_3 and C_4 plants (Schneckenberger and Kuzyakov 2007).

Microbial biomass derived from recent rhizo-C ($C_{4\text{-}MB}$) and SOM ($C_{3\text{-}MB}$) was calculated as follows:

$$C_{4-MB} = C_{MB}{}^{*} f_{C4} \quad (6)$$

$$C_{3-MB} = C_{MB}{}^{*} f_{C3} \quad (7)$$

where C_{MB} is the total amount of SOM-derived C in microbial biomass.

Relative contribution of rhizo-C

The relative contributions of rhizo-C to CO_2 emission, microbial biomass, and DOM were estimated based on the contribution of rhizo-C to SOM, CO_2, microbial biomass, and DOM. In order to evaluate the availability of rhizo-C that entered the soil within 4 weeks and to compare it with the availability of SOM that had entered the soil before maize cropping, the ratios of C_4- to C_3-derived C in CO_2, microbial biomass, and DOM were related to that in SOM. The respective C_4/C_3 ratios were calculated using linear two-source isotopic mixing models (Phillips and Gregg 2001).

$$\text{Relative contribution} = f_{C4}(\text{pool}) \Big/ f_{C4}(\text{SOM}) \quad (8)$$

where f_{C4} (pool) is the contributions of the C_4 source to total CO_2, microbial biomass, or DOM and f_{C4} (SOM) is the contribution of the C_4 source to total SOM.

Mean residence time of rhizo-C and SOM

The cumulative C mineralization data for rhizo-C and SOM were fitted individually to the following first-order one C-pool model (Kuzyakov 2011):

$$C(t) = C^{*}\left(1-e^{-kt}\right) \quad (9)$$

where $C(t)$ is the cumulative CO_2 emission from rhizo-C and SOM during the 56-day incubation, C is the initial size of the C pools, and k is the decomposition rate. Mean residence time (MRT) was calculated as reciprocal to decomposition rates.

Statistics

The significant differences of cumulative CO_2, microbial biomass, and DOM under N fertilization are shown as least significance difference (LSD) (5%) estimated by one-way ANOVA. The values presented in the figures and tables are given as means ± standard errors (SEs).

Results

The $\delta^{13}C$ dynamics in CO_2 and microbial biomass during incubation

The growth of maize (C_4) on a C_3 soil increased the $\delta^{13}C$ in all soil C pools (Fig. 1). The increase was much stronger in CO_2 (1.6–4.6‰) and microbial biomass (3.0–5.0‰) than in SOM (0.3‰), indicating fast processing of rhizo-C by microorganisms. The $\delta^{13}C$ of CO_2 and microbial biomass in C_3-C_4 soil varied from −22 to −26‰. The $\delta^{13}C$ of microbial biomass decreased from −22.2 to −24.2‰ during the 56-day incubation and that of CO_2 decreased from −22.6 to −25.5‰ (Fig. 1). The $\delta^{13}C$ of CO_2 and microbial biomass increased with N fertilization, showing the higher contribution of rhizo-C in the microbial pool with N fertilization. The $\delta^{13}C$ of the DOM pool varied within a narrow range of −26.1 to −27.3‰ and was almost unaffected by N fertilization (Fig. 1). The $\delta^{13}C$ of CO_2 and microbial biomass all decreased with time, which shows reduced utilization of exudates within 56 days. The $\delta^{13}C$ of DOM pool, however, was always similar to that of SOM and only showed small changes over time. Overall, N fertilization increased the $\delta^{13}C$ of CO_2 and microbial biomass and smoothed the time effect.

Soil respiration, microbial biomass, extractable C, and N

N fertilization decreased CO_2 emissions by 27–42% compared to the control without N (298 μg C g^{-1}; Fig. S1). The cumulative PE during 56 days was always negative and became more strongly negative with increasing N fertilization. N addition had no effects on microbial biomass and DOM over the 56 days ($P < 0.05$; Fig. S1). The microbial biomass varied from 68.1 to 96.5 μg C g^{-1}, while DOM decreased by a factor of 2.5 from 48.7 to 18.4 μg C g^{-1} during incubation. Overall, N fertilization decreased the intensities of C turnover (CO_2 release), even though microbial biomass remained nearly constant.

Contribution of rhizo-C to total CO_2 emission, microbial biomass, and DOM

Based on the $\delta^{13}C$ difference, we calculated the contribution of rhizo-C and SOM to all C pools (Eq. 6). Only 1.8% of SOM

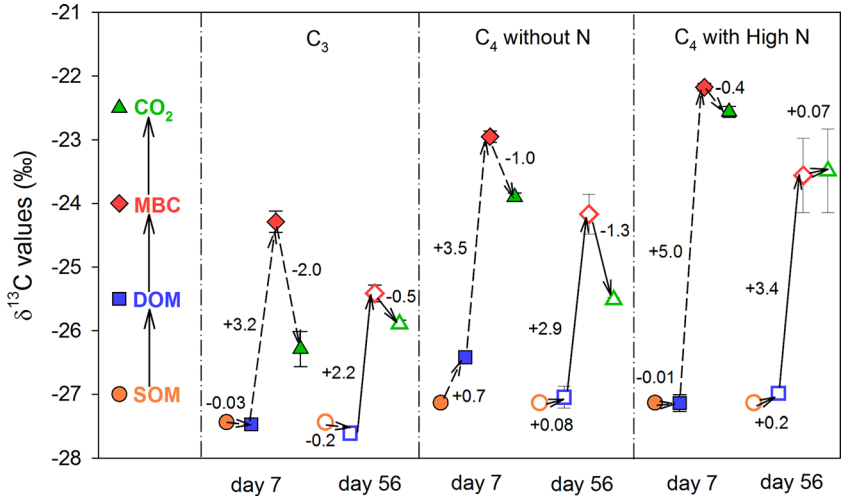

Fig. 1 Contribution of ^{13}C fractionation and preferential substrate utilization to changes in the $\delta^{13}C$ signature of C pools in soil after 7 and 56 days of incubation. The ^{13}C fractionation values are presented based on $\delta^{13}C$ of the C_3 reference soil (*left*), soil after the C_3-C_4 vegetation change without N (*middle*), and with high N (300 kg N ha^{-1}) fertilization (*right*). We assumed that the $\delta^{13}C$ transformations go in the following direction: SOM (*yellow*) ➔ DOM (*red*) ➔ MBC (*blue*) ➔ CO_2 (*green*). Therefore, we use *arrows* to show these transformations. Values show the $\delta^{13}C$ changes between C pools and fluxes

were derived from rhizo-C, whereas it was much higher in CO_2 (7–28%), microbial biomass (6–22%), and DOM (2–5%). The contribution of rhizo-C to CO_2 emission decreased from 16.6–27.6% at day 3 to 6.9–13.2% at day 56 of incubation across all treatment. N fertilization increased the contribution of rhizo-C to CO_2 emission, which varied from 37 to 91% compared to 0 N (Fig. 2c). N fertilization decreased the SOM incorporation into CO_2 but did not affect rhizo-C; accordingly, the rhizo-C contribution to CO_2 increased ($P < 0.05$). However, N fertilization increased the rhizo-C and decreased the SOM to microbial biomass; this caused an increase in rhizo-C contribution ($P < 0.05$; Fig. 3). The contribution of rhizo-C to DOM slightly decreased from 3–5 to 2–3% during incubation and was also slightly decreased by N addition (Fig. 3). Rhizo-C contributed only a small proportion (<6%) to DOM, which was about ten times smaller than that in microbial biomass and CO_2. Overall, N fertilization increased the contribution of rhizo-C to CO_2 emission and microbial biomass, and both of them latter decreased within 2 months. The DOM pool was not sensitive to N fertilization.

Relative contribution of rhizo-C to CO_2 emission, microbial biomass, and DOM

The relative contribution of rhizo-C—estimated by relating the contribution of rhizo-C to CO_2 (microbial biomass or DOM) to its contribution to SOM (Eq. 8)—demonstrated relative incorporation of rhizo-C into pools and fluxes. The ratio of C_4 to C_3 was 0.02 in SOM, 0.06 in DOM, 0.07 in microbial biomass, and 0.18 in CO_2 (Fig. 4). Thus, the relative contribution of rhizo-C increased from 3 (0.06 / 0.02 = 3) and 4 (0.08 / 0.02 = 4) to 10 (0.18 / 0.02 = 10) for DOM, microbial biomass, and CO_2, respectively (Fig. 4). We designate this parameter as a "relative contribution of rhizo-C," which helps to compare the changes in contribution of rhizo-C from DOM, microbial biomass, and CO_2 to SOM. With N fertilization, the relative contribution of rhizo-C to microbial biomass increased by five times (from 3.7 to 19.8) at the early stage and by two times (from 4.8 to 8.9) at the late stage (Fig. 4). Similarly, the relative contribution of rhizo-C to CO_2 was increased by two times with N fertilization, from 10.0 to 18.4 at the early stage and from 3.2 to 7.2 at the late stage. The relative contribution of CO_2 and microbial biomass both decreased with time (Fig. 4, bottom). Overall, the fractions of rhizo-C in CO_2, microbial biomass, and DOM all decreased with time, reflecting the C_4-derived C consumption during incubation. The response of rhizo-C to N fertilization was stronger for microbial biomass and CO_2 than for the DOM pool.

Discussion

Dynamics of isotopic composition of soil C pools

The ^{13}C enrichment of CO_2 (1.6–6.9‰) and microbial biomass (3.0–6.8‰) was comparable with the 3.6–5‰ ^{13}C enrichment found for CO_2 in Inceptisol using ^{13}C natural abundance methods (Formánek and Ambus 2004). This indicates that mainly young pools (^{13}C–enriched, including sugars, starch, cellulose) were used for mineralization. The ^{13}C fractionation between SOM as a substrate and CO_2 respired by microorganisms is the sum of the ^{13}C fractionation during microbial uptake of the substances and during respiration.

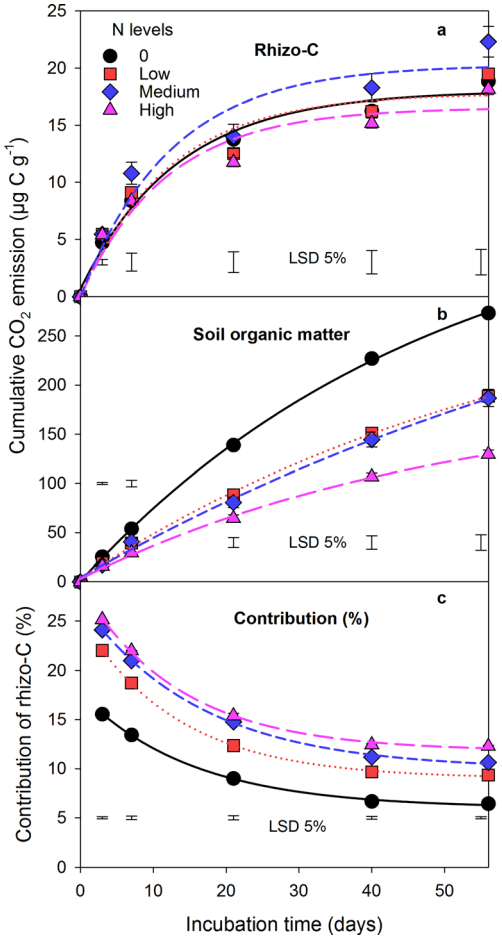

Fig. 2 The amount of rhizo-C (C_4) (**a**) and soil organic matter (C_3) (**b**) to CO_2 and the relative contribution of rhizo-C to CO_2 (**c**) during the 56-day incubation of C_3-C_4 rhizosphere soil with increasing N fertilization (0, 75, 150, and 300 kg N ha^{-1}). N fertilization increased the contribution of rhizo-C to CO_2 emission, which varied from 37 to 91% compared to 0 N. Values are means ± standard error (n = 4). The significant difference between N fertilization for each time is shown as LSD (5%). Cumulative CO_2 emissions for rhizo-C and C_3-derived SOM were fitted with a one-pool decay model, CO_2 (t) = P*(1 − exp(−k*t)). The *black*, *red*, *blue*, and *purple lines* represent the cumulative CO_2 and contribution of rhizo-C under 0, low, medium, and high N fertilization, respectively

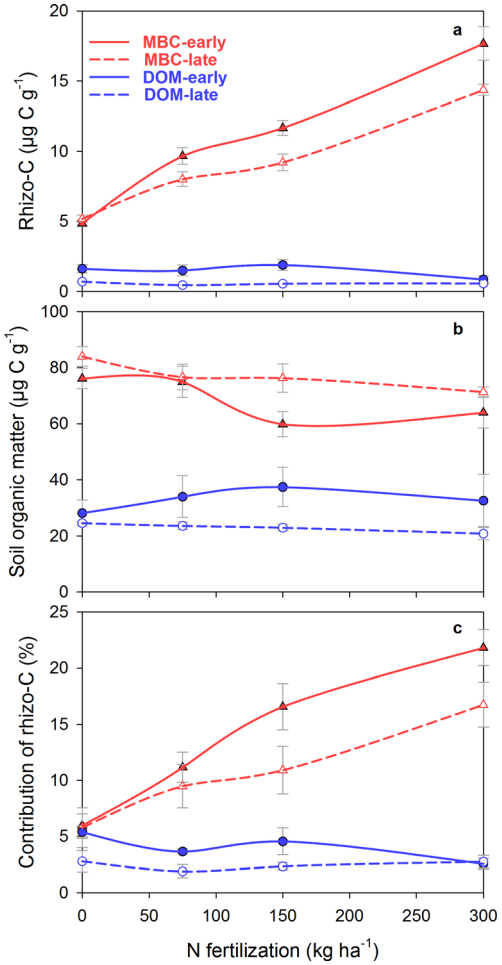

Fig. 3 The amount of rhizo-C (C_4) (**a**) and soil organic matter (C_3) (**b**) in microbial biomass (*red line*) and DOM (*blue line*) and the contribution of rhizo-C to microbial biomass and DOM (**c**) at early (0–7 days) and late (21–56 days) stages of the C_3-C_4 soil depending on increased N fertilization (0, 75, 150, and 300 kg N ha^{-1}). Values are means ± standard error (n = 4). The *red* and *blue lines* show the relative contribution of rhizo-C to microbial biomass and DOM, respectively. The *solid* and *dotted lines* represent the data obtained at early and late stages of incubation

This ^{13}C enrichment partly reflects the preferential use of certain organic compounds during metabolism (Werth and Kuzyakov 2010), and the ^{13}C enrichment is due to new maize rhizodeposition (C_4 signature). These low molecular weight organics deposited by maize roots such as amino acids and sugars can be very rapidly taken up by microorganisms, within several minutes (Fischer et al. 2010). The preferential use of this ^{13}C-enriched SOM fraction (rhizo-C) leads to a more rapid loss of ^{13}C than ^{12}C during decomposition, causing ^{13}C depletion in the remaining SOM. Hence, microbial biomass was ^{13}C enriched because of preferential utilization of rhizodeposits (C_4), but respired CO_2 was depleted in ^{13}C by −0.9‰ on average during incubation (yet still remaining enriched compared to SOM) (Fig. 1). This was comparable with the −0.1 to −7.4‰ depletion in microbial biomass during 30 or 40 days of incubation elsewhere (Blagodatskaya et al. 2011).

The $\delta^{13}C$ of CO_2 and microbial biomass in the C_3-C_4 soil became increasingly negative with incubation time, demonstrating a depletion of more readily available rhizo-C compared to C_3-derived organics. CO_2 produced at the beginning mainly originated from those compounds with fast decomposition rates, whereas compounds with lower decomposition rates contributed more to the CO_2 over time (Werth and Kuzyakov 2008; Pausch and Kuzyakov 2012). The $\delta^{13}C$ of CO_2 and of microbial biomass increased with N fertilization (Fig. 1), because fertilization induced relatively more rhizo-C incorporated into the C cycle.

The $\delta^{13}C$ increase caused by C_4 rhizodeposition was pronounced in microbial biomass and CO_2, while it was rather

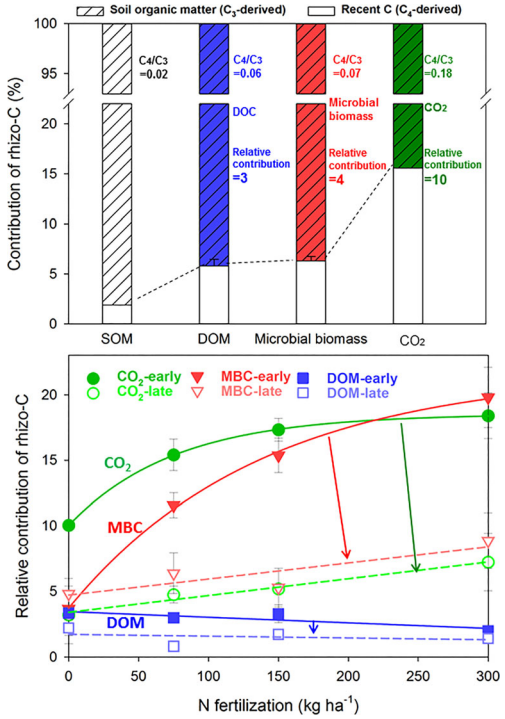

Fig. 4 The contribution of rhizo-C (C_4) and C_3-C to SOM, DOM (*blue*), microbial biomass (*red*), and CO_2 (*green*) at the start of incubation of C_3-C_4 soil without N fertilization (*top*). The relative contribution of rhizo-C to DOM, microbial biomass, and CO_2 at 3 and 56 days after the incubation of C_3-C_4 soil depending on N fertilization rates (0, 75, 150, and 300 kg N ha^{-1}) (*bottom*). Values are means ± standard error (n = 4). The relative contribution of rhizo-C estimated as ration of the contribution of rhizo-C to CO_2, microbial biomass, and DOM and its contribution to SOM (Eq. 8). The *green*, *red*, and *blue lines in the bottom figure* show the relative contribution of rhizo-C to CO_2, microbial biomass, and DOM, respectively. The *solid* and *dotted lines* represent the data obtained at 3 and 56 days after incubation. The *arrows in the bottom figure* show the time effect on relative contribution of rhizo-C

weak for the DOM (Fig. 1). Even with increasing N fertilization, the $\delta^{13}C$ values of DOM remained almost stable during incubation. This can be explained as the quick selective microbial uptake of available substrates from DOM originating from root exudates and plant residues (Esperschütz et al. 2009). Thus, rhizo-C is rapidly incorporated into microbial biomass (see the $\delta^{13}C$ shift in Fig. 1) (Bol et al. 2009). Similarly, root exudates were quickly taken up by microorganisms during rice growth, while DOM remained unchanged (Yuan et al. 2016). The remaining soluble C originating from SOM (C_3-derived) was ^{13}C depleted compared to microbial biomass. A similar higher enrichment of microbial biomass compared to the soluble C pool has been observed in rhizosphere soil (Esperschütz et al. 2009). This indicates that the higher microbial biomass and activities in the rhizosphere lead to rapid consumption of rhizo-C. Accordingly, most of the DOM originated from native SOM and not from the rhizodeposits, which is reflected by similar $\delta^{13}C$ values for DOM and SOM (Fig. 1).

Priming effect and contribution of rhizo-C to soil C pools

N fertilization strongly decreased (by 27–42%) the CO_2 emissions and induced pronounced negative PE in the rhizosphere soil (Fig. S1). These findings correspond to the 8–15% decrease in CO_2 emissions after N fertilization elsewhere (Janssens et al. 2010; Liu and Greaver 2010). Previous studies also found that mineral N additions reduced microbial biomass by 15–20% and decreased soil CO_2 emissions (Treseder 2008; Liu and Greaver 2010; Ramirez et al. 2012). In our study, however, microbial biomass was not affected by N addition ($P < 0.05$; Fig. S1). This probably reflects sufficient labile C (e.g., exudates) that is readily available for microorganisms in the rhizosphere (Kuzyakov et al. 2007). Thus, the microbial biomass in the rhizosphere remained constant under N fertilization, in contrast to studies with bulk soil.

A high contribution of rhizo-C to CO_2 and to microbial biomass, and the moderate contribution to DOM, was in contrast to the much lower fraction of rhizo-C in SOM (Figs. 2 and 3). These results again illustrate the very rapid uptake of easily available rhizo-C by microorganisms (Bol et al. 2009; Fischer and Kuzyakov 2010). A higher contribution of recent C to CO_2 (40–79%) and to microbial biomass (56–82%) and a lower contribution to SOM and to DOM (29–32%) were already detected elsewhere (Pausch and Kuzyakov 2012; Luo et al. 2017). Even after 37 years of maize cropping, the contribution of C_4-derived C accounted for only 15% of total SOM but for about 58% of CO_2 (Flessa et al. 2000). Despite a slow asymptotic increase of C_4-C in the SOM, its portion in the CO_2 increased much faster (Kuzyakov 2011). This means that after a short period (4 weeks) of C_4 rhizodeposition, despite the low portion of C_4-C to SOM, its contribution to the CO_2 efflux can amount to more than 20% (Fig. 5) and therefore cannot be ignored.

N fertilization retarded the decomposition of old and stable SOM but did not affect newly deposited C ($P < 0.05$; Fig. 2). This confirmed the hypothesis 2. Microorganisms reduce the mining of recalcitrant SOM (lower N requirements) and shift towards labile C under N fertilization (Chen et al. 2014; Vogel et al. 2015). Similarly, N addition stimulates enzymes that degrade labile C and inhibits enzyme activities that contribute to decomposing recalcitrant C (Hobbie et al. 2012; Riggs et al. 2015). Thus, the retardation mechanism of stabile SOM decomposition under N fertilization is explained by intensive microbial metabolism of the easily available C of rhizodeposits when N is not limited. The decreased decomposition of SOM can also be interpreted as preferential substrate use, which has already been hypothesized as the reason for a suppressed decomposition of native SOM under elevated atmospheric CO_2 (Cheng 1999; Cardon et al. 2001). According to one C-pool model, N fertilization increased the MRT of SOM but did not affect the MRT of rhizo-C (Table 1). The MRT in the current study was shorter than the MRT for both

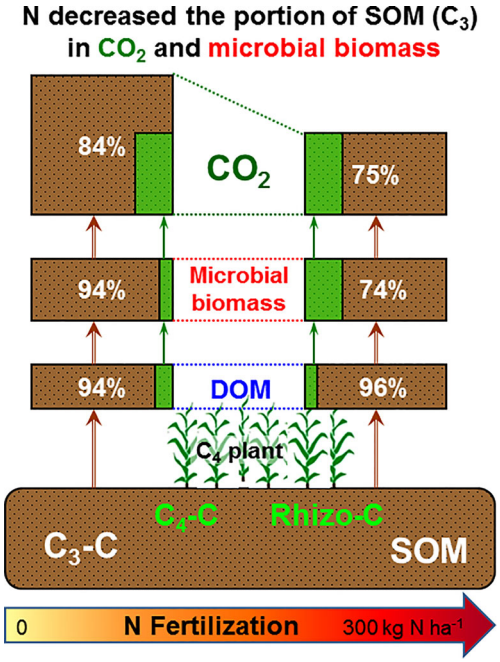

Fig. 5 Contribution of rhizo-C (C_4) and C_3-C to soil organic matter (SOM), dissolved organic matter (DOM), microbial biomass, and CO_2 emissions under increasing N fertilization. The *green color* of C pools and CO_2 fluxes represents the contribution of rhizo-C (C_4), and the *brown color* represents the contribution of the SOM (C_3). The percentage shows the contribution of old C to DOM, microbial biomass, and CO_2 emissions. Maize (C_4) was planted on a soil from a long-term wheat (C_3) field and grown 4 weeks to obtain C_4 rhizodeposit enrichment in this C_3 soil. The *wide arrow on the bottom* shows the increasing N fertilization (up to 300 kg ha^{-1}). The *green* and *brown arrows* show the transformation of rhizo-C and C_3-C from SOM to CO_2, respectively. See further explanations in the text

recent C (21 days) and old C (64 days) in 12 years after C_3-C_4 vegetation change (Blagodatskaya et al. 2011). The shorter MRT reflects the fact that the decomposition rate of very recent (4 weeks) rhizo-C was two times faster than that of moderately recent C (several years old) (Blagodatskaya et al. 2011), the latter having already been microbially processed and turned over many times within soil C pools. The decomposition rate of rhizo-C was 3.7 times faster than SOM, and it increased 6.5 times under high N fertilization. Moreover, the qCO_2 fall with N fertilization in both rhizo-C (by three times) and SOM (by two times) (Table 2). The qCO_2 is a function of substrate availability, microbial community structure (e.g., fungal-to-bacterial ratio), maintenance requirements, and carbon use efficiency (CUE) (Insam and Haselwandter 1989; Wardle and Ghani 1995). The three former parameters, however, are assumed to remain static under microbial biomass steady-state conditions, when the qCO_2 is mainly dependent on the CUE. As no changes in microbial biomass were observed between days 21 and 40, the microbial metabolism efficiency estimated by specific respiration (qCO_2) may reflect the physiological characteristics (Blagodatskaya et al. 2014). Thus, we can assume that N fertilization increased microbial CUE in both rhizo-C and SOM based on the decreased qCO_2. N fertilization reduces the cumulative CO_2 emission due to the higher efficiency of microbial C reutilization as compared with N-limited conditions (Blagodatsky et al. 1998). Thus, we demonstrated the applicability of both mechanisms, which are preferential substrate utilization and increased CUE under N fertilization (Figs. 1 and 4).

N addition had no effects on total microbial biomass and DOM over 56 days ($P < 0.05$; Fig. S1). Remarkably, N fertilization increased recent labile C incorporated into microbial biomass, which may be due to the increased dominance of fast-growing microorganisms benefiting from labile C supported by N input (Fontaine et al. 2003). Microorganisms reduced the decomposition of recalcitrant C because their N requirements were met by N fertilization (Fig. 3). The more readily available portion of C_4-derived DOM was intensively involved in microbial metabolism (Pelz et al. 2005). Therefore, the small proportion of C_4-derived DOM (decreasing from 5 to 2% during incubation) indicated microbial metabolic activity and rapid rhizo-C decomposition (Fig. 3). The decreased rhizo-C in microbial biomass and DOM during the incubation also confirmed the fast turnover of newly deposited C versus SOM. Overall, N fertilization strongly increased the

Table 1 The decomposition rates and mean residence time (MRT) of rhizo-C and C_3-C

	Rhizo-C		C_3-C	
	k (day^{-1})	MRT (day)	k (day^{-1})	MRT (day)
0 N	0.083	12.0	0.023	44.2
Low N	0.084	11.9	0.021	48.3
Medium N	0.081	12.3	0.018	57.1
High N	0.085	11.7	0.013	75.8

Parameters obtained for the fitted one-pool, first-order kinetic model (CO_2 (t) = P*(1 − exp(−k*t))) to cumulative CO_2 emission data from rhizo-C and C_3-C. In the model, k is the decomposition rate (day^{-1}) of C_4- or C_3-derived SOC. MRT was calculated as reciprocal to decomposition rates

Table 2 CO_2 produced per time and microbial biomass unit (qCO_2; μg C mg^{-1} biomass-C day^{-1}) of rhizo-C, C_3-C, and total C

	Total C	Rhizo-C	C_3-C
0 N	52.5 ± 0.7	31.4 ± 0.6	54.8 ± 1.0
Low N	39.7 ± 3.2	26.2 ± 2.4	43.6 ± 3.5
Medium N	42.5 ± 3.6	23.6 ± 2.0	48.3 ± 4.1
High N	25.4 ± 1.0	11.4 ± 0.5	31.0 ± 1.3

Values are means ± standard error (n = 4). Note that the estimation of qCO_2 values was based on data for the period from 21 to 40 days of incubation as microbial steady state (Fig. S1). Under such steady-state conditions, the estimated efficiency of microbial metabolism by specific respiration (qCO_2) can be used as a physiological characteristic

contribution of rhizo-C to CO_2 (37–91%) and microbial biomass (0.6–2.6 times), depending on the duration and the amount of N added (Fig. 5). All these N effects declined with time.

Relative contribution of rhizo-C to soil C pools

The relative contribution of rhizo-C to CO_2, microbial biomass, and DOM at the early stage was 10, 4, and 5, respectively (Fig. 4). The relative contribution of this very recent C to microbial biomass and CO_2 was much higher here than in other studies, in which the defined "recent C" was already microbially processed and turned over many times within soil pools. For example, the relative contribution of maize-derived C (<1 year) to CO_2 flux was about seven times higher than that of SOM stabilized in soil for longer than 1 year (Pausch and Kuzyakov 2012). The turnover rate of recent rhizo-C (<12 years) was 6 and 3.7 times higher than SOM in CO_2 and microbial biomass, respectively (Blagodatskaya et al. 2011). After 37 years of maize cropping, the relative contribution of recent C was about 4 (Flessa et al. 2000). These decreases in the relative availability of recent C with increasing age reflect the greater stability of C after long-term turnover.

N increased the relative contribution of rhizo-C in microbial biomass (two to five times) and CO_2 (two times) but had a minor effect on DOM. This confirmed the hypothesis 3 that the relative availability of rhizo-C will increase with N availability. Inorganic N accelerates the decomposition of soluble carbohydrates but may well have the opposite effect on more recalcitrant organics (Fog 1988). The increased fraction of rhizo-C in CO_2 under increasing N fertilization was mainly caused by inhibited native SOM mineralization; at the same time, the increase in microbial biomass was caused by both an increase of rhizo-C and a decrease of SOM incorporation. This is confirmed by the lower qCO_2 and higher MRT of rhizo-C under N fertilization (Tables 1 and 2), which means increased turnover and CUE of rhizo-C.

The availability of soil C decreased with time, as shown by the higher relative contribution of rhizo-C to C pools and fluxes at the early versus late stage (Fig. 4). The decrease in the relative contribution of rhizo-C to CO_2 and to microbial biomass with time was much stronger than in the DOM pool, possibly because of the very small pool size of DOM, which mainly originated from native SOM. The decrease in the relative contribution of rhizo-C over time mainly reflected the reduction of labile recent C in the soil without a permanent input of fresh C_4-C. The decreased rhizo-C in the microbial biomass may show a reutilization of the C_4-microbial pool. Nonetheless, according to the decreased rhizo-C in the microbial biomass, the maximum reutilized C_4-C (1.6–3.3 μg g^{-1}) was insufficient to have a strong effect on C_4-CO_2 emissions (Figs. 2 and 3). Overall, the time effect of the relative contribution of rhizo-C to CO_2, microbial biomass, and DOM significantly correlated with the recent C pool size and incubation time.

Conclusions

N fertilization increased the $\delta^{13}C$ of CO_2 and microbial biomass due to preferential utilization of C that was newly deposited from maize roots (^{13}C-enriched) versus SOM (relatively ^{13}C-depleted) sources. N fertilization did not affect microbial biomass ($P > 0.05$) but decreased the CO_2 emissions (27–42%) and caused negative PE —a decrease of SOM decomposition. The negative PE occurred due to higher CUE by microorganisms, as confirmed by the lower qCO_2. The contribution of rhizo-C was much higher to the CO_2 efflux (17–28%) and to microbial biomass (9–38%) compared to DOM (4–7%) and SOM (2%). This indicates that soluble organics (here originated from maize roots, C_4 signature) are preferably used and decomposed by microorganisms as compared to SOM. N fertilization increased the contribution of rhizo-C to CO_2 emissions and to microbial biomass, reflecting acceleration of its turnover. The availability of rhizo-C for microorganisms was up to 10 times higher than C older than 4 weeks. N fertilization increased the relative contribution of rhizo-C to microbial biomass by two to five times and to CO_2 by about two times. DOM was not sensitive to N because only a very small part was originated from maize rhizodeposits, while the main part originating from SOM was less decomposed under N fertilization. The fraction of rhizo-C in CO_2, microbial biomass, and DOM decreased over time, demonstrating a high preference of rhizo-C for microbial utilization compared to C from SOM. The turnover of very recent rhizo-C (within 4 weeks) was 3.7 times faster than that of SOM and increased with N fertilization. We conclude that N fertilization facilitates C sequestration in agricultural soils by decreasing SOM decomposition mainly through an increase in the turnover of newly deposited C and through an improved CUE of rhizo-C.

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