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# Interactive effects of crop residue quality and nitrogen fertilization on soil organic carbon priming in agricultural soils

Qian Ma<sup>1</sup>  $\cdot$  Tetsuhiro Watanabe<sup>1,2</sup>  $\cdot$  Jinsen Zheng<sup>1,3</sup>  $\cdot$  Shinya Funakawa<sup>1,2</sup>

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## Abstract

Purpose Soil organic carbon (SOC) priming affects C sequestration in soils, the intensity of which differs depending on residue quality. N fertilization could also alter SOC priming. However, the interaction of crop residue quality and N fertilization on the SOC priming is still not clear. To address this gap in knowledge, we conducted this study.

Materials and methods We undertook a 110-day laboratory incubation experiment to evaluate the SOC priming and sequestration induced by maize shoot and root residues with and without the application of mineral fertilizer-N in two types of agricultural soils (Andisol and Entisol). Application rates of maize residue and N were 3 g C kg<sup>-1</sup> soil and 60 mg N kg<sup>-1</sup> soil, respectively.  $13C$ -labeled maize residue allowed quantifying residue decomposition and calculating SOC priming and sequestration.

Results and discussion After 110 days of incubation, the cumulative intensity of priming effect was higher for root residue than shoot residue. Addition of N results in contrasting effects on the priming effect induced by root and shoot residue in both types of soils; with root residue, it reduced the intensity of priming effect and resulted in a higher net C sequestration because of reduced N mining, whereas it had little effect with shoot residue, where co-metabolism is the likely explanation for the positive priming effect.

Conclusion Crop residue quality and N fertilization can interactively affect the SOC priming. N fertilization is beneficial for soil C sequestration when soil is treated with low-quality crop residue (e.g., root residue) because of lowering of the intensity of priming effect and crop residue decomposition.

Keywords Crop residue return  $\cdot$  Crop residue quality  $\cdot$  N fertilization  $\cdot$  Priming effect  $\cdot$  Soil C sequestration

# 1 Introduction

Crop residues are the by-products of agriculture and the main C source for arable soils. In general, it is recommended that

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- <sup>2</sup> Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
- <sup>3</sup> Center for Ecological Research, Kyoto University, Shiga 520-2113, Japan

crop residues are returned to the soil to increase soil organic carbon (SOC) storage and maintain soil fertility (Liu et al. [2014;](#page-11-0) Jin et al. [2020](#page-11-0)). Fresh organic matter (FOM) inputs to soil may alter native SOC mineralization; this changes in the SOC mineralization caused by the FOM added to the soil is called priming effect (Kuzyakov et al. [2000](#page-11-0)). The increase or decrease in SOC mineralization, compared with soil without FOM addition, is termed as positive or negative priming effect, respectively. Most of the previous studies have shown that FOM inputs have high potential to accelerate soil organic matter (SOM) mineralization (Wang et al. [2015;](#page-11-0) Lenka et al.  $2019$ ), thus resulting in a higher  $CO<sub>2</sub>$  emission and adversely affecting on global climate change (Kuzyakov [2010](#page-11-0); Zhang et al. [2013\)](#page-12-0). Further, it has been reported that the crop residue return may also result in a decrease in the SOC (Fontaine et al. [2004;](#page-10-0) Kirkby et al. [2014\)](#page-11-0), as the loss of native SOC through priming effect can exceed the newly formed SOC (Fontaine et al. [2004](#page-10-0)). To design effective strategies of crop residue management for avoiding adverse impact of the priming effect

 $\boxtimes$  Oian Ma [maqian6677@gmail.com](mailto:maqian6677@gmail.com)

<sup>&</sup>lt;sup>1</sup> Graduate School of Global Environmental Studies, Kyoto University, Kyoto 606-8502, Japan

on SOC mineralization, an improved understanding of how crop residue return affects the intensity of priming effect, and the subsequent net C balance is needed.

Different types of crop residue differ in quality, which is defined by chemical composition (e.g., lignin content) and stoichiometry (C/N ratio) and plays an important role in SOC mineralization through the priming effect (i.e., SOC priming) (Wang et al. [2015;](#page-11-0) Schmatz et al. [2016\)](#page-11-0). However, the intensity of priming effect induced by residues with different qualities does not seem to be consistent; higher-quality FOM (which usually have lower C/N ratio and more labile compounds) added to soil can lead to either a lower (Shahbaz et al. [2017](#page-11-0)), an equal (Guenet et al. [2010](#page-11-0); Xu et al. [2018\)](#page-12-0), or a higher (Mwafulirwa et al. [2019](#page-11-0)) positive priming effect than lower-quality FOM which has higher C/N ratio and more recalcitrant compounds. These inconsistent findings of crop residue quality on priming effect may come from the different durations of incubation and soil N availability (Wang et al. [2016;](#page-11-0) Xu et al. [2016](#page-12-0)). Considering that the residue quality changes during decomposition and the recalcitrance of residue increases over time (Hadas et al. [2004\)](#page-11-0), the priming effect in later slow decomposition stage of residue may be different from that in their early intensive decomposition stage. Thus, a relatively long-term incubation (e.g., several months) covering the slow decomposition stage of residue is necessary to precisely estimate the priming effect compared with shortterm incubation study covering only intensive decomposition stage of residue.

Nutrient availability, especially N, can be another key factor influencing SOC priming. Yet the SOC priming as a function of N availability has not been fully understood (Blagodatskaya et al. [2009](#page-10-0)). Globally, agroecosystems are experiencing increasing N inputs because of N deposition and fertilization (e.g.,  $NH_4^+$ -N and  $NO_3^-$ -N) (Galloway et al. [2008](#page-11-0)). Increased N availability can significantly influence SOC cycling because they have close interaction (Janssens et al. [2010\)](#page-11-0). Previous studies have revealed that N addition significantly decreases the positive priming effect induced by maize stalk addition (Wang et al. [2016\)](#page-11-0) and can even result in a negative priming effect (Qiu et al. [2016\)](#page-11-0). On the other hand, Chen et al.  $(2014)$  $(2014)$  and Meng et al.  $(2017)$  $(2017)$  showed that N addition had a minimal effect on the intensity of positive priming effect induced by maize residue addition. In fact, most previous studies have only examined the effect of N addition on priming effect using a single crop residue, making it difficult to compare this effect among different types of crop residue and thus preventing a full examination of the potential interactions between N availability and crop residue quality in altering SOC priming.

Based on our current understanding, microbial N mining and co-metabolism are frequently used explanations for observations of positive priming effect with FOM addition. Microbial N mining is the process where microorganisms

enhance the activity of extracellular enzymes to acquire N from SOM when N is limited for supporting microbial growth in the soil system. This, therefore, promotes SOM mineralization (Fontaine et al. [2011;](#page-10-0) Chen et al. [2014\)](#page-10-0). Co-metabolism is the enhancement of SOC mineralization due to acceleration of microbial growth and activity with the addition of FOM (Blagodatskaya and Kuzyakov [2008\)](#page-10-0); microorganisms feeding on FOM could also decompose similar compounds in SOM and then enhance SOM mineralization. Among these two mechanisms, microbial N mining is closely related to the availability of N (Craine et al. [2007](#page-10-0); Aye et al. [2018\)](#page-10-0).

To better understand the effect of crop residue return on SOC priming and sequestration, it is necessary to investigate the combined effects of crop residue quality and N fertilization on SOC turnover over a long time frame including slow decomposition stage of crop residue. The objective of this study was to assess the interactive effects of maize (Zea mays L.) residue quality (shoot residue vs. root residue) and mineral N addition on the SOC priming in two types of agricultural soils (Andisol and Entisol) through a laboratory incubation experiment that covered slow decomposition stage of residue. Maize shoot has lower C/N ratio and more labile compounds compared with the root, and thus they were used to represent crop residues with different qualities.  $^{13}$ C-labeling of these residues allowed distinguishing between mineralized C from maize residue and native SOC to quantify SOC priming and sequestration. We hypothesized that (1) the addition of N would reduce the positive priming effect induced by residue treatment through decreasing N mining from SOM, and this reducing effect would be less apparent for shoot residue treatment because of its higher N content, and (2) maize shoot residue would cause higher priming effect in the early phase of incubation because of co-metabolism due to its high decomposability, while the root residue would cause higher priming effect over time because of its slower decomposition and microbial N mining stimulated by its lower N content.

# 2 Materials and methods

#### 2.1 Soil collection and characteristics

Soil samples (0–10 cm) were taken from two farmlands used for vegetable cultivation. One was located in the Nagano Prefecture, Japan (36° 31′ N, 138° 21′ E), where the soil was derived from volcanic ash and classified as Andisol (Soil Survey Staff [2014](#page-11-0)). The other was located in the Kyoto Prefecture, Japan (35° 3' N, 135° 48' E), where the soil was classified as Entisol (Soil Survey Staff [2014\)](#page-11-0). After air-drying, soils were sieved  $(\leq 2$  mm), and visible organic residues were eliminated prior to the incubation experiment. Selected physicochemical properties of the two soil types are shown in Table [1.](#page-2-0)

<span id="page-2-0"></span>Table 1 Properties of soils and maize residues used for the incubation experiment



EOC water-extractable organic carbon, EN water-extractable nitrogen

# 2.2 Production of <sup>13</sup>C-labeled maize residue

Maize plants were cultivated in potted trays filled with perlite and vermiculite, irrigated with Hoagland's nutrient solution (N, 210 mg L<sup>-1</sup>; P, 31 mg L<sup>-1</sup>; K, 234 mg L<sup>-1</sup>; Ca, 200 mg L<sup>-1</sup>; Mg, 48 mg L<sup>-1</sup>; S, 64 mg L<sup>-1</sup> in addition to micronutrients) once a week after germination, and grown at 25 °C (12 h day/12 h night) in a biotron (NC350HC; Nippon Medical & Chemical Instruments Co. Ltd., Osaka, Japan), which provided light intensity at 800-µmol photons  $m^{-2}$  s<sup>-1</sup>. The pulse labeling of the maize plants with  ${}^{13}CO_2$  (99 atom  $%$  $13$ C, Tomoe Shokai Co. Ltd., Japan) started from 10 days after germination and was conducted twice a week for 1 month. In each pulse labeling event, the plants were transferred to a portable labeling chamber which was sealed airtight with silicone rubber. Pulse of  ${}^{13}CO_2$  was generated by injecting 120 mL of  ${}^{13}CO_2$  (99 atom %) with a 60-mL syringe. The chamber air was circulated using two battery-operated minifans. The chamber air was sampled several times (5 mL, using a gas-tight syringe) for monitoring the  $CO<sub>2</sub>$  concentration (using a gas chromatograph; GC-2014, Shimadzu Inc., Kyoto, Japan), which temporarily reached 700–900 ppm and then decreased. To maximize the uptake of  ${}^{13}CO_2$  in each pulse labeling, the chamber was kept sealed for 6–8 h with an additional injection of  ${}^{12}CO_2$  (60–120 mL) in between to maintain a proper  $CO<sub>2</sub>$  concentration for maize growth.

After harvest, maize shoots and roots were washed, dried at 70 °C for 1 week, and milled to pass through a 2-mm sieve prior to incubation. A subsample of about 10 mg of the residue was used for the determination of C and N contents and  $^{13}$ C isotope abundance using an elemental analyzer connected to an isotope ratio mass spectrometer (EA-IRMS) (Delta V advantage, Thermo Fisher Scientific, MA, USA). For measuring extractable organic C (EOC) and extractabe N (EN) in the maize residue, 20-mL deionized water was added to 1.0 g of the residue, shaken for 2 h at 120 rpm and then filtered through a filter paper (No. 6, Advantec, Tokyo, Japan) (Surey et al. [2020\)](#page-11-0). The obtained extracts were analyzed for EOC and EN content using a TOC analyzer (Shimadzu TOC- $V_{CSH}$ , Shimadzu Inc.). The characteristics of the maize shoot and root residues are given in Table 1.

#### 2.3 Soil incubation and experiment design

The experiment was established in 275-mL jars (Toyo Glass Co. Ltd., Japan) with a gas-tight lid, each containing 45 g of air-dried soil. The soil was pre-incubated at 55% of its water holding capacity for 7 days to avoid a flush in microbial respiration induced by rewetting (Shi and Marschner [2017\)](#page-11-0). Nine treatments with three replicates in each type of soil were set up: neither N nor maize residue was added (control), NH<sub>4</sub><sup>+</sup>-N amended soil (NH<sub>4</sub>), NO<sub>3</sub><sup>-</sup>-N amended soil (NO<sub>3</sub>), maize shoot residue amended soil (SR), maize shoot residue +  $NH_4^+$ -N amended soil (SR + NH<sub>4</sub>), maize shoot residue +  $NO<sub>3</sub><sup>-</sup>-N$  amended soil  $(SR + NO<sub>3</sub>)$ , maize root residue amended soil (RR), maize root residue + NH<sub>4</sub><sup>+</sup>-N amended soil ( $RR + NH<sub>4</sub>$ ), and maize root residue +  $NO<sub>3</sub><sup>-</sup>-N$  amended soil  $(RR + NO<sub>3</sub>)$ . The two N sources,  $NO<sub>3</sub><sup>-</sup>-N$  and  $NH<sub>4</sub><sup>+</sup>-N$ , were applied at 60 mg N kg<sup>-1</sup> soil as KNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, respectively. The source of C was either the maize shoot residue or the root residue, which were applied at 3.00 g C kg<sup>-1</sup> soil (i.e., 0.326 g of shoot residue or 0.327 g root residue in each glass jar). The soil was thoroughly mixed with the maize residue after injecting the solution that contained the N sources corresponding to each treatment; soil water content was then adjusted to 60% of the water holding capacity. Each glass jar included a plastic bottle containing 10-mL 1- M NaOH solution to trap  $CO<sub>2</sub>$  derived from the mixed soil and a glass vial containing 5-mL 5-mM HCl to retain soil moisture. Five glass jars with plastic bottle and glass vial but without soil were treated as blanks. The jars were tightly closed with an airtight cap and incubated in the dark at 25 °C throughout the 110 days of the experiment in an incubator (LTI-1200, Eyela, Tokyo, Japan). The airtight cap was used to ensure the full trapping of the mineralized C derived from SOC and maize residue in the NaOH solution without being interfered by the atmospheric  $CO<sub>2</sub>$  during the incubation (the potential contamination of atmospheric  $CO<sub>2</sub>$  during sampling was corrected by the blanks; see below in detail). Even though the jars were sealed, the  $O<sub>2</sub>$  content in the sealed glass jars was sufficient for soil microorganisms during the incubation period according to our pre-experiment (see the details in Text S1 and Fig. S1).

## 2.4  $CO<sub>2</sub>$  sampling and analysis

Mineralized C  $(CO<sub>2</sub>)$  derived from the maize residue and the soil was trapped in 10 mL of 1-M NaOH in the plastic bottle placed inside each jar. The trap solution was replaced on day 2, 4, 7, 14, 21, 28, 48, 68, and 90 of incubation. At each replacement, we took half of the removed NaOH solution (5 mL) to titrate carbonate ion using a potentiometric automatic titrator (COM-1600, Hiranuma Sangyo Co. Ltd., Ibaraki, Japan); the volume of 0.1-M HCl consumed for changing pH from 8.2 to 4.2 was used to calculate the amount of carbonate ion. To correct for any  $CO<sub>2</sub>$  contamination from the atmosphere during the operation procedure (e.g., opening and closing lids), three blanks (i.e., empty jar containing only 10-mL 1-M NaOH and 5-mL 5-mM HCl) were simultaneously sampled and analyzed at each sampling event. Values from the blanks were then subtracted from each treatment. For  $^{13}$ C analyses, the carbonate remaining in the other half of the NaOH solution (5 mL) was precipitated with  $1-M$  SrCl<sub>2</sub>. The NaOH solution containing  $S<sub>1</sub>CO<sub>3</sub>$  was repeatedly centrifuged (2000 rpm, 5 min) and washed after each round of centrifugation with deionized water until NaOH was removed and the pH reached 7 (Blagodatskaya et al.  $2011$ ). The SrCO<sub>3</sub> precipitate was then dried at 70  $^{\circ}$ C, and the <sup>13</sup>C abundance was determined using the EA-IRMS (Delta V advantage, Thermo Fisher Scientific).

## 2.5 Soil analysis

After air-drying and sieving the soils that were collected from the fields, they were analyzed for selected physicochemical properties including soil pH, soil texture, total C (TC) and its  ${}^{13}C$  abundance, soil inorganic C, total N (TN), and mineral N ( $NH_4^+$ -N and  $NO_3^-$ -N) (Table [1\)](#page-2-0). Soil pH was measured at a soil to water ratio of 1:5 by using a pre-calibrated pH electrode (Benchtop pH meter F-70 Series, Horiba, Kyoto, Japan). For the soil texture analysis, firstly, the organic matter in soil samples was removed using  $H_2O_2$ , the pH was then adjusted to between 9 and 10, and then the samples were ultrasonicated. The sand (0.05–2 mm), silt (2–50  $\mu$ m) and clay  $(< 2 \text{ um})$  fractions were determined by the sieving, sieve-pipette, and pipette method, respectively (Gee and Or [2002\)](#page-11-0). The air-dried and sieved soils were dried at 100 °C, fineground, and analyzed for TC, TN, and  $^{13}$ C abundance using the EA-IRMS. Based on our pre-experiment, the relatively high temperature (100 °C) for soil drying did not affect the determination of TC and TN content for our soils (as compared with 70 °C drying and freeze-drying, see Table S1). Soil inorganic carbon (calcium carbonate) was measured by rapid titration method (Piper [1966](#page-11-0)). For mineral N measurement, 5 g soil was extracted with  $25$ -mL 0.05-M  $K_2SO_4$ (soil/extractant = 1:5) and shaken for 1 h on a reciprocal shaker. The suspension was centrifuged (2000 rpm, 10 min) and filtered through a filter paper (No. 6, Advantec), and  $NH_4^+$ and  $NO<sub>3</sub><sup>-</sup>$  in the obtained extracts were determined by colorimetric analysis using an automated flow injection analyzer (AQLA-700 Flow Injection Analyzer, Aqualab, Tokyo, Japan).

At the end of incubation (110 days), soils from the experimental jars were destructively sampled for the analysis of microbial biomass C (MBC), dissolved organic C (DOC), and mineral N  $(NH_4^+$ -N and  $NO_3^-$ -N). MBC was measured by the fumigation extraction method, as described by Vance et al. ([1987](#page-11-0)). Briefly, 16 g of the soil sample was equally divided into two subsamples, and one subsample was fumigated for 24 h at 25 °C with ethanolfree CHCl<sub>3</sub>. Fumigated and non-fumigated soils were extracted with 40-mL 0.05-M  $K_2SO_4$  (soil/extractant = 1:5) and shaken for 1 h on a reciprocal shaker. The suspension was centrifuged (2000 rpm, 10 min) and filtered through a filter paper (No. 6, Advantec). The obtained extracts were analyzed for total C content using a TOC analyzer (Shimadzu TOC-V<sub>CSH</sub>, Shimadzu Inc.).  $NH_4^+$  and  $NO_3^$ in the non-fumigated  $K_2SO_4$  extracts were determined by colorimetric analysis using the automated flow injection analyzer. MBC was calculated as  $EC/k_{EC}$ , where  $EC$ (mg C kg<sup>-1</sup> soil) was the difference between the amounts of organic C from fumigated and non-fumigated soils, and  $k_{\text{EC}} = 0.45$  (Wu et al. [1990](#page-11-0)). The remaining extracts from the fumigated and non-fumigated samples were freezedried, and the  $^{13}$ C abundance was measured using the EA-IRMS.

To quantify gross C sequestration (residue-derived C incorporation into the soil), at the end of the incubation, we removed the remaining maize residues and recovered the soils using the water washing method (Wang et al. [2018\)](#page-11-0). Briefly, a 30-mL deionized water was added to 10.0 g of the soil residue mixture and shaken for 30 min at 120 rpm. The washed sample was collected and dried at 100 °C and then analyzed for total C content and the  $^{13}$ C abundance using the EA-IRMS.

#### <span id="page-4-0"></span>2.6 Calculations

The proportion of maize residue-derived C (Pres) in  $CO<sub>2</sub>$ emissions,  $K_2SO_4$  extracts, or water-washed soil residues was calculated according to a two-source mixing model, using Eq. 1 (Shahbaz et al. [2017](#page-11-0)):

$$
Pres = (Vtr-Vc)/(Vr-Vc)
$$
 (1)

where *Vtr* represents <sup>13</sup>C values (%) of either CO<sub>2</sub>-C trapped in NaOH, C in the fumigated or non-fumigated  $K_2SO_4$  extract, or SOC in water-washed soil residues from maize residue amended soil; Vr represents  ${}^{13}C$  values (%) of the maize shoot or root residue before incubation; and  $Vc$  represents  $^{13}$ C values (%) of each corresponding pool in the control soil.

The amount of C derived from residue  $(C_{res\text{-}derived})$  in various pools was calculated using Eq. 2 (Poirier et al. [2013](#page-11-0)):

$$
C_{res-derived} = Pres \times [C]
$$
 (2)

where [C] represents either total  $CO_2$  emissions (mg C kg<sup>-1</sup>), C content (mg C kg<sup>-1</sup>) in fumigated (TOC<sub>F</sub>) or non-fumigated (TOC<sub>NF</sub>) K<sub>2</sub>SO<sub>4</sub> extract, or C content (mg C kg<sup>-1</sup>) in waterwashed soil residues.

MBC derived from residues was calculated using the following equation (Eq. (3); Paterson and Sim [2013\)](#page-11-0):

$$
MBC_{res-derived}
$$
  
= [(Pres<sub>F</sub> × TOC<sub>F</sub>)-(Pres<sub>NF</sub> × TOC<sub>NF</sub>)]/K<sub>EC</sub> (3)

where  $Pres_F$  and  $Pres_{NF}$  represent the proportion of C derived from residue in the freeze-dried extract of fumigated and nonfumigated samples, respectively.

The intensity of priming effect (mg  $CO_2$ -C kg<sup>-1</sup> soil) was calculated based on the following equation (Eq. (4); Blagodatskaya et al. [2011\)](#page-10-0).

$$
Priming effect = (CO2 total-CO2 res-derived)-CO2 control (4)
$$

where CO<sub>2</sub> total, CO<sub>2</sub> res-derived, and CO<sub>2</sub> control represent CO<sub>2</sub> amounts (mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) coming from the residue amended soil, maize residue, and control soil, respectively.

The net C sequestration was then determined as the difference between the amounts of residue-derived C incorporation into the soil (gross C sequestration, see above) and the SOC primed.

#### 2.7 Statistical analysis

All data were tested for normality and homogeneity of variance. Two-way analysis of variance (ANOVA) was used to assess the effects of residue type (maize shoot and maize root), N application (without N addition,  $NH_4^+$ -N, and  $NO_3^-$ -N addition), and their interactions on cumulative native soil mineralization, cumulative maize residue decomposition, priming effect, gross C sequestration, net C sequestration, mineral N content, MBC derived from soil at the end of the incubation, and cumulative priming effect in the early phase (0–28 day) and later phase (29–110 day). For cumulative native soil mineralization and MBC derived from soil, the effect of residue type contains three patterns (without residue addition, maize shoot addition, and maize root addition). Multiple comparisons of means with a Tukey test was conducted to examine the differences in the mean values among treatments. Differences with  $p < 0.05$  were considered statistically significant unless stated otherwise. Statistical analysis was conducted with the SPSS Statistics (version 20.0, SPSS Inc., Chicago, IL, USA). Figures were generated using SigmaPlot 12.5 (SYSTAT Software, CA, USA).

## 3 Results

#### 3.1 Maize residue decomposition

The cumulative maize residue decomposition during the 110 day incubation (Fig. 1) was significantly affected by residue



Fig. 1 Cumulative maize residue decomposition under different treatments in Andisol and Entisol during the 110 days of incubation. Error bar represents standard error of the mean  $(n = 3)$ . Different letters

at the end of the line indicate significant differences  $(p < 0.05)$  between the treatments after 110 days of incubation. SR, shoot residue; RR, root residue

<span id="page-5-0"></span>type, N application, and their interaction in both Andisol and Entisol (Table 2). The cumulative decomposition of shoot residue was significantly higher  $(p < 0.01)$  than that of root residue after 110 days of incubation in both Andisol and Entisol (1450 vs. 1240 mg C kg<sup>-1</sup> soil in Andisol; 1440 vs. 1350 mg C kg−<sup>1</sup> soil in Entisol). In Andisol, shoot residue decomposition was slightly reduced to 1380 mg C kg<sup>-1</sup> soil by  $NH_4^+$ -N addition ( $p < 0.05$ ), but was not affected by  $NO<sub>3</sub><sup>-</sup>-N$  addition; root residue decomposition was significantly ( $p < 0.01$ ) reduced to 1100 and 1150 mg C kg<sup>-1</sup> soil with  $NH_4^+$ -N and  $NO_3^-$ -N addition, respectively. In Entisol, shoot residue decomposition was not affected by N addition  $\sim$  1400 mg C kg<sup>-1</sup> soil), while root residue decomposition was significantly decreased  $(p < 0.01)$  to 1220 and 1170 mg C  $\text{kg}^{-1}$  soil with NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N addition, respectively. The decomposition patterns of maize residue can be described by two distinct phases characterized by high (0–28 day) and slow decomposition rates (29–110 day).

## 3.2 Soil organic carbon mineralization

The cumulative mineralization of native SOC after 110 day incubation (Fig. [2\)](#page-6-0) was significantly affected by the residue type, N application, and their interaction in both Andisol and Entisol (Table 2). In the control treatments, the cumulative SOC mineralization was 852 and 649 mg C kg<sup>-1</sup> soil in Andisol and Entisol, respectively. N addition decreased native SOC mineralization; in Andisol,  $NH_4^+$ -N and  $NO_3^-$ -N addition significantly reduced  $(p < 0.01)$  SOC mineralization to 738 and 833 mg C kg<sup> $-1$ </sup> soil, respectively. Similarly, in Entisol,  $NH_4^+$ -N and  $NO_3^-$ -N addition significantly decreased  $(p < 0.01)$  SOC mineralization to 555 and 571 mg C kg<sup>-1</sup> soil, respectively. Addition of maize residue alone enhanced native SOC mineralization as expected, and root residue stimulated more native SOC mineralization than shoot residue (27.8% vs. 15.8% in Andisol; 43.7% vs. 15.8% in Entisol).

When inorganic N was added to residue amended soil of Andisol, SOC mineralization was significantly decreased  $(p < 0.01)$  compared with treatment with maize residue alone, and this negative effect was stronger in root amended soil than shoot amended soil (14.4% and 10.5% in root amended soil, and 4.5% and 1.7% in shoot amended soil for  $NH_4^+$ -N and  $NO_3^-$ -N additions, respectively). In Entisol, under shoot residue treatments, N addition did not alter SOC mineralization ( $\sim$  750 mg C kg<sup>-1</sup> soil); under root residue treatments, on the contrary, N addition significantly reduced  $(p < 0.01)$  SOC mineralization by 15.7% and 18.9% with  $NH_4^+$ -N and  $NO_3^-$ -N addition, respectively, when compared with treatments with root residue alone.



j

For  $CO_2-C_{\text{scal}}$ , the factor of residue has three patterns (without residue addition, maize shoot residue, and maize root residue addition)

or  $CO_2-C_{\text{coll}}$ , the factor of residue has three patterns (without residue addition, maize shoot residue, and maize root residue addition)

<span id="page-6-0"></span>Fig. 2 Cumulative soil C mineralization in Andisol and Entisol after 110 days of incubation. Error bar represents standard error of the mean  $(n = 3)$ . Different letters above bars indicate significant differences between treatments ( $p < 0.05$ ). SR, shoot residue; RR, root residue



## 3.3 Priming effect and soil C balance

The cumulative priming effect at the end of the incubation was positive across all the treatments (Fig. 3), and it was significantly affected by the residue type, N application, and their interaction in both Andisol and Entisol (Table [2](#page-5-0)). After the 110-day incubation, the cumulative priming effect was significantly higher in root than shoot residue alone treatment (237 vs.135 mg C kg<sup>-1</sup> soil in Andisol; 301 vs. 103 mg C kg<sup>-1</sup> soil in Entisol). Under shoot residue treatments, the intensity of priming effect was not affected  $(p > 0.05)$  by N addition in both Andisol and Entisol. Under root residue treatments,  $NH_4^+$ -N and  $NO_3^-$ -N addition significantly reduced  $(p < 0.01)$  the intensity of priming effect by 66.2 and 48.5%, respectively, when compared with root residue alone treatment in Andisol. Similarly, in Entisol,  $NH_4^+$ -N and  $NO_3^-$ -N addition significantly reduced ( $p < 0.01$ ) the intensity of priming effect by 17.9 and 37.9%, respectively, when compared with root residue alone treatment.

The cumulative priming effect over time showed two distinct phases that were characterized by a switch from fast and positive priming in the early stage (0–28 day) to slow and either positive (observed in the root residue alone treatment in Andisol and all the root residue treatments in Entisol) or negative priming (observed in the root residue plus N

treatments and all the shoot residue treatments) in the later stage (29–110 day) of the incubation (Fig. 3). In the early stage  $(0-28 \text{ day})$ , the cumulative priming effect was significantly higher  $(p < 0.01)$  in shoot than root residue alone treatment (168 vs. 141 mg C kg<sup>-1</sup> soil) in Andisol but was significantly higher ( $p < 0.01$ ) in maize root than shoot residue alone treatment (157 vs. 115 mg C kg<sup>-1</sup> soil) in Entisol. In Andisol, the addition of  $NO_3$ <sup>-</sup>-N did not affect the intensity of priming effect induced by shoot or root residue ( $p > 0.05$ ). In Entisol, the addition of  $NO_3^-$ -N significantly reduced ( $p < 0.01$ ) the priming effect under the root residue treatment but not in the shoot residue treatment  $(p > 0.05)$  and in the treatment with root residue plus NH<sub>4</sub><sup>+</sup>-N. In the later stage, the cumulative priming effect was significantly higher  $(p < 0.01)$  in root residue alone treatment than shoot residue alone treatment in both Andisol (96 vs.  $-33$  mg C kg<sup>-1</sup> soil) and Entisol (114 vs. − 42 mg C kg<sup>-1</sup> soil), and N addition significantly decreased  $(p < 0.01)$  the priming effect in root residue treatments but not in shoot residue treatments  $(p > 0.05)$  in both Andisol and Entisol.

Gross and net C sequestrations under each treatment are shown in Fig. [4.](#page-7-0) After the 110-day incubation, the net C sequestration was positive in all of the treatments (Fig. [4c and d\)](#page-7-0). In the residue alone treatments, gross and net C sequestrations were higher in root than shoot residue treatments in Andisol

Fig. 3 Cumulative priming effect under different treatments in Andisol and Entisol during 110 days of incubation. Error bar represents standard error of the mean  $(n = 3)$ . Different letters in the box and at the end of the line indicate significant differences between treatments after 28 days and 110 days of incubation, respectively  $(p < 0.05)$ . SR, shoot residue; RR, root residue



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(Fig.4a and c). In Entisol, the root residue alone treatment was not significantly different from the shoot residue alone treatment for gross C sequestration (Fig. 4b) but showed a lower net C sequestration (Fig. 4d). Under shoot residue treatments, gross and net C sequestrations were not affected by the addition of N in either Andisol or Entisol. On the other hand, under root residue treatments, N addition significantly enhanced gross and net C sequestrations in both Andisol and Entisol. Thus, we did not find any effect of N addition on the gross and net C sequestrations.

## 3.4 Soil microbial biomass C and mineral N

Microbial biomass C derived from residue and soil in each treatment after the 110-day incubation in Andisol and Entisol is shown in Fig. [5](#page-8-0). MBC derived from soil was significantly affected by the residue type, N application, and their interac-tion (Table [2](#page-5-0)). The addition of N did not affect ( $p > 0.05$ ) the amount of MBC derived from the soil in shoot amended treatments in both Andisol and Entisol, while it significantly

decreased  $(p < 0.01)$  the MBC derived from the soil in root amended treatments in both Andisol and Entisol.

Mineral N in each treatment after 110 days of incubation in Andisol and Entisol is shown in Fig. [6.](#page-8-0) Mineral N was higher in residue amended soils compared with the control in Andisol, while mineral N was depleted in residue amended soils in Entisol, even in treatments where N was added.

## 4 Discussion

# 4.1 Effects of crop residue quality and N fertilization on the decomposition of maize residue

The decomposition rate of crop residue was controlled by the crop residue quality; the shoot residue had higher decomposition rate in the early phase and was more decomposed during the 110-day incubation than the root residue in both Andisol and Entisol (Fig. [1](#page-4-0)). The higher EOC content and lower C/N ratio in shoot residue compared with root residue (Table [1](#page-2-0))



Fig. 4 Gross carbon sequestration (maize residue-derived C incorporation into the soil) and net carbon sequestration (maize residue-derived C incorporation into the soil minus primed soil C) in Andisol (a, c) and Entisol (b, d) after 110 days of incubation. Error bar represents standard

error of the mean  $(n = 3)$ . Different letters above bar indicate significant differences between treatments ( $p < 0.05$ ). SR, shoot residue; RR, root residue

<span id="page-8-0"></span>

Fig. 5 Microbial biomass C (MBC) in different treatments after 110 days of incubation in Andisol and Entisol. Total MBC in residue and residue combined with N treatments was separated into MBC derived from residue and soil. Vertical bars are standard errors  $(n = 3)$ . Different letters in

dark gray bar (lower case letters in white color) indicate significant differences ( $p < 0.05$ ) of MBC derived from soil between treatments. SR, shoot residue; RR, root residue

could be the reasons for the higher decomposability of the shoot residue. This is in line with previous studies, which have reported that fast decomposition occurred in FOM with high available C content and low C/N ratio (Freschet et al. [2013](#page-11-0); Mwafulirwa et al. [2019\)](#page-11-0). In our study, the decomposed maize residue after 110 days of incubation accounted for 37–49% of the input amount. This proportion was comparable to a previous study (Shahbaz et al. [2017](#page-11-0)) which reported that about 30– 60% of maize residue had been decomposed after 120 days of incubation of Luvisol.

The crop residue decomposition rate decreased with incubation time. The different decomposition rates between the early and later phases of incubation (Fig. [1\)](#page-4-0) are attributed to the decline of more labile organic compounds in maize residues which were quickly utilized by microbes during the early phase of the incubation (Brandstatter et al. [2013\)](#page-10-0), indicating that its recalcitrance increased over time.

The effect of N fertilization on maize residue decomposition depended on the residue type. In general, N addition retarded the decomposition of root residue but did not affect the decomposition of shoot residue after the 110-day incubation (Fig. [1](#page-4-0)). Root residue had high C/N ratio (Table [1\)](#page-2-0), which might be an indication of the higher content of recalcitrant compounds such as lignin and phenols (Freschet et al. [2013;](#page-11-0) Barel et al. [2019](#page-10-0)). Further, the addition of inorganic N could reduce the N mining from maize root residue as N addition suppresses the production of the lignindegrading enzyme and decreases the abundance of microbes responsible for recalcitrant C decomposition (Austin and Ballare [2010;](#page-10-0) Carreiro et al. [2000](#page-10-0)). These could lead to lower root residue decomposition. Our result was consistent with a previous study which showed that N addition tends to retard the decomposition of FOM with lower quality (higher lignin content and C/N ratio) (Knorr et al. [2005\)](#page-11-0). The suppressed decomposition of maize root residue leads to a greater C sequestration, which is beneficial for arable cropping systems.

#### 4.2 SOC priming with maize residue and N addition

Fig. 6 Mineral N  $(NH_4^+$ -N +  $NO_3$ <sup>-</sup>-N) content (mg N kg<sup>-1</sup> soil) under different treatments in Andisol and Entisol after 110 days of incubation. Vertical bars are standard errors  $(n = 3)$ . SR, shoot residue; RR, root residue



The root residue induced more intense priming effect than shoot residue, probably because the low-quality residue stimulates N mining. The cumulative priming effect after the 110-

day incubation was higher in the maize root than shoot residue alone treatment in both Andisol and Entisol (Fig. [3\)](#page-6-0). The root residue showed a higher C/N ratio and lower EOC content compared with shoot residue (Table [1\)](#page-2-0), which could result in an inadequate supply of N to cover the requirements of microorganisms (Recous et al. [1995](#page-11-0); Moorhead and Sinsabaugh [2006](#page-11-0)) and therefore stimulate N mining from soils. Furthermore, root residue containing a relatively high amount of recalcitrant compounds (Lian et al. [2016\)](#page-11-0) is more beneficial for the growth of K-strategists (Fontaine et al. [2003\)](#page-10-0), which can feed on SOM (Kuzyakov et al. [2000;](#page-11-0) Shahbaz et al. [2017\)](#page-11-0), therefore stimulating more SOM mineralization. This result indicates that the susceptibility of SOM to mineralization increased when decaying roots are present (Shahbaz et al. [2017\)](#page-11-0).

As we hypothesized, N addition weakens N mining in the root residue-treated soils, and the effect of N addition was not apparent in the shoot residue-treated soils. N fertilization reduced cumulative priming effect in the root residue treatments in both Andisol and Entisol after the 110-day incubation (Fig. [3\)](#page-6-0). The increased N availability through external N supply could suppress the enzyme production and decrease the N mining from SOM (Chen et al. [2014\)](#page-10-0), thereby reducing the priming effect. This explanation is supported by the fact that the addition of N reduced the amount of MBC derived from the soil in the root residue treatments (Fig. [5](#page-8-0)). In contrast to the root residue treatments, N addition did not affect the cumulative priming effect in the shoot residue treatments after 110 days of incubation (Fig. [3\)](#page-6-0) because the shoot residue with a low C/N ratio and high EOC content (Table [1\)](#page-2-0) is conducive to the growth of fast-growing r-strategists which preferred to use more available substrates rather than recalcitrant SOM, especially after N addition (Chen et al. [2014\)](#page-10-0). Our results reveal that the priming effect is interactively affected by crop residue quality and N addition and highlight that the combined input of N fertilizer and crop residue with low quality (e.g., root residue) can effectively reduce the native SOC loss through priming effect.

The intensity and direction of priming effect change with the duration of incubation are controlled by the residue quality and N availability. Fast and positive priming effect occurred in the intensive phase of maize residue decomposition (0– 28 day) (Fig. [3\)](#page-6-0) because the presence of labile compounds in shoot and root residue can boost the growth of microorganisms (Hu et al. [1999\)](#page-11-0). The increasing microbial biomass promoted the production of extracellular enzymes and consequently enhanced the mineralization of SOC based on cometabolism mechanism (Kuzyakov et al. [2000](#page-11-0); Fang et al. [2018\)](#page-10-0). Shoot residue having a higher EOC content and lower C/N ratio (Table [1](#page-2-0)) could stimulate more growth of microorganisms, therefore inducing a higher positive priming effect in Andisol during the early phase (Fig. [3](#page-6-0)). Higher priming effect was found in root residue treatments rather than in shoot residue treatments in Entisol (Fig. [3](#page-6-0)); the reason for this could be

that N mining also contributed to the SOC priming in Entisol due to the lower N availability (Table [1\)](#page-2-0) as  $NO<sub>3</sub><sup>-</sup>-N$  addition significantly reduced the intensity of priming effect in root residue treatments in Entisol at this stage (0–28 day) (Fig. [3,](#page-6-0) Table [2\)](#page-5-0).

In the later stage (29–110 day) of the incubation, different residue qualities caused divergent direction of priming effect change; negative priming effect occurred with shoot residue and persisted almost till the end of the incubation, and a slow positive priming effect continued with root residue (Fig. [3\)](#page-6-0). The negative priming effect with shoot residue can be attributed to the preferential utilization of microbial necromass, which has a lower C/N ratio than that of the remaining residue and SOM (Fontaine et al. [2011](#page-10-0)). Our explanation is supported by the short turnover time of microbes  $\left(\sim 30 \text{ days}\right)$ ; Blagodatskaya et al. [2009](#page-10-0), [2011](#page-10-0)). For the root treatments, the positive priming effect was mainly attributed to the microbial N mining, especially in Entisol, which had a higher SOM priming (114 mg C kg<sup>-1</sup> soil) than Andisol (96 mg C kg<sup>-1</sup> soil) (Fig. [2](#page-6-0) and Table [2](#page-5-0)) due to the lower N availability in Entisol soils (Fig. [6\)](#page-8-0). Moreover, N fertilization significantly decreased the cumulative priming effect in the later stage of incubation in root residue-treated soils of both Andisol and Entisol (Fig. [3](#page-6-0) and Table [2\)](#page-5-0), which could be a reflection of the N addition mitigating N limitation and consequently reducing the microbial N mining from SOM.

Affirming our second hypothesis, the priming effect can have two phases that are controlled by different mechanisms over the incubation of several months, which could lead to opposite effects on the priming effect. Higher priming effect can occur with high-quality residue in short-term incubations (i.e., during 2 to 3weeks of incubation in this study) due to cometabolism. In contrast, higher priming effect can also occur with low-quality residue under N-limited conditions, especially in slow decomposition stage of residues (i.e., after 28 days of incubation in this study) due to N mining. Additionally, the intensity of priming effect in soils treated with low-quality crop residue could decrease under high N availability conditions by reducing N mining. These findings may explain the inconsistent results of crop residue quality on the intensity of priming effect in previous studies (Shahbaz et al. [2017;](#page-11-0) Mwafulirwa et al. [2019\)](#page-11-0) and suggest that relatively longterm (e.g., several months) experiments should be conducted to better capture the priming effect dynamics (i.e., intensity and direction) and the underlying mechanisms after crop residue addition.

# 4.3 C balance of maize residue C sequestration and SOC priming

The combined application of maize root residue and N fertilizer is beneficial for SOC sequestration. After 110 days of incubation, net C sequestration was higher with root residue

<span id="page-10-0"></span>than with shoot residue in Andisol (Fig. [4c\)](#page-7-0), despite the fact that root residue induced higher priming effect than shoot residue (Fig. [3](#page-6-0)). The higher gross C sequestration with root residue addition contributed to the higher net C sequestration due to the lower decomposition rate of root residue (Fig. [1](#page-4-0)). In Entisol, net C sequestration was significantly lower in root than shoot residue treatments because of the higher intensity of priming effect in maize root residue amended soil (Fig. [3\)](#page-6-0). N addition significantly enhanced the net C sequestration in maize root residue treatments because of the reduced intensity of priming effect (Fig. [3\)](#page-6-0) and resulted in higher net C sequestration for the combined application of maize root residue and mineral N application than in the shoot application (Fig. [4](#page-7-0)). N forms did not affect net C sequestration (Fig. [4c and d](#page-7-0)). Considering that  $NO_3$ <sup>-</sup>-N is susceptible to leaching,  $NH_4$ <sup>+</sup>-N is recommended as the mineral N fertilizer in terms of C sequestration.

## 5 Conclusions

Our study revealed the interactive effects of maize residue quality and N fertilization on SOC priming. N addition decreased priming effect, which was induced by the application of maize root residue as well as root residue decomposition during the 110-day incubation. This was not found in the maize shoot residue treatments. Thus, N addition significantly increased soil C sequestration in the roottreated soils. Such decreased priming effect and maize root residue decomposition could be attributed to the reduction of microbial N mining. We further demonstrated the importance of relatively long-term incubation for several months for the evaluation of priming effect, the intensity of which varied over time as it was controlled by different mechanisms; co-metabolism is more evident in the first month (i.e., intensive decomposition stage of maize residue) and N mining in the later months (i.e., slow decomposition stage of maize residue), if at all, especially under low N condition. This study highlights that N fertilization is beneficial to soil C sequestration when soil is treated with low-quality crop residue (e.g., maize root residue) because of lowering of the intensity of priming effect and crop residue decomposition. Future studies conducted under field conditions are needed to verify our findings before they can be applied in actual agricultural fields.

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Code availability Not applicable.

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#### Compliance with ethical standards

Conflicts of interest The author declares that they have no conflict of interest.

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