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Nitrogen fertilization decreases the decomposition of soil organic matter and plant residues in planted soils

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ABSTRACT

Nitrogen fertilization may affect the decomposition of soil organic matter (SOM) and plant residues in soil, but this effect is still very uncertain and depends on living plants. We investigated the effects of mineral N (N_{min}) availability on SOM and plant residue decomposition in wheat (*Triticum aestivum* L.) growing soils in a pot experiment. Five treatments were assessed: (1) Control [no maize (*Zea Mays* L.) residues and no N fertilization]; (2) ^{15}N-urea addition; (3) ^{15}N maize leaves; (4) ^{15}N maize leaves + urea; and (5) ^{15}N-urea + maize straw. The decomposition of SOM and plant residues was traced by the changes of N and C in the light fraction (density < 1.80 g cm^{-3}) during the 127 days. Urea fertilization decreased the decomposition of SOM and maize residues, as indicated by remaining N and C in the light fraction compared to soil without urea. The C decomposition was tightly coupled to that of N in the light fraction SOM. In soils with maize residues, both maize- and SOM-derived light fractions decomposed slowly with N fertilization. Soil microbial biomass N content was increased by maize residues but was unaffected by urea addition. Under low soil N_{min} levels, microbes met their N demand by increasing an acquisition from accelerated decomposition of organic sources. To mine N in the N_{min} limited soils, soil microbes might have directly taken up more N-containing organics and thus facilitated SOM decomposition. For such an acceleration of SOM decomposition, the presence of N uptake by living plants was especially important, which decreased the N_{min} in soil and so, increased N limitation for microorganisms. We concluded that N fertilization decreases SOM decomposition and increases the efficiency of C sequestration in soil through higher portion of un-decomposed crop residues.

1. Introduction

Aboveground crop residues are byproducts of agriculture. One benefit of returning crop residues to soil is C sequestration and soil organic matter (SOM) formation. The higher C/N ratio of crop residues than the soil microbial biomass implies that mineral N (N_{min}) availability may affect the microbial decomposition of crop residues (Sinsabaugh et al., 2013; Cyle et al., 2016; Zang et al., 2016). N fertilization may impact the efficiency of C sequestration through crop residue incorporation. Numerous studies (Recous et al., 1995; Mary et al., 1996; Henriksen and Breland, 1999; Neff et al., 2002; Potthoff et al., 2005) have suggested that high N_{min} level stimulates the decomposition of plant residues and SOM. Some studies (Neff et al., 2002; Hobbie, 2005; Hobbie et al., 2012; Kaspari et al., 2008) have suggested that the effect of the N_{min} level on plant residues and SOM decomposition is variable, depending on N content in residues and soil, abundances of other nutrients, organic compound's composition, N leaching and microbial community structure. N fertilization reduces microbial biomass in many ecosystems (Treseder, 2008) and decreases soil CO_2 emissions (Treseder, 2008; Janssens et al., 2010; Spohn et al., 2016; Zang et al., 2016). The decrease in the N_{min} content changes the decomposer community and accelerates SOM mineralization, resulting in reduced SOM accumulation (Fontaine and Barot, 2005).

The effects of soil N_{min} on SOM and plant residue decomposition may be biased by the study approach. Current studies on the effects of N_{min} level on SOM and plant residue decomposition have two limitations: (1) the most of these studies have been conducted in short-term incubation experiments, with mineralization dynamics deduced from CO_2 efflux and N_{min} changes. In the short-term, the releases of CO_2 and N_{min} reflect the decomposition kinetics of readily labile compounds (Gunina and Kuzyakov, 2015; Cyle et al.,

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2016), and thus poorly represent the complex components, which dominate plant residues and SOM. (2) In all incubation studies, the decomposition proceeds in soils without plant growth. In terrestrial ecosystems, microbial decomposition and plant nutrient uptake take place simultaneously, with yielded N_{min} being removed continuously from decomposition sites.

The present paper quantified the effects of soil N_{min} availability on the decomposition of SOM and pant residues in the presence of root N uptake. We hypothesized that N fertilization would decrease SOM and plant residue decomposition in soils with growing plants. Despite the fact that soil microbes preferentially use N_{min}, N_{min} deficiency leads to organic N uptake by microbes (Hobbie, 2005; Geisseler et al., 2009, 2010, 2012), which would facilitate N mineralization of SOM and plant residues. The increased N mineralization under low N_{min} would be linked with increasing organic C mineralization (Jonasson et al., 1999; Manzoni et al., 2010). We investigated the dynamics of various soil N pools, N uptake by wheat (*Triticum aestivum* L.) and traced the fate of N from applied plant residues and urea fertilizer by using ^{15}N as a tracer. The decomposition of SOM and plant residues was traced by the changes in light fraction N (LFN) and organic C (LFOC) during 127 days.

2. Materials and methods

2.1. Experimental design

The experiment was conducted from March to July 2016 in a large rainproof shelter at the Yuzhong Experimental Station (35°51′N, 104°7′S, altitude of 1620 m above sea level) of Lanzhou University. Soil was collected from the 0–20 cm depth in a cropland with wheat and soybean (Glycine max L. Merrill) growing for four years after conversion from native C3 grassland (no C4 species). The soil was developed from loess and had silt loam texture, with a pH value of 8.38 (water: soil = 2.5). Total SOC content was 7.43 g kg^{-1}, total N 0.64 g kg^{-1}, total P 0.70 g kg^{-1}. The C/N ratio of SOM was 11.6. Soil N_{min} and Olsen P were 12 and 6 mg kg^{-1}, respectively.

The pot experiment had five treatments: (1) Control (no plant residues and no N fertilization); (2) ^{15}N-urea (amended with ^{15}N labeled urea at 129 mg N kg^{-1} soil); (3) ^{15}N-leaves [amended with ^{15}N labeled maize (*Zea Mays* L.) leaves at 115 mg N kg^{-1} soil]; (4) ^{15}N-leaves plus urea (amended with ^{15}N labeled maize leaves at 115 mg N plus urea at 129 mg N kg^{-1} soil); and (5) ^{15}N-urea plus straw (amended with ^{15}N labeled urea at 129 mg N plus maize straw at 108 mg N kg^{-1} soil). In the fifth treatment (^{15}N-urea plus straw), we used the maize straw (mixture of maize leaves and stems) instead of maize leaves, because it corresponds more to the agriculture practice. The ^{15}N labeled maize leaves and (non-labeled) straw were collected at maturity, respectively from two field experiments conducted in 2015 at a high-altitude site (2013 m a. s. l.). In that ^{15}N labeling experiment, the maize was fertilized at sowing with ^{15}N labeled urea (^{15}N abundance: 10.15%). The ^{15}N labeled maize leaves contained 1.40% N (^{15}N abundance: 1.97%) and 45.2% C, with a C/N ratio of 32.4. The (non-labeled) maize straw had N content of 1.10% and C 43.0%, with a C/N ratio of 39.1. Maize residue materials were pulverized into powders (<1 mm). The urea application rate in this pot experiment was equivalent to 290 kg N ha^{-1}, maize leaf and straw application rates were equivalent to 19 and 22 t plant residues ha^{-1}, respectively.

Air-dried soil (equivalent to 6.72 kg oven-dry weight, sieved through 5 mm) was mixed with maize residue and/or fine urea powders (for Control, without residues or no N fertilizers) and then put in a plastic pot (height 22.5 cm; volume 6.8 L), resulting in a bulk density value of about 1.2 g cm^{-3}. In order to avoid the potential phosphorus deficiency for wheat growth, KH_2PO_4 (monopotassium phosphate) was added at 20 mg P kg^{-1} soil to all treatments (including control). Each treatment had five replicated pots (5 treatments × 5 replicates = 25 pots in total). After setup, all pots were watered. Twenty days later, 10 wheat (cultivar: Dingxi 24) seeds were sown in each pot. At the two-leaf stage, the crops were thinned to five seedlings per pot. Throughout the pot experiment, from setup to the time of wheat maturity, soil moisture was maintained at 70% of field capacity, with supplementation of distilled water every 1–3 days according to the water loss estimated by weighing. At day 63 after sowing (booting stage), a 1-cm layer of polythene polyfoam balls (3–4 mm in diameter) was placed on the soil surface and kept until the wheat's harvest time, to reduce soil water evaporation. Twenty-five pots were randomly placed in an area of 4 m^2 in the rainproof shelter. During the experiment, the air temperature measured daily at 09:00 varied from 10 °C to 36 °C.

2.2. Soil sampling

Five pots of each treatment were randomly divided into two groups: sampling group (three pots) and supplemental group (two pots). At days 32 (seedling stage), 63 (booting) and 107 (maturity) after sowing, soil samples were taken using an auger (inner diameter: 20 mm) from three pots of each sampling group, for measuring soil N_{min}, microbial biomass N (MBN) and LFN content, and ^{15}N abundances in all these pools. In each sampling pot, four sub-samples (from four points) were mixed to form a composite sample. After each sampling at days 32 and 63, the holes in the three sampling pots, formed due to the soil sampling, were filled with soils taken from two supplemental pots of the same treatment using the same auger. Plugging those holes with the substitute soils from the supplemental group reduced potential influence of disturbance of soil sampling on microbial activity and wheat growth.

For measuring the initial LFN and LFOC contents at the beginning of the experiment (at 20 days before wheat sowing), samples were taken from treated soils prior to being put into plastic pots. Each 100-g air-dried sample from those relative to the sampling group was placed in a beaker and incubated for 24 h under room temperature, after being moistened to 70% of field capacity. All incubated soils were then air-dried and prepared for analyses.

2.3. Soil analyses

Total N_{min} and MBN were determined from fresh soil samples. N_{min} was extracted using 0.5 M K_2SO_4 and total N_{min} in the extracts was distilled using Kjeldahl apparatus, in the presence of MgO (magnesium oxide) and Devarda alloy (Keeney and Nelson, 1982). Ammonium N in the distillate was titrated using H_2SO_4 solution. After titration, the distillate was condensed under acidic conditions in a water bath to 2–3 ml, to analyse ^{15}N isotope ratio via a gas isotope mass spectrometer (MAT-271, Thermo Fisher, America). The soil N_{min} derived from ^{15}N labeled sources (^{15}N-N_{min}) was calculated as:

$$^{15}N - N_{min} = \text{total } N_{min} \times (^{15}N\%_{treatment} - {}^{15}N\%_{control}) / \times (^{15}N\%_{source} - {}^{15}N\%_{control})$$

where $^{15}N\%_{treatment}$ and $^{15}N\%_{control}$ were ^{15}N abundances of total N_{min} in the ^{15}N source-added treatment and (non-added) control, respectively; $^{15}N\%_{source}$ was ^{15}N abundance in the ^{15}N source. The soil N_{min} derived from non-labeled sources was the difference between total N_{min} and ^{15}N-N_{min}.

Total soil MBN was extracted via the chloroform

fumigation–extraction method (Brookes et al., 1985a). The fumigated and non-fumigated samples were shaken for 1 h in 0.5 M K_2SO_4. Total (ammonium and organic) N in the K_2SO_4 extracts was distilled after Kjeldahl digestion, exactly following the procedure described by Brookes et al. (1985b). After titration of total NH_3 by using H_2SO_4, the distillate was condensed in a water bath for ^{15}N isotope ratio analysis. Total soil MBN was calculated by the difference between total N in the digested K_2SO_4 extracts from the fumigated and non-fumigated samples, and 0.54 for extract correction factor (Brookes et al., 1985a; Joergensen and Mueller, 1996). The soil MBN derived from labeled sources (^{15}N-MBN) was calculated:

$$^{15}\mathrm{N}-\mathrm{MBN}=\left(\text{total}\,^{15}\mathrm{N}_{\text{fumigated}}-\text{total}\,^{15}\mathrm{N}_{\text{non-fumigated}}\right)/0.54$$

where total $^{15}N_{fumigated}$ and total $^{15}N_{non\text{-}fumigated}$ were total N derived from ^{15}N source in the digested K_2SO_4 extracts from fumigated and non-fumigated samples, respectively. The total $^{15}N_{fumigated}$ or total $^{15}N_{non\text{-}fumigated}$ was calculated using an equation similar to that for calculating $^{15}N\text{-}N_{min}$. The soil MBN derived from non-labeled sources was the difference between total MBN and ^{15}N-MBN.

Soil light fraction organic matter (LFOM, density < 1.8 g cm^{-3}) was separated using dense NaI solution (Hai et al., 2010). A 50-g air-dried soil sample (<2 mm) was placed in a 250-ml centrifuge bottle, to which 125 ml of NaI was added. The sample was stirred for 1 min with a glass stick. Material adhering to the wall of the bottle was rinsed into the solution with an additional 10 ml of NaI. After standing overnight, the sample was centrifuged for 30 min at 3000 rpm. Immediately after centrifugation, the supernatant was filtered through a 0.45-μm hydrophilic polyvinylidene fluoride filter under vacuum, and material retained on the filter (LFOM) was rinsed with distilled water until the water ran clear. After being oven-dried to constant weight at 60 °C, the separated soil LFOM was ground to fine powder. About 200 mg LFOM powders was digested in concentrated H_2SO_4 and H_2O_2 (Lu, 2000), and the N in the digest was distilled using micro Kjeldahl apparatus. After titration, the distillate was condensed in a water bath for ^{15}N isotope ratio analysis. The ^{15}N source derived soil LFN (^{15}N-LFN) was calculated using a formula also similar to that for calculating $^{15}N\text{-}N_{min}$. Non-labeled soil LFN was the difference between total LFN and ^{15}N-LFN.

We used the $^{13}C/^{12}C$ ratio of the total LFOC pool to distinguish maize-from SOM-derived LFOC sub-fractions at the beginning and end of the experiment. The C content in the LFOM was measured using an Elementar Analyzer (Vario Macro Cube, Germany), and the $^{13}C/^{12}C$ ratio was analyzed using an Isotope Ratio Mass Spectrometer (DELTA V Advantage, USA). The $\delta^{13}C$ values of LFOM samples were expressed relative to the isotopic ratio of $^{13}C/^{12}C$ in Pee Dee Belemnite (0.011237). The maize-derived LFOC was calculated as

$$\text{Maize}-\text{derived LFOC}=\text{total LFOC}\times\left(\delta^{13}\mathrm{C}_{\text{treatment}}-\delta^{13}\mathrm{C}_{\text{control}}\right)/\left(\delta^{13}\mathrm{C}_{\text{residue}}-\delta^{13}\mathrm{C}_{\text{control}}\right)$$

where $\delta^{13}C_{treatment}$ and $\delta^{13}C_{control}$ represented the $\delta^{13}C$ values of LFOM in the maize residue (leaves or straw) amended treatments and in the control, respectively. $\delta^{13}C_{residue}$ was the $\delta^{13}C$ value of maize leaves or straw. The SOM-derived LFOC was the difference between total LFOC and maize-derived LFOC.

2.4. Plant sampling and analysis

At day 63 after wheat sowing, the aboveground biomass and roots were destructively sampled from pots of supplemental groups. At the time of wheat maturity, grains, stems and leaves (including glumes) and roots were collected from all pots in the sampling group. Roots were washed of soil with a sieve (aperture: 0.15 mm). All plant organs were oven-dried at 60 °C to a constant weight and ground to powders. Total N in various plants was digested in concentrated H_2SO_4 and H_2O_2 (Lu, 2000) and total N was distilled using micro Kjeldahl apparatus. After titration, the distillate was condensed for ^{15}N isotope ratio analysis.

2.5. Statistics

One-way analysis of variance (ANOVA) was used to assess the effects of treatments on wheat N uptake by the booting stage and harvest time of wheat. One-way ANOVA was also used to assess the treatment effects on the decomposition percentages of LFN and LFOC and their sub fractions. Two-way ANOVA, using treatment and sampling time as two fixed factors, was applied to assess variations in the measured soil N_{min}, MBN, LFN, LFOC and their sub-fraction contents. The least significant difference was used to identify the significance of variation between treatments at $P \leq 0.05$. All statistical analyses were performed in GenStat 17.0 (VSN International Ltd. Rothamsted, England).

3. Results

3.1. Wheat nitrogen uptake

Compared to control, ^{15}N-leaves decreased the total N uptake in wheat biomass (including roots) in the early growth. However, by the time of harvest, total N uptake increased by 71% in the soil with ^{15}N-leaves compared to the control (Fig. 1a and b). By either wheat booting or maturity stage, the total N uptake by wheat was greater in ^{15}N-leaves plus urea and ^{15}N-urea plus straw soils, respectively, than in ^{15}N-leaves soil (Fig. 1a and b).

Urea slightly increased the wheat N use from the ^{15}N-leaves and prominently increased the N uptake from the other sources (including urea) by booting stage. However, urea increased the wheat N uptake only from the other sources (including urea) by wheat maturity (Fig. 1c, d, e, f). In the two soils with ^{15}N-urea added, maize straw amendment decreased the wheat N uptake from the ^{15}N-urea but increased the wheat N uptake from the other sources (including straw) in the early and at the end of growth (Fig. 1c, d, e, f).

3.2. Dynamics of soil microbial biomass and mineral nitrogen

There were significant interactions between treatment and sampling time on total N and ^{15}N in microbial biomass (Fig. 2a, b, c) ($P < 0.001$). During wheat growth, only residue amendments increased total soil MBN content compared to the control (Fig. 2a). This indicated that only C availability limited soil microorganisms. In the soil with ^{15}N-leaves added only, total soil MBN dropped sharply from 51 mg N kg^{-1} at day 63 after wheat sowing, to 25 mg N kg^{-1} at harvest (Fig. 2a), reflecting N redistribution from microorganisms to the plants.

In the two soils with ^{15}N-leaves added, urea addition decreased the microbial use of N from the ^{15}N-leaves, but increased the N uptake from other sources (including urea) (Fig. 2b and c). In the two soils with added ^{15}N-urea, straw addition increased the microbial use of N from both ^{15}N-urea and other sources (including straw) compared to no straw addition (Fig. 2b and c). However, the

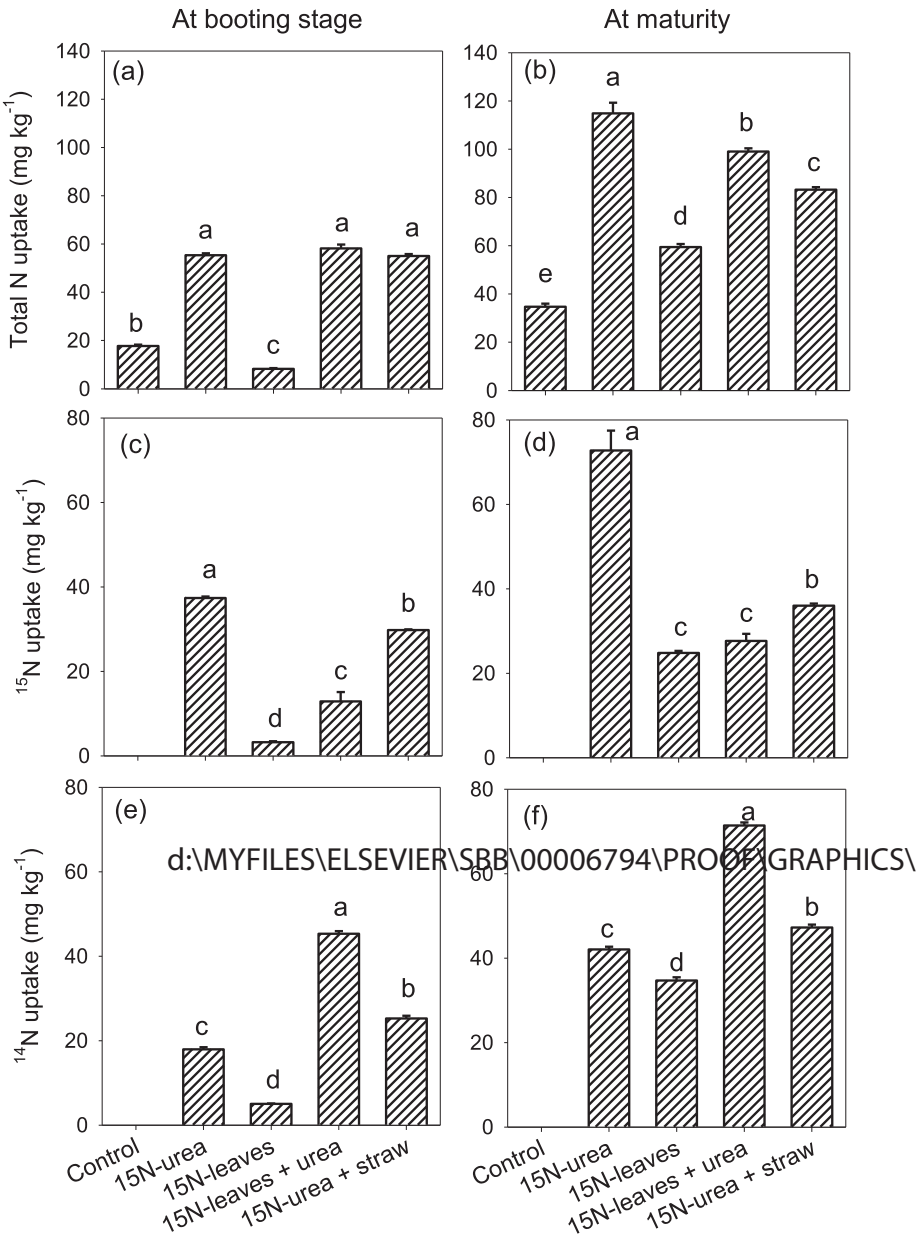

Fig. 1. Effects of N and plant residue addition on wheat N uptake and its partitioning by ^{15}N tracer at wheat booting and maturity. (a) and (b): total N uptake; (c) and (d): N uptake from ^{15}N-labeled sources; (e) and (f): N uptake from non-labeled sources. Mean values with lower-case letters indicating significant differences between the treatments at $P \leq 0.05$. Bars are the means ± 1 standard error ($n = 2$ at booting stage and 3 at maturity).

increment from other sources (mainly organic N) was greater than that from the ^{15}N-urea (Fig. 2b and c).

The total N_{min} concentration during wheat growth was highest in the soil with urea, followed by those in the soils with urea combined with plant residues and lowest in the soil of control or with ^{15}N-leaves added only (Fig. 2d). Urea addition increased ^{14}N-N_{min} content in the soils with ^{15}N-leaves added (Fig. 2e). Straw addition decreased ^{15}N-N_{min} content in the soils with ^{15}N-urea added (Fig. 2f).

3.3. Dynamics of C and N in the light organic fraction

The dynamics of total LFN and its sub-fractions during the experiment are shown (Fig. 2g, h, i). Total LFN and ^{14}N-LFN decreased in all soils with time (Fig. 2g, i). ^{15}N enrichment of LFN decreased in the two soils with ^{15}N-leaves added but increased with time in the other two soils with ^{15}N-urea added, (Fig. 2h). This showed that the organic N release from the LFOM and the N_{min} immobilization in the LFOM occurred simultaneously.

Over the 127-day period, total LFN decomposition (difference in the content between the beginning and end of the experiment) was 58 mg N kg^{-1} in the control soil; in contrast with 10 mg N kg^{-1} in the soil with ^{15}N-urea added only (Fig. 2g). The total LFN decomposition was 72 mg N kg^{-1} in the soil with ^{15}N-leaves added only, compared to 56 and 44 mg N kg^{-1} in the soils with ^{15}N-leaves plus urea and ^{15}N-urea plus straw, respectively (Fig. 2g).

There were significant interactions between treatment and sampling time on total soil LFOC content and the C/N ratio of LFOM ($P < 0.001$) (Fig. 3a and b). Similar to that of total LFN, the decomposed total LFOC during the 127-day period was greater in the soils without urea than in the soils with urea addition, regardless of the presence of maize residues (Fig. 3a). By the end of

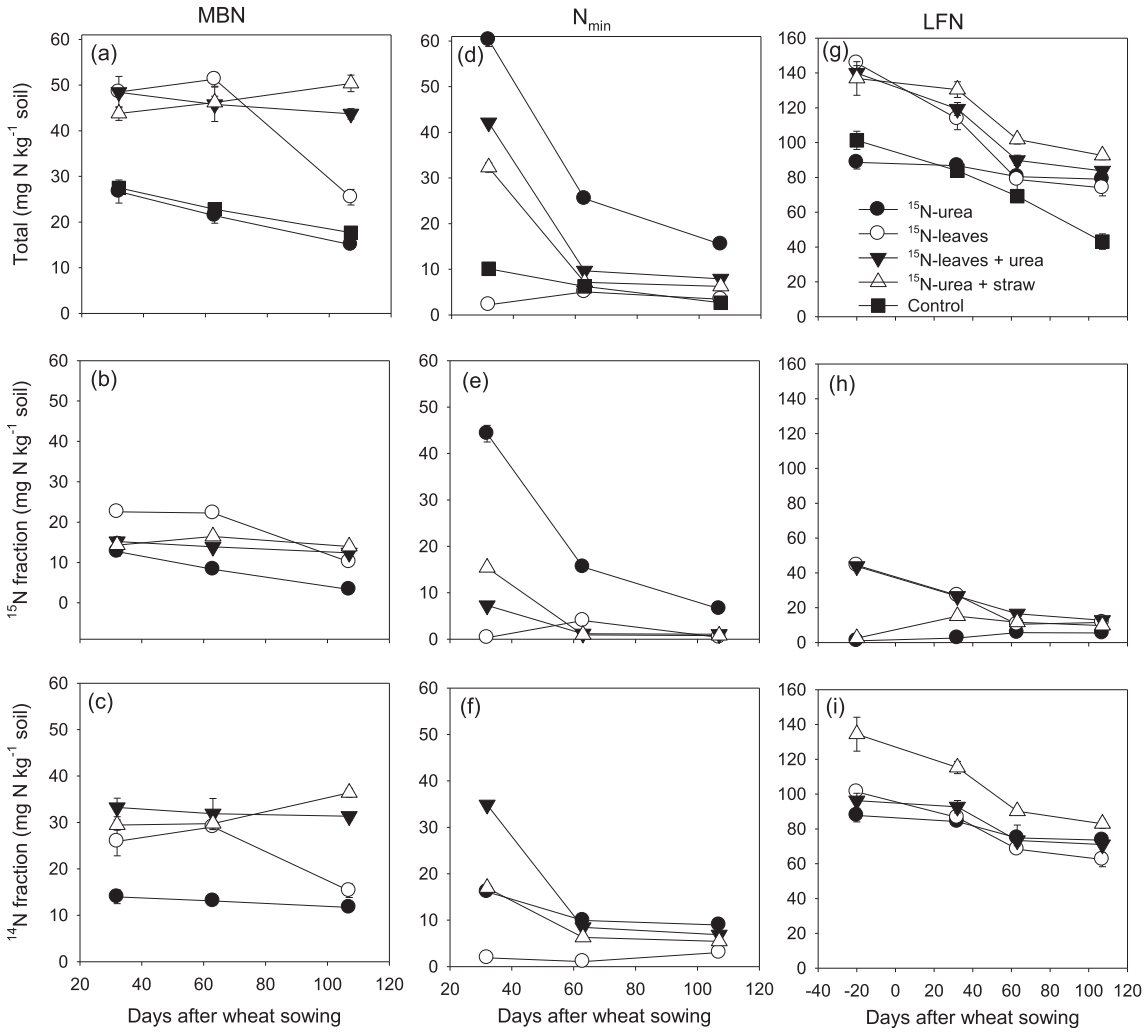

Fig. 2. Dynamics of soil microbial biomass N (MBN), mineral N (N_{min}), and light fraction N (LFN), and their partitioning by ^{15}N tracer during wheat growth. (a), (d) and (g): total pools; (b), (e) and (h): fractions derived from ^{15}N-labeled sources; (c), (f) and (i): fractions derived from Non-labeled sources. Bars are the means ± 1 standard error ($n = 3$).

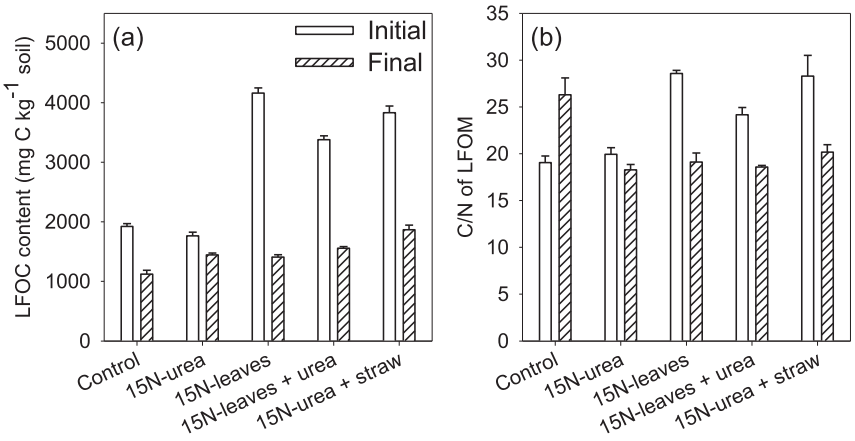

Fig. 3. Effects of N and plant residue addition and sampling time on light fraction organic carbon (LFOC) content and C/N ratio of light fraction organic matter (LFOM). (a): LFOC content; (b): C/N ratio of LFOM. Initial: at the beginning of experiment; Final: at wheat harvest. Bars are 1 standard error ($n = 3$).

the experiment, the control soil had the C/N ratio of total FLOC increased but other soils had the C/N ratio decreased (Fig. 3b).

The decomposed portions of total LFN and LFOC were both smaller in the soils with urea than without urea, independent on residue addition (Fig. 4a and b). The source partitioning based on ^{15}N and ^{13}C/^{12}C ratio showed that the urea-fertilization decreased the decomposition of both maize- and SOM-derived LFOM fractions (Fig. 4c, d, e, f). The decomposition percentage of total LFN was

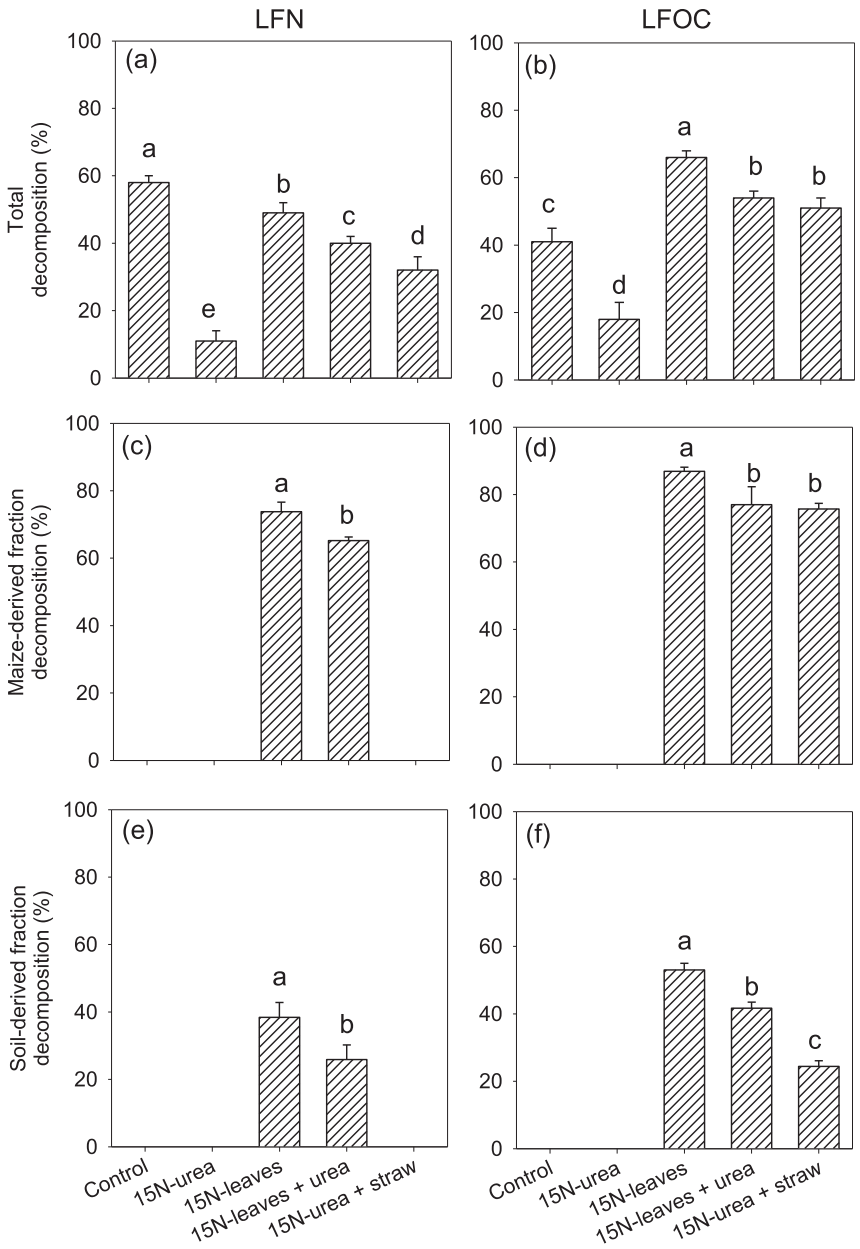

Fig. 4. Effects of N and plant residue addition on the decomposition percentages of light fraction N (LFN) and organic C (LFOC) and their separations partitioned by ^{15}N tracer and $^{13}C/^{12}C$ ratio, over the initial amounts. (a) and (b): total pools; (c) and (d): maize-derived fractions; (e) and (f): soil-derived fractions. Different lower-case letters show that mean values are significantly different from one another at $P \leq 0.05$. Bars are 1 standard error ($n = 3$).

negatively correlated to total N uptake by wheat at maturity, whereas it was positively correlated to that of total LFOC (Fig. 5a and b).

4. Discussion

4.1. Wheat nitrogen uptake

In the soil with ^{15}N-leaves addition, the decrease in the total wheat N uptake by booting stage was ascribed to the lower N_{min} availability compared to the control soil (Fig. 1a; Fig. 2d). Organic N may slightly contribute to plant N uptake (Persson and Näsholm, 2001; Näsholm et al., 2009), but the quantitative importance is negligible (Inselsbacher et al., 2010; Biernath et al., 2008; Rasmussen and Kuzyakov, 2009; Rasmussen et al., 2010). Moran-Zuloaga et al. (2015) and Huygens et al. (2016) even suggested that plants depend exclusively on N_{min} forms made available through microbial N mineralization of plant residues and SOM. The decreased N_{min} availability in the soil with ^{15}N-leaves added only resulted from microbial community increase (i.e., MBN content) compared with the control (Fig. 2a; Fig. 2d). The incorporation of crop residues in soil usually decreases N_{min} due to microbial immobilization, and thus intensifies the competition for N_{min} between microorganisms and plants (Williams et al., 1968; Recous et al., 1995; Corbeels et al., 2000).

By the time of wheat harvest, the total N uptake by wheat from the soil with ^{15}N-leaves added-only increased compared to that in the control soil (Fig. 1b). The occupation of soil volume by growing roots increased, and so, made plants more competitive for N_{min} compared to microbes (Xu et al., 2011; Kuzyakov and Xu, 2013). In

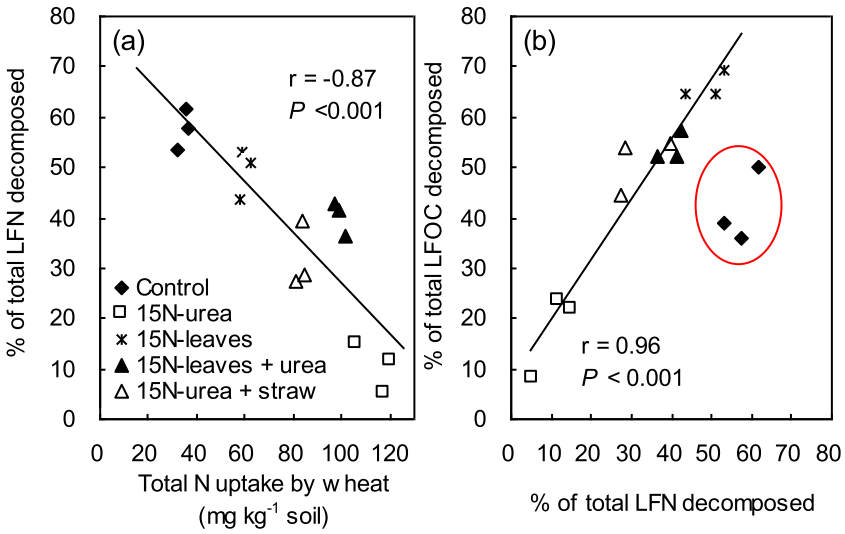

Fig. 5. Correlations of total light fraction N (LFN) decomposition with total N uptake by wheat and total light fraction organic carbon (LFOC) decomposition across all pots during the experiment. (a): Percentage of total LFN decomposed versus total N uptake by wheat; (b): percentage of total LFOC decomposed versus that of total LFN. The points in oval of the figure (b) show that the Control soil (without urea and no maize residue addition) is strongly different from the other soils (with urea and/or maize residues), in the extent of synchronization between LFN and LFOC decomposition.

addition, N assimilated in microbial biomass during the early wheat growth might have been released through microbial turnover in the late growth (Kuzyakov and Xu, 2013). Microbial necromass is more decomposable than plant residue in residue incorporated soils (Jensen, 1994).

In all soils with maize residues, an increase in the total wheat N uptake after urea fertilization was obviously due to the increased soil N_{min} (Fig. 1a and b; Fig. 2d). In the two soils with ^{15}N-urea, the decreased ^{15}N use by wheat after straw addition was due to ^{15}N immobilization in microbial biomass (Fig. 2b, e).

4.2. Soil organic matter decomposition

The present study clearly showed that N fertilization decreased SOM decomposition in soils with plants. This finding was indicated by the decreased decomposition of LFOM in urea amended than in non-amended soils, regardless of whether maize residues were added (Fig. 2g; Fig. 3a; Fig. 4a and b). In the soils with maize residues, both maize- and SOM-derived LFOM fractions decomposed less, under high than under low N_{min} levels (Fig. 4c, d, e, f). The N amount recovered in the wheat biomass is a suitable indictor for soil N_{min} availability. Thus, LFN decomposition was negatively correlated to N uptake by wheat (Fig. 5a). The density fractionation is often used in SOM and plant residues decomposition studies (Francois et al., 1991; Neff et al., 2002; Wichern et al., 2006; Zareitalabad et al., 2010; Gunina and Kuzyakov, 2014). LFOM generally holds a small but the most labile portion of the total SOM. In the soil used for the present study, LFOC and LFN accounted for 26% and 16% of the total SOC and N, respectively. This LFOM is more readily available for microbes than are the mineral-associated SOM pools. The LFOM is also sensitive to management practices; consequently, it is an early indicator for management effects (Gregorich et al., 1994; Haynes, 2005; Von Lützow et al., 2007).

The decrease of SOM decomposition with increasing N availability in the present study was contradictory to a stimulating effect of an increase in soil N_{min} on the decomposition of plant residues or SOM, as suggested by some other studies (Recous et al., 1995; Mary et al., 1996; Henriksen and Breland, 1999; Neff et al., 2002; Potthoff et al., 2005). In fact, the effect of mineral N on plant residues or SOM decomposition is variable (Neff et al., 2002; Hobbie, 2005; Hobbie et al., 2012) and negative effects are frequently reported (Hobbie, 2005; Bradford et al., 2008; Janssens et al., 2010; Rousk et al., 2011; Riggs et al., 2015). After a close examination of these results, Zang et al. (2016) concluded that increasing addition of N as NO_3^- or NH_4^+ exponentially decreases SOM decomposition.

In the present study, MBN was unaffected by soil N_{min} availability (Fig. 2a, b, c, d, f). On the contrary, the microbial biomass affected N_{min} level in soil. Urea addition decreased the microbial use of N from ^{15}N-leaves in soils with ^{15}N-leaves added, where total MBN was similar in the early and middle wheat growth. Straw-addition induced increase in the total MBN in the whole wheat growth originated more from organic sources than form ^{15}N-urea in soils with ^{15}N-urea added. ^{15}N-urea addition increased N_{min}, but straw addition decreased N_{min} level in soil. Therefore, under low N_{min} levels, soil microorganisms used more N from organic sources than from inorganic sources. Soil microorganisms use both organic N and N_{min} (Barraclough, 1997; Geisseler et al., 2009, 2010, 2012; Inselsbacher et al., 2010). Soil microorganisms take up either N_{min} after mineralization (through mineralization–immobilization turnover route, MIT route) or low-weight organic N-containing molecules (direct route) (Drury et al., 1991; Mary et al., 1996; Barraclough, 1997; Geisseler et al., 2009, 2010, 2012). Under low N_{min} availability, the direct route is a preferred pathway for microbial assimilation of N (Hobbie, 2005; Geisseler et al., 2009, 2010, 2012).

Thus, in the N_{min} limited soils, the direct uptake of N-containing organic compounds helps microbes to increase or maintain the size of their community. After microbial death, the fate of debris-N includes being (i) re-used by microbes through direct and/or MIT routes, (ii) taken up by plants after N mineralization, and (iii) incorporated into soil mineral-associated and/or LFOM fractions (Francois et al., 1991; Vogel et al., 2015; Cyle et al., 2016). The plant uptake of N mineralized from organic sources would reduce the N_{min} supply for microbes (Fig. 6). This would in turn push microbes to increase their mineralization of SOM and direct use of N-containing organics. The lower the N_{min} availability in soil, the stronger the shift of microbial N uptake towards the direct route and thus, the greater amount of organic N-containing compounds was

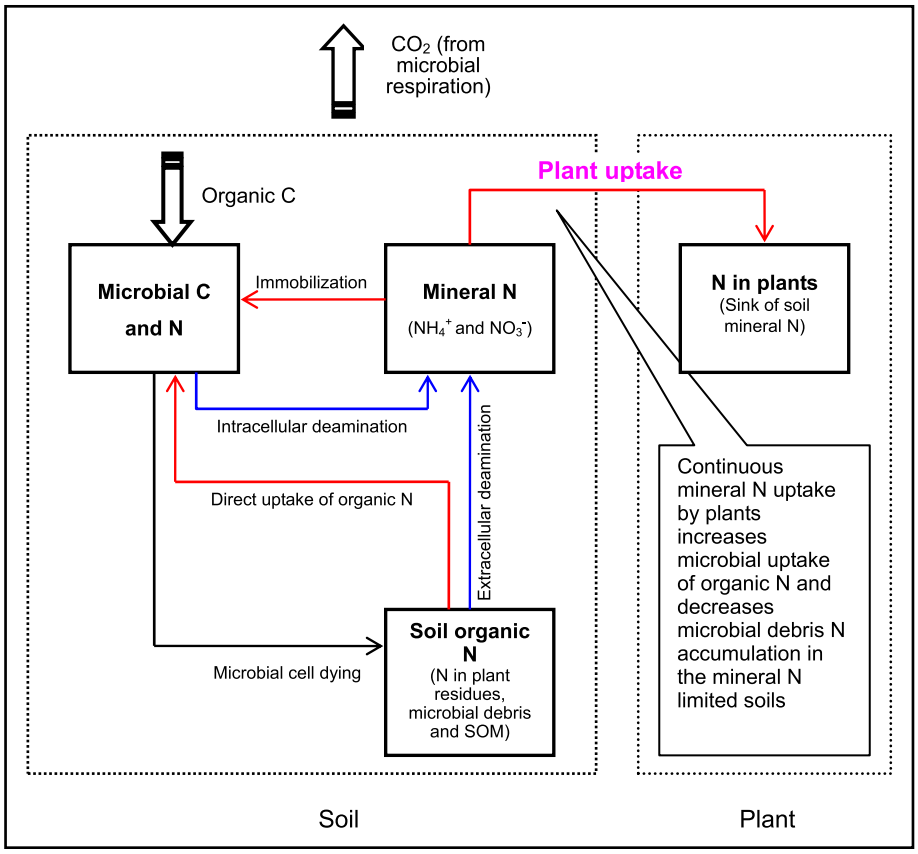

Fig. 6. A hypothesized mechanism to explain the effect of mineral N availability on N mineralization in plant growing soils. To mine N in the N_{min} limited soils, soil microbes might have directly taken up more N-containing organics and thus facilitated SOM decomposition. For such an acceleration of SOM decomposition, the presence of living plants was especially important, which decreased the N_{min} in soil and so, increased N limitation for microorganisms.

mineralized (Fig. 6).

The close correlation in the decomposition between LFN and LFOC (Fig. 5b) indicated that the C decomposition was tightly coupled to that of N in the present study. Riggs et al. (2015) and Spohn (2015) proposed that high N_{min} availability leads to the lower microbial utilization of C for respiration and higher C allocation to growth. Spohn et al. (2016) further suggested that under high N availability, microbes do not only respire less but also take up less C, resulting in an increase in the microbial C use efficiency.

The synchronization between decomposition of LFN and LFOC in the control soil was different from that in the other treatments, with the C/N ratio in the LFOM increased after decomposition (Fig. 3b; Fig. 5b). This result indicates that without exogenous N input to planted soil, the direct microbial use of N-containing organics (with a low C/N ratio) during decomposition led to a small C loss per unit of N, leaving the increase C/N ratio of LFOM remains. Specific microbial species might have survived and met their both N and C demands relying on an assimilation of N-containing organics (Goddard and Bradford, 2003; Geisseler et al., 2009). The increased C/N ratio of LFOM in the soils without N fertilization compared to the N-fertilized soils has been frequently reported in long-term field experiments (Hai et al., 2010; E et al., 2012).

In conclusion, urea fertilization decreased the decomposition of plant residues and SOM in soils with growing plants. Microbial use of N was unaffected by N_{min} level. Under low N_{min} levels, the microorganisms used more N originated from organic sources than from added fertilizer. We speculated that the microbial direct uptake of N-containing organic molecules might have facilitated SOM decomposition in the N_{min} limited soils. The mechanisms of the coupling between LFN and LFOC decomposition need to be further investigated. Continuous plant uptake of N_{min} stimulated SOM and plant residue decomposition. Therefore, studies on SOM mineralization should be done in the presence of living plants.

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