Effects of Crop Residue Quality and Nitrogen Fertilization on Priming of Soil Organic Carbon Mineralization

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ABSTRACT

Soil is the largest C pool in terrestrial ecosystems, even a little change of soil organic carbon (SOC) could have a large effect on climate change. Fresh organic matter (FOM) inputs to soil may increase or suppress native SOC mineralization; this phenomenon is called priming effect. Priming effect is one of the most critical interactions between soil C input and SOC mineralization. However, our current knowledge of the underlying mechanisms of priming effect is still unclear. Its direction or intensity could be affected by many factors, which can be divided into two groups: FOM properties and soil properties. However, how these factors affect the intensity of the priming effect is still limited. In agroecosystems, crop residues are the common source for FOM. Changes in the quality of crop residue or soil nutrient availability induced by crop residue and fertilization all could affect priming effect in agroecomsystems. Study on how these factors (crop residue quality and N fertilization) directly and interactively affect the priming effect provides an opportunity to explore the underlying mechanisms of priming effect and quantify the magnitude of priming effect. Such understanding would help design effective management strategies of crop residue return and N fertilization for decreasing SOC loss through priming effect and improving SOC storage in agricultural soils. Here I conducted two incubation experiments, using the ^{13}C tracer technique, to clarify how crop residue quality and N fertilization affect the priming of SOC mineralization in agricultural soils.

(1) To evaluate the SOC priming induced by the interaction of crop residue quality and N fertilization on SOC priming, a 110-day laboratory incubation experiment by adding ^{13}C-labeled maize (Zea mays L.) shoot and root residues with and without mineral fertilizer-N was conducted in two types of agricultural soil (Andisol, AND; Entisol, ENT). After 110 days of incubation, 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. 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.

(2) To investigate the importance of priming effect in the subsoil compared with topsoil. I used ^{13}C-labeled maize residue with contrasting quality (shoot vs. root) incorporated to topsoil (0–10 cm) and subsoil (40–60 cm) of two types of agricultural soil (Andisol, AND; Entisol, ENT) with and without N fertilization through a 110-day incubation experiment. These two different types of arable soils yielded consistent results. After 110-day incubation, maize residue addition induced a positive priming effect, and this positive priming effect directly related to maize residue quality (shoot > root) in the subsoil, which was in contrast to that in topsoil (root > shoot). Meanwhile, in the subsoil, the higher priming effect occurred with shoot residue through microbial growth induced by the addition of labile residue with a low C/N ratio. In contrast, in the topsoil, N additions reduced the priming effect under the root treatment but did not alter that under the shoot treatment. The contrasting effects of crop residue quality on the intensity of priming effect between soil layers suggested that microbial N-mining could dominate the contribution to higher priming effect in the topsoil, while the microbial co-metabolism would play a more critical role in the subsoil. The relative priming effect (% of SOC mineralization) in subsoil was 2.7-22.0 times higher than in topsoil across two soil types. The results suggest a higher vulnerability to SOC loss through priming effect in subsoil compared to topsoil.

Key words: crop residue quality, fresh organic matter availability, nitrogen fertilization, nitrogen availability, priming effect

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Chapter 1

Introduction

1.1 Role of soil organic carbon in the carbon cycle

Soil is the largest C pool in terrestrial ecosystems, storing around 2,344 Pg C at depths of up to 3m, larger than the combination of C in atmosphere and plant, and about three times as much the atmosphere (Jobbagy and Jackson, 2000). Soil organic carbon (SOC) plays an essential role in soil health and fertility (Smith et al., 2015), and it is also a significant source and sink of atmospheric greenhouse gases (Lal, 2010; Lehmann and Kleber, 2015). Although soil organic matter (SOM) mineralization offers available nutrients for plant uptake in ecosystems, it provides a CO_2 emission flux equivalent to 7 times of the anthropogenic CO_2 emissions (Prentice, 2001; Solomon et al., 2007), which could cause a large positive impact on global warming (Lal, 2010). It is clearly important to understand the turnover of SOC since it plays a crucial role in soil ecosystem functioning and climate change.

1.2 Role of agriculture in the carbon cycle and the definition of priming effect

Farmers have a vested interest in maintaining and increasing SOC for agricultural fields because soil quality and yield improve when the SOC level increases. Simultaneously, the agricultural sector can affect the carbon cycle on a large scale, often through the SOC mineralization. In the past 200 years, a global C debt due to land-use change to agriculture was 133 Pg C for the top 2 m of soil, and about 25% of this loss attributed to SOC mineralization (Houghton, 2012; Sanderman and Berhe, 2017). Hence, the loss of SOC in agroecosystems has received increasing attention in recent years. Crop residues as the byproducts of agriculture are the primary C source for agricultural soils; returning these byproducts to the soil is generally recommended to increase SOC stock and maintain soil fertility (Jin et al., 2020; Liu et al., 2014). However, these fresh organic matters (FOMs) as an energy and nutrient source for soil microbes may influence their catabolic and anabolic processes, and further affect native SOM mineralization, which is known as priming effect (Kuzyakov et al., 2000).

1.3 Controlling factors and underlying mechanisms of priming effect

Priming effect is one of the most critical interactions between C input and SOC mineralization in soils. Thus, understanding the direction and intensity of priming effect is essential for long-term SOC storage and climate change mitigation. The supply of FOM can either increase (positive priming effect), suppress (negative priming effect) or have no effect (no priming effect) on SOM mineralization (Zhang et al., 2013). The schematization of the organic substrate addition on priming of SOM mineralization is shown in Fig. 1.1. In general, the FOM addition always promoted SOM mineralization and resulted in positive priming effect (Fontaine et al., 2011; Shahzad et al., 2012), by 37% on average (Luo et al., 2016). However, the intensity of priming effect is variable ranging from -95.1% to 1207% compared to soil without FOM addition. It has been reported that the crop residue return even resulted in a reduction of SOC (Kirkby et al., 2014), as the SOC loss through priming effect exceeded the newly formed SOC (Fontaine et al., 2004). Although the importance of priming effect in SOC cycle has been widely recognized, the mechanism of priming effect is still controversial. Besides, the existing research findings on the priming effect are diverse, showing that its direction and intensity are affected by many factors, including FOM properties and soil properties.

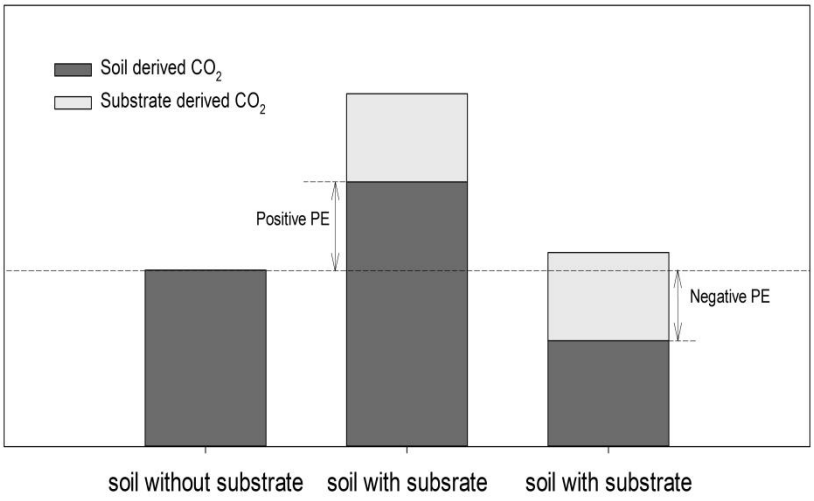

Fig. 1.1 Schematization of the organic substrate addition on priming of soil organic matter (SOM) mineralization. The increase of SOM mineralization represents positive priming effect (PE), while decrease of SOM mineralization reflects negative PE (modified after Kuzyakov et al. 2000).

1.3.1 Controlling factors on priming effect

The direction (acceleration or retardation of SOC decomposition) and magnitude of priming effect in response to FOM additions are not easy to predict because several factors are involved. The factors can be divided into two groups: FOM properties and soil properties (e.g., crop residue quality and soil N availability).

Crop residues are the common source for FOM in agricultural soils. The crop residue quality plays an important role in SOC turnover and it generally defined by complexity (e.g., lignin content) and stoichiometry (C:N ratio) of compounds in substrates and varies among plant organs (e.g., shoot and root); root usually contains more recalcitrant compounds and has higher C:N ratio than shoot (Barel et al., 2019; Freschet et al., 2013). Our current knowledge on the effect of crop residue quality on priming effect is still under debate. Previous studies showed that higher quality FOM added to soil lead to a lower (Shahbaz et al., 2017a; Shahbaz et al., 2017b), an equal (Chen et al., 2014), or a higher (Mwafulirwa et al., 2019) positive SOM priming than lower quality FOM. These inconsistent findings of crop residue quality on priming effect may come from the different predominant mechanism of positive priming effect under different types of soils, durations of incubation, and soil N availability (Wang et al. 2016; Xu et al. 2016).

Nutrient availability, especially N, can play key role in SOC priming, yet the SOC priming as a function of N availability has not been fully understood (Blagodatskaya et al., 2009). Globally, the agroecosystems are experiencing increasing N input by N deposition and fertilization (e.g. NH_4^+-N and NO_3^--N) (Galloway et al., 2008). The increased N availability would significantly influence SOC cycling due to their close interaction (Janssens et al., 2010). Previous studies revealed that N addition significantly decreased the positive priming effect induced by maize stalk addition (Wang et al., 2018), and even resulted in a negative priming effect (Qiu et al., 2016). Yet, Chen et al. (2014) and Meng et al. (2017) showed that N addition had 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 with single crop residue, making it difficult to compare this effect among different types of crop residue and thus prevented a full examination on the potential interactions between N availability and crop residue quality in altering SOC priming.

The importance of crop residue return on priming of SOC mineralization is mostly considered for topsoil, while the information for subsoil is scarce (Gregory et

al. 2014; Ogle et al. 2005). Priming effect in subsoil mostly occurs through mechanical input of FOM to subsoil and deep rooting growth (Lorenz et al., 2011; Lynch and Wojciechowski, 2015). SOC in subsoil is considered more stable and has higher mean resistance time compared to topsoil. However, in contrast to the general assumption that subsoil is less affected by management, Khan et al. (2007) showed more serious losses of SOM below the plough layer in a silty-loam soil. A possible explanation of subsoil C loss can be due to priming effect. When energy rich FOM was added to subsoil, subsoil C could induce SOC loss through priming effect (Fontaine et al. 2007). However, there are only a few studies that have examined SOC loss through priming effect in subsoil with FOM addition, the findings of which are contradictory (Fontaine et al., 2007; Salomé et al., 2010). Therefore, the effect of FOM addition on priming effect in the subsoil is still not clear.

1.3.2 Mechanisms of priming effect

Several mechanisms have been proposed to explain positive priming effect. Such as co-metabolism, microbial N mining and microbial stoichiometric decomposition. Co-metabolism is that SOC mineralization can be enhanced due to an acceleration of microbial growth and activity with the FOM addition (Blagodatskaya and Kuzyakov, 2008); microorganisms feeding on FOM could also decompose similar compounds in SOM and then enhance SOM mineralization. Microbial N mining is that when N is limited for supporting microbial growth in soil system, microorganisms enhance activity of extracellular enzyme to acquire N from SOM, therefore promote SOM mineralization (Chen et al. 2014; Fontaine et al. 2011). The microbial stoichiometry decomposition assumes that microbial stoichiometry of FOM is a driving force on SOM mineralization, meaning that the microbial activity and SOM mineralization could be stimulated when high quality FOM or both C and N inputs matches microbial demands (Chen et al., 2014; Craine et al., 2007). Negative priming effect can be explained by the preferential utilization of more available substrates rather than SOM in soil systems: microorganisms keep their energy acquiring habit and enzymatic activities on more energy-rich substrate resulting in a decrease in SOM mineralization (Kuzyakov, 2010; Qiao et al., 2014).

1.4 Study objectives and dissertation organization

The objective of this study was to evaluate how the crop residue quality and N fertilization affect the priming of SOC mineralization in agricultural soils. The clarification of the effect of these factors on the priming effect is beneficial for understanding the underlying mechanisms of priming effect and designing the

appropriate management strategies of crop residue return and N fertilization in agroecosystems to improve soil quality and reducing SOC loss through priming effect. I evaluated the effects of crop residue quality with and without N fertilization on the priming effect in agricultural soils (topsoil and subsoil) using the ^{13}C tracer technique.

The thesis comprises the following chapters. After this general introduction in Chapter 1, Chapter 2 describes the soils and maize residues used in the incubation experiment. Chapter 3 examines the interactive effects of crop residue quality and N fertilization on priming effect in two different soil types of agricultural soil. Chapter 4 investigates the differences of priming effect induced by crop residue addition in terms of crop residue quality in subsoil compared to topsoil in two types of agricultural soil. Chapter 5 is a general discussion. Finally, Chapter 6 presents the concluding remarks.

Chapter 2

Description of Soil Sampling and the Maize Residue Production

2.1 Soil sampling

The soils (0–20 cm and 40–60 cm) were taken from two farmlands used for vegetable cultivation in the year of 2018. One is located in Nagano prefecture, Japan (36°31′N, 138°21′E); the soil is derived from volcanic ash and classified as Andisol (AND) (Soil Survey Staff, 2014). The other is located in Kyoto prefecture, Japan (35°3′N, 135°48′E); the soil is classified as Entisol (ENT). After air-drying, all soils samples were sieved (≤ 2mm) to eliminate organic residues prior to the incubation experiment. The physico-chemical properties of soils are shown in Table 2.1.

2.2 Production of ^{13}C-labled maize residue

^{13}C-labeled maize residue (shoot and root) used in Chapter 3 and Chapter 4 were taken from ^{13}C-labeled maize plants. Maize plants were cultivated in potted trays filled up with perlite and vermiculite, irrigated with Hoagland's nutrient solution (N: 210, P: 31, K: 234, Ca: 200, Mg: 48, S: 64 mg L^{-1} plus 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^{-1}. The pulse-labeling of maize plant 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 one month. In each pulse-labelling event, the plants were transferred to a portable labeling chamber which was air tightly sealed 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 by two battery operated mini-fans. The chamber air was sampled several times (5 mL, using a gas-tight syringe) for monitoring the CO_2 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 labelling, 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_2 concentration for maize growth.

After harvest, maize shoot and root were washed and dried at 70°C for one week and milled to pass through a 2 mm sieve prior to incubation. Around 10 mg of residue subsample was used for the determination of the 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 extractable N (EN) in maize residue, 20 mL deionized water was added to 1.0 g maize residue, shaken for 2 hours at 120 rpm and then filtered through filter paper (No. 6, Advantec, Tokyo, Japan). 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 2.2.

Table 2.1 Description of soil characteristics.

	Andisol topsoil (0–10 cm)	Entisol topsoil (0–10 cm)	Andisol subsoil (40–60 cm)	Entisol subsoil (40–60 cm)
Total C (%)	8.36	2.87	3.66	0.87
Total N (%)	0.57	0.27	0.26	0.06
C:N	14.5	10.5	13.9	13.5
^{13}C (%)	1.09	1.09	1.09	1.09
NH_4^+-N (mg kg^{-1})	20.3	25.6	23.8	15.1
NO_3^--N (mg kg^{-1})	112	18.6	76	13.7
Sand (%)	22	60	30	63
Silt (%)	50	25	42	22
Clay (%)	28	15	28	14
pH (H_2O)	6.5	7.2	6.3	5.8

Table 2.2 Description of maize residue characteristics

	Maize shoot residue	Maize root residue
Total C (%)	41.4	41.3
Total N (%)	3.32	1.60
C:N	12.5	25.8
^{13}C (%)	7.13	5.42
EOC(g kg^{-1})	11.4	6.2
EN(g kg^{-1})	4.6	3.2

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

Chapter 3

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

Abstract

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, I conducted this study. I 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^{-1} soil and 60 mg N kg^{-1} soil, respectively. ^{13}C-labeled maize residue allowed quantifying residue decomposition and calculating SOC priming and sequestration. After 110 days of incubation, 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. 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.

3.1 Introduction

Crop residues are the byproducts of agriculture and the main C source for arable soils. In general, it is recommended that crop residues are returned to the soil to increase soil organic carbon (SOC) storage and maintain soil fertility (Jin et al., 2020; Liu et al., 2014). 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). The increase or decrease in SOC mineralization, compared to soil without FOM addition, 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 (Lenka et al., 2019; Wang et al., 2015a), thus resulting in a higher CO_2 emission and adversely affecting on global climate change (Kuzyakov, 2010; Zhang et al., 2013). Further, it has been reported that the crop residue return may also result in a decrease in the SOC (Fontaine et al., 2004; Kirkby et al., 2014), as the loss of native SOC through priming effect can exceed the newly formed SOC (Fontaine et al., 2004). To design effective strategies of crop residue management for avoiding adverse impact of the priming effect 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) (Schmatz et al., 2016; Wang et al., 2015a). 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., 2017a), an equal (Guenet et al., 2010; Xu et al., 2018), or a higher (Mwafulirwa et al., 2019) 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., 2016a; Xu et al., 2016). Considering that the residue quality changes during decomposition and the recalcitrance of residue increases over time (Hadas et al., 2004), 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 to short-term 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). 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). Increased N availability can significantly influence SOC cycling because they have close interaction (Janssens et al., 2010). Previous studies have revealed that N addition significantly decreases the positive priming effect induced by maize stalk addition (Wang et al., 2018), and can even result in a negative priming effect (Qiu et al., 2016). On the other hand, Chen et al. (2014) and Meng et al. (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; Chen et al. 2014). 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); 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 (Aye et al., 2018; Craine et al., 2007).

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 to 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. I 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.

3. 2 Materials and methods

3.2.1 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). 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). After air-drying, soils were sieved (≤ 2 mm), and visible organic residues were eliminated prior to the incubation experiment. Selected physico-chemical properties of the two soil types are shown in Table 1.

3.2.2 Production of ^{13}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^{-1}, P: 31 mg L^{-1}, K: 234 mg L^{-1}, Ca: 200 mg L^{-1}, Mg: 48 mg L^{-1}, S: 64 mg L^{-1} 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^{-1}. 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 one 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 mini-fans. The chamber air was sampled several times (5 mL, using a gas-tight syringe) for monitoring the CO_2 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_2 concentration for maize growth.

After harvest, maize shoots and roots were washed, dried at 70 °C for one 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 two hours at 120 rpm and then filtered through a filter paper (No. 6, Advantec, Tokyo, Japan) (Surey et al., 2020). 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 3.1.

Table 3.1 Properties of soils and maize residues used for the incubation experiment

	Andisol	Entisol	Maize shoot residue	Maize root residue
Total C (%)	8.36	2.87	41.4	41.3
Total N (%)	0.57	0.27	3.32	1.60
C:N ratio	14.5	10.5	12.5	25.8
^{13}C (%)	1.09	1.09	7.13	5.42
Inorganic C (%)	0.04	0.05		
EOC (g kg^{-1})			11.4	6.2
EN(g kg^{-1})			4.6	3.2
NH_4^+-N (mg kg^{-1})	20.3	25.6		
NO_3^--N (mg kg^{-1})	112.0	18.6		
Sand (%)	22	60		
Silt (%)	50	25		
Clay (%)	28	15		
pH(H_2O)	6.5	7.2		

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

3.2.3 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 seven days to avoid a flush in microbial respiration induced by rewetting (Shi and Marschner, 2017). Nine treatments with three replicates in each type of soil were set up: neither N nor maize residue was added (control), NH_4^+-N amended soil (NH_4), NO_3^--N amended soil (NO_3), maize shoot residue amended soil (SR), maize shoot residue + NH_4^+-N amended soil (SR + NH_4), maize shoot residue + NO_3^--N amended soil (SR + NO_3), maize root residue amended soil (RR), maize root residue + NH_4^+-N amended soil (RR + NH_4), maize root residue + NO_3^--N amended soil (RR + NO_3). The two N sources, NO_3^--N and NH_4^+-N were applied at 60 mg N kg^{-1} soil as KNO_3 and $(NH_4)_2SO_4$, respectively. The source of C was either the maize shoot residue or the root residue, which were applied at 3.00 g C kg^{-1} 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_2 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_2 during the incubation (the potential contamination of atmospheric CO_2 during sampling was corrected by the blanks, see below in detail). Even though the jars were sealed, the O_2 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 S3.1 and Fig.S3.1).

3.2.4 CO_2 sampling and analysis

Mineralized C (CO_2) 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, I 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_2 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 $SrCl_2$. The NaOH solution containing $SrCO_3$ 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 $SrCO_3$ precipitate was then dried at 70 °C, and the ^{13}C abundance was determined using the EA-IRMS (Delta V advantage, Thermo Fisher Scientific).

3.2.5 Soil analysis

After air-drying and sieving the soils that were collected from the fields, they were analyzed for selected physico-chemical 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 4.1). 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 μm) and clay (< 2 μm) fractions were determined by the sieving, sieve-pipette and pipette method, respectively (Gee and Or 2002). The air-dried and sieved soils were dried at 100 °C, fine-ground, 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 to 70 °C drying and freeze-drying, see Table S3.1). Soil inorganic carbon (calcium carbonate) was measured by rapid titration method (Piper 1966). 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), NH_4^+ and NO_3^- 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), soil 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). 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 ethanol-free $CHCl_3$. 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 filter paper (No. 6, Advantec). The obtained extracts were analyzed for total C content using a TOC analyzer (Shimadzu TOC-V_{CSH}, 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^{-1} soil) was the difference between the amounts of organic C from fumigated and non-fumigated soils, and k_{EC} = 0.45 (Wu et al. 1990). The remaining extracts from the fumigated and non-fumigated samples were freeze-dried, 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). Briefly, 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.

3.2.6 Calculations

The proportion of maize residue derived C (*P*res) in CO_2 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):

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

where, *Vtr* represents ^{13}C values (%) of either CO_2-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).

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

where, [C] represents either total CO_2 emissions (mg C kg^{-1}), C content (mg C kg^{-1}) in fumigated (TOC_F) or non-fumigated (TOC_{NF}) K_2SO_4 extract, or C content (mg C kg^{-1}) in water-washed soil residues.

MBC derived from residues was calculated using the following equation (Eq. (3); Paterson and Sim, 2013)

$MBC_{res\text{-}derived} = [(P\text{res}_F \times TOC_F) - (P\text{res}_{NF} \times TOC_{NF})] / K_{EC}$ (3)

where, $P\text{res}_F$ and $P\text{res}_{NF}$ represent the proportion of C derived from residue in the freeze-dried extract of fumigated and non-fumigated samples, respectively.

The intensity of priming effect (mg CO_2-C kg^{-1} soil) was calculated based on the following equation (Eq. (4); Blagodatskaya et al., 2011).

Priming effect = $(CO_{2\ total} - CO_{2\ res\text{-}derived}) - CO_{2\ control}$ (4)

where, $CO_{2\ total}$, $CO_{2\ res\text{-}derived}$, and $CO_{2\ control}$ represent CO_2 amounts (mg CO_2-C kg^{-1} soil) coming from 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.

3.2.7 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 SPSS Statistics (version 20.0, SPSS Inc., Chicago, IL, USA). Figures were generated using SigmaPlot 12.5 (SYSTAT Software, CA, USA.).

3. 3 Results

3.3.1 Maize residue decomposition

Cumulative maize residue decomposition during the 110-day incubation (Fig. 3.1) was significantly affected by residue type, N application, and their interaction in both Andisol and Entisol (Table 3.2). 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^{-1} soil in Andisol; 1440 vs. 1350 mg C kg^{-1} soil in Entisol). In Andisol, shoot residue decomposition was slightly reduced to 1380 mg C kg^{-1} soil by NH_4^+-N addition ($p < 0.05$), but was not affected by NO_3^--N addition; root residue decomposition was significantly ($p < 0.01$) reduced to 1100 and 1150 mg C kg^{-1} soil with NH_4^+-N and NO_3^--N addition, respectively. In Entisol, shoot residue decomposition was not affected by N addition (~1400 mg C kg^{-1} soil), while root residue decomposition was significantly decreased ($p < 0.01$) to 1220 and 1170 mg C kg^{-1} soil with NH_4^+-N and NO_3^--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).

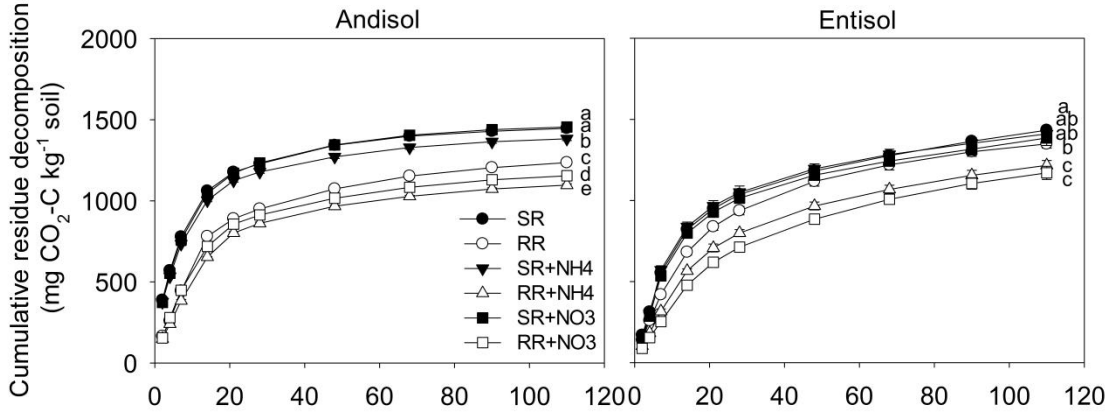

Fig. 3.1 Cumulative maize residue decomposition under different treatments in Andisol and Entisol during the 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters at the end of the line indicate significant differences ($p < 0.05$) between the treatments after 110 days of incubation. SR, shoot residue; RR, root residue

Table 3.2 *F* values from two-way ANOVA showing the effects of residue type (maize shoot residue and maize root residue) and N application (without N addition, NH_4^+-N, and NO_3^--N addition) on the cumulative SOC mineralization (CO_2-C_{soil}), cumulative crop residue decomposition (CO_2-C_{res}), cumulative priming effect (CO_2-C_{primed}), gross C sequestration (C_{gross}), net C sequestration (C_{net}), MBC derived from soil (MBC_{soil}) after 110 days of incubation, and cumulative priming effect in the early phase (0–28 day) (CO_2-$C_{primedearly}$) and later phase (29-110 day) (CO_2-$C_{primedlater}$) in Andisol and Entisol. Note: for CO_2-$C_{soil,}$ the factor of residue has three patterns (without residue addition, maize shoot residue, and maize root residue addition)

Soil type		CO_2-C_{soil} (mg C kg^{-1} soil)	CO_2-C_{res} (mg C kg^{-1} soil)	CO_2-$C_{primedearly}$ (mg C kg^{-1} soil)	CO_2-$C_{primedlater}$ (mg C kg^{-1} soil)	CO_2-C_{primed} (mg C kg^{-1} soil)	C_{gross} (mg C kg^{-1} soil)	C_{net} (mg C kg^{-1} soil)	MBC_{soil} (mg C kg^{-1} soil)
Andisol	residue	3135.9***	2295.3***	22.94***	32.90***	5.9*	1012.5***	255.6***	6.1**
	N	831.8***	114.3***	11.99***	2.44 ns	19.8***	14.2***	23.8***	17.7***
	residue × N	128.0***	24.9***	1.85 ns	10.55**	7.1**	10.3**	10.9**	3.3*
Entisol	residue	881.8***	104.1***	18.14***	197.00***	300.7***	83.6***	2.15 ns	27.6***
	N	43.5***	17.0***	17.25***	12.37***	17.7***	28.3***	39.7***	26.2***
	residue × N	15.6**	6.3*	12.00***	5.32*	14.9***	10.1**	19.8***	11.6***

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns, no significant difference

3.3.2 Soil organic carbon mineralization

Cumulative mineralization of native SOC after 110-day incubation (Fig. 3.2) was significantly affected by the residue type, N application, and their interaction in both

Andisol and Entisol (Table 3.2). In the control treatments, cumulative SOC mineralization was 852 and 649 mg C kg^{-1} 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^{-1} 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^{-1} 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 to 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 (~750 mg C kg^{-1} 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 to treatments with root residue alone.

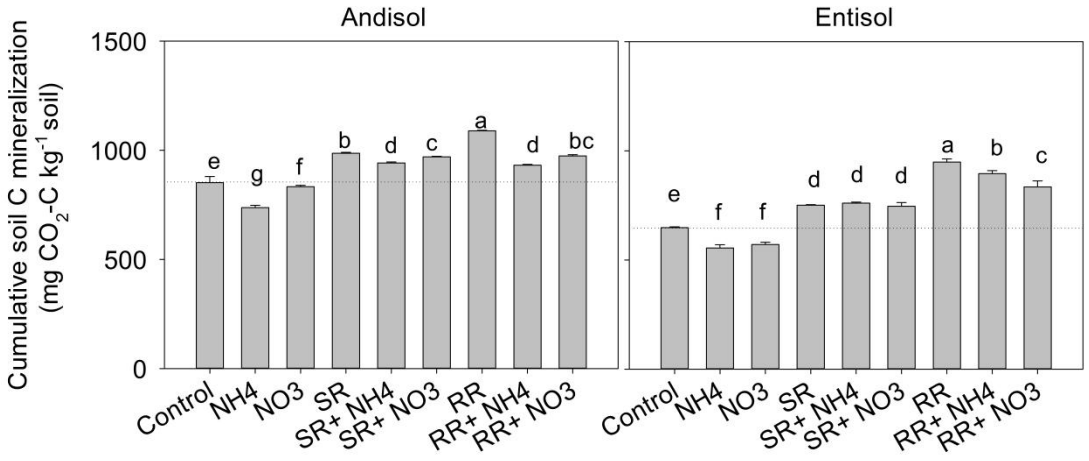

Fig. 3.2 Cumulative soil C mineralization in Andisol and Entisol after 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters above bars indicate significant differences between treatments ($p < 0.05$). SR, shoot residue; RR, root residue

3.3.3 Priming effect and soil C balance

The cumulative priming effect at the end of the incubation was positive across all the treatments (Fig. 3.3), and it was significantly affected by the residue type, N application, and their interaction in both Andisol and Entisol (Table 3.2). 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^{-1}soil in Andisol; 301 vs. 103 mg C kg^{-1} 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 to 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 to 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. 3.3). In the early stage (0–28 day), the cumulative priming effect was significantly higher ($p < 0.01$) in shoot than root residue alone treatment (168 vs. 141 mg C kg^{-1} soil) in Andisol but was significantly higher ($p < 0.01$) in maize root than shoot residue alone treatment (157 vs. 115 mg C kg^{-1} soil) in Entisol. In Andisol, the addition of NO_3^--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_4^+-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^{-1} soil) and Entisol (114 vs. −42 mg C kg^{-1} 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.

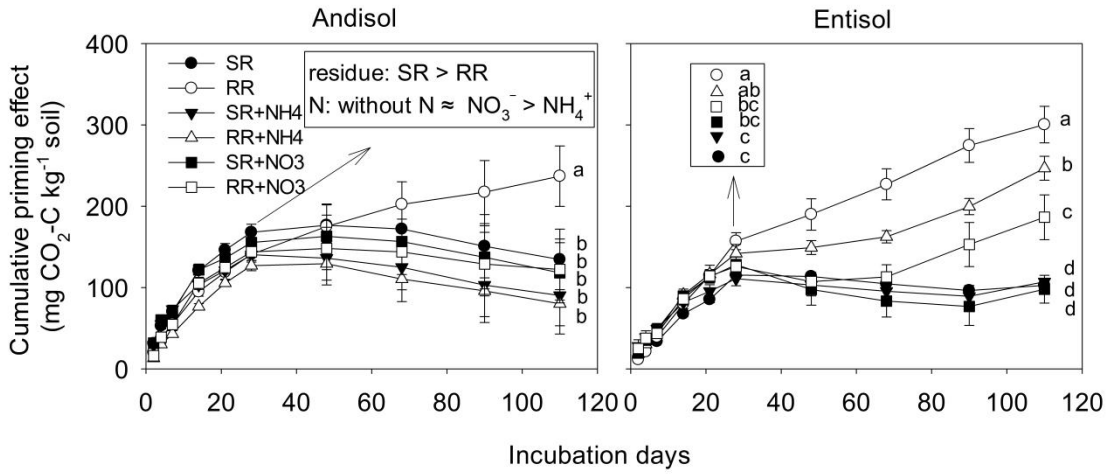

Fig. 3.3 Cumulative priming effect under different treatments in Andisol and Entisol during 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters in the box and at the end of the line indicate significant differences between treatments after 28 days and 110 days of incubation, respectively ($p < 0.05$). SR, shoot residue; RR, root residue

Gross and net C sequestrations under each treatment are shown in Fig. 3.4. After the 110-day incubation, the net C sequestration was positive in all of the treatments (Figs. 3.4c and d). In the residue alone treatments, gross and net C sequestrations were higher in root than shoot residue treatments in Andisol (Figs. 3.4a and c). In Entisol, the root residue alone treatment was not significantly different from the shoot residue alone treatment for gross C sequestration (Fig. 3.4b) but showed a lower net C sequestration (Fig. 3.4d). 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, I did not find any effect of N addition on the gross and net C sequestrations.

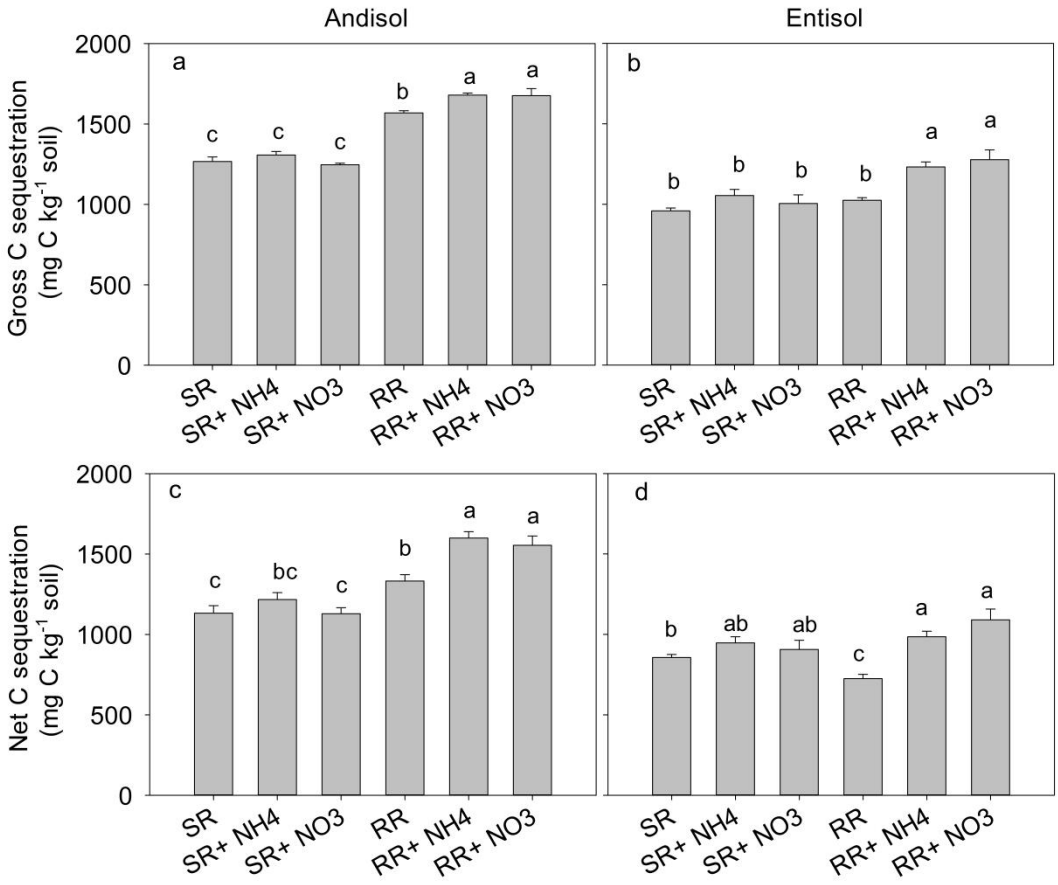

Fig. 3.4 Cumulative priming effect under different treatments in Andisol and Entisol during 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters in the box and at the end of the line indicate significant differences between treatments after 28 days and 110 days of incubation, respectively ($p < 0.05$). SR, shoot residue; RR, root residue

3.3.4 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. 3.5. MBC derived from soil was significantly affected by the residue type, N application, and their interaction (Table 3.2). 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. 3.6. Mineral N was higher in residue amended soils compared to the control in Andisol, while mineral N was depleted in residue amended soils in Entisol, even in treatments where N was added.

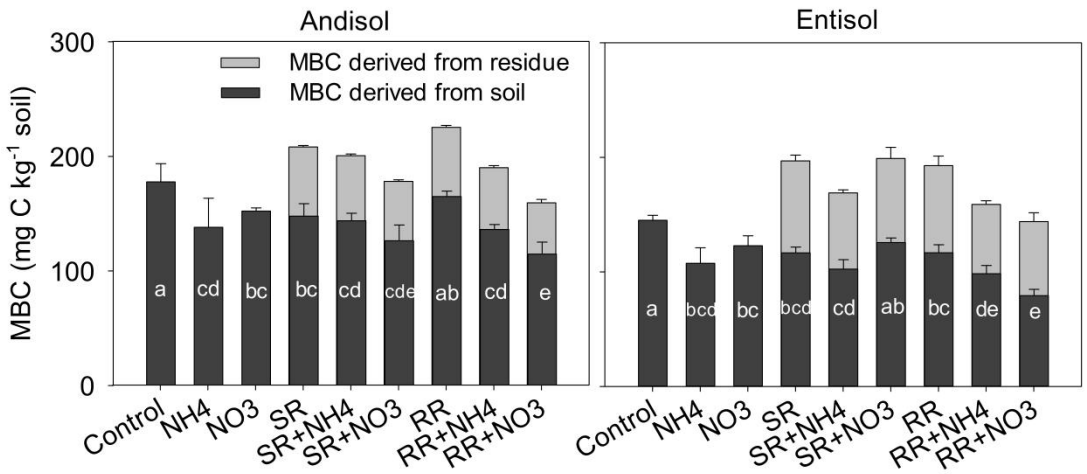

Fig. 3.5 Microbial biomass C (MBC) in different treatments after 110 days of incubation in Andisol and Entisol. Total MBC in residue and residue combined with N treatments was separated into MBC derived from residue and soil. Vertical bars are standard errors (n = 3). Different letters in dark gray bar (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

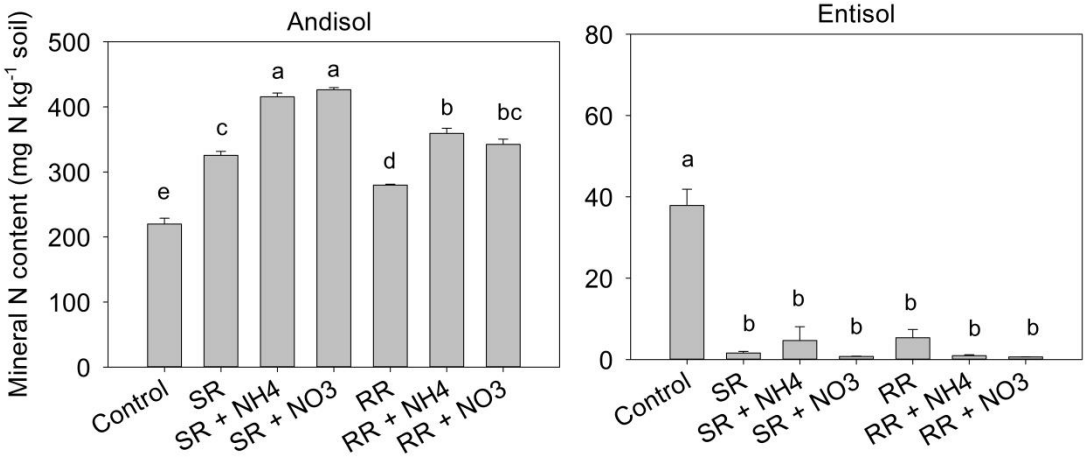

Fig. 3.6 Mineral N (NH_4^+-N + NO_3^--N) content (mg N kg^{-1} soil) under different treatments in Andisol and Entisol after 110 days of incubation. Vertical bars are standard errors (n = 3). Different letters above bars indicate significant differences ($p < 0.05$) among treatments. SR, shoot residue; RR, root residue SR, shoot residue; RR, root residue

3.4 Discussion

3.4.1 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. 3.1). The higher EOC content and lower C:N ratio in shoot residue compared to root residue (Table 3.1) 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; Mwafulirwa et al., 2019). 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) 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. 3.1) is 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), 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. 3.1). Root residue had high C:N ratio (Table 3.1), which might be an indication of the higher content of recalcitrant compounds such as lignin and phenols (Barel et al., 2019; Freschet et al., 2013). Further, the addition of inorganic N could reduce the N mining from maize root residue as N addition suppresses the production of the lignin-degrading enzyme and decreases the abundance of microbes responsible for recalcitrant-C decomposition (Austin and Ballare, 2010; Carreiro et al., 2000). These could lead to lower root residue decomposition. Our result was consistent with a previous study which showed that N addition tend to retard the decomposition of FOM with lower quality (higher lignin content and C:N ratio) (Knorr et al., 2005).

The suppressed decomposition of maize root residue leads to a greater C sequestration, which is beneficial for arable cropping systems.

3.4.2 SOC priming with maize residue and N addition

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. 3.3). The root residue showed a higher C:N ratio and lower EOC content compared to shoot residue (Table 3.1), which could result in an inadequate supply of N to cover the requirements of microorganisms (Moorhead and Sinsabaugh, 2006; Recous et al., 1995), and therefore stimulate N mining from soils. Furthermore, root residue containing a relatively high amount of recalcitrant compounds (Lian et al., 2016) is more beneficial for the growth of K-strategists (Fontaine et al., 2003), which can feed on SOM (Kuzyakov et al. 2000; Shahbaz et al. 2017), 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).

As I hypothesized N addition weakens N mining in the root residue treated soils, and the effect of N addition was not apparent in the shoot reside treated soils. N fertilization reduced cumulative priming effect in the root residue treatments in both Andisol and Entisol after the 110-day incubation (Fig. 3.3). The increased N availability through external N supply could suppress the enzyme production and decrease the N mining from SOM (Chen et al. 2014), 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. 3.5). 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. 3.3) because the shoot residue with a low C:N ratio and high EOC content (Table 3.1) 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). 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 and 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. 3.3) because the presence of labile compounds in shoot and root residue can boost the growth of microorganisms (Hu et al., 1999). The increasing microbial biomass promoted the production of extracellular enzymes and consequently enhanced the mineralization of SOC based on co-metabolism mechanism (Fang et al., 2018; Kuzyakov et al., 2000). Shoot residue having a higher EOC content and lower C:N ratio (Table 3.1) could stimulate more growth of microorganisms, therefore inducing a higher positive priming effect in Andisol during the early phase (Fig. 3.3). Higher priming effect was found in root residue treatments rather than in shoot residue treatments in Entisol (Fig. 3.3); the reason for this could be that N mining also contributed to the SOC priming in Entisol due to the lower N availability (Table 3.1) as NO_3^--N addition significantly reduced the intensity of priming effect in root residue treatments in Entisol at this stage (0–28 day) (Fig. 3.3, Table 3.2).

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. 3.3). 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). Our explanation is supported by the short turnover time of microbes (~30 days; Blagodatskaya et al. 2009; 2011). 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^{-1} soil) than Andisol (96 mg C kg^{-1} soil) (Fig. 3.2 and Table 3.2) due to the lower N availability in Entisol soils (Fig. 3.6). 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. 3.3 and Table 3.2), 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 two to three weeks of

incubation in this study) due to co-metabolism. 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; Mwafulirwa et al. 2019) and suggest that relatively long-term (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.

3.4.3 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 than with shoot residue in Andisol (Fig. 3.4c), despite the fact that root residue induced higher priming effect than shoot residue (Fig. 3.3). 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. 3.1). 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. 3.3). N addition significantly enhanced the net C sequestration in maize root residue treatments because of the reduced intensity of priming effect (Fig. 3.3) 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. 3.4). N forms did not affect net C sequestration (Figs. 3.4c and 3.4d). Considering that NO_3^--N is susceptible to leaching, NH_4^+-N is recommended as the mineral N fertilizer in terms of C sequestration.

3.5 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 root treated soils. Such decreased priming effect and maize root residue decomposition could be

attributed to the reduction of microbial N mining. I 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.

Supplementary materials

Text S3.1 I conducted a pre-experiment to verify if the O_2 content in the sealed glass jars is sufficient for soil microorganisms during period incubation. The source of C was glucose, which was applied at 1.4, 2.8, 4.2, 5.6, 8.5, 17.1, 25.6 and 34.1 g C kg^{-1} soil. The pre-incubated Andisol (45 g) was thoroughly mixed with the glucose in each glass jar and 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_2 derived from the mixed soil and a glass vial containing 5 mL 5 mM HCl to retain soil moisture. Three 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 7 days of the incubation in an incubator (LTI-1200, Eyela, Tokyo, Japan). The incubation conditions were the same as our experiment using maize residue. CO_2 was released in proportional to added glucose until ~1300 mg CO_2-C kg^{-1} soil (Fig. S1). Our sampling frequency (replacement of the trap solution, NaOH, at day 2, 4, 7, 14, 21, 28, 48, 68, and 90 of incubation, allowing for aeration) guaranteed that O_2 content in the sealed jars was sufficient for microorganisms in maize residue treated soils between each sampling event in our experiment, because no results from any single sampling event showed higher mineralized-C than 500 mg CO_2-C kg^{-1} soil.

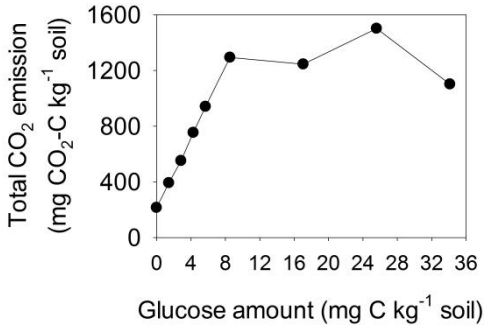

Fig. S3.1 Total CO_2 released from glucose treated Andisol

Table S3.1 The total C (TC) and total N (TN) content in freeze-dried and oven-dried (at 70°C and 100°C) Andisol and Entisol. Same letter indicates no significant differences among the treatments ($p > 0.05$).

	TC （mg C kg^{-1} soil）			TN （mg N kg^{-1} soil）		
	Freeze-drying	70°C drying	100°C drying	Freeze-drying	70°C drying	100°C drying
Andisol	84.4±2.3a	82.3±0.9a	83.9±3.4a	5.8±0.3a	5.6±0.1a	5.7±0.3a
Entisol	27.1±0.6a	28.4±0.7a	27.6±1.3a	2.5±0.1a	2.7±0.1a	2.7±0.1a

Chapter 4

Distinct effects of shoot- and root-residues on the priming effect in subsoil versus topsoil in agroecosystems

Abstract

Priming effect is one of the most important interactions between organic C input and soil organic carbon (SOC) mineralization. However, the impacts of crop residue quality on PE in the subsoil is still not clear, despite more than 50% of SOC is stored in the subsoil. This study aimed to compare the effect of crop residue quality on priming effect between subsoil and topsoil. I conducted a 110-day laboratory incubation experiment by adding 3 g C kg-1 soil of 13C-labeled maize shoot or root residue with and without N addition to topsoil (0–10 cm) and subsoil (40–60 cm) of two types of agricultural soil (Andisol; Entisol). Our results showed that after 110-day incubation, maize residue addition induced positive PE. In the subsoil, the shoot residue induced higher priming effect than root, whereas the trend was reversed in the topsoil. Meanwhile, in the topsoil, N additions reduced the priming effect under the root treatment but did not altered that under the shoot treatment, while in the subsoil, the higher priming effect occurred with shoot residue through microbial growth induced by the addition of labile residue with low C/N ratio. Relative priming effect (% of SOC mineralization) in the subsoil was 2.7–22 times higher than in the topsoil across two soil types. The contrasting effects of residue quality (shoot vs. root) on priming effect intensity between soil layers, suggested that microbial N-mining could dominate the contribution to higher priming effect in the topsoil, while the microbial co-metabolism would play a more important role in the subsoil. Higher relative priming effect in the subsoil points to the vulnerability of SOC to decomposition upon FOM addition (particularly with high-quality FOM) in subsoil. Our results highlight that subsoil C is not as stable as previously perceived, and could be even more easily destabilized than that in the topsoil after FOM addition. Furthermore, the priming effect differently induced by high- and low-quality residues between topsoil and subsoil should be considered for more efficient residue management in agroecosystems.

4. 1 Introduction

Soil is the largest C pool in terrestrial ecosystems, storing around 2,344 Gt C at depths of up to 3 m, which is three times as much as in the atmosphere (Jobbagy and Jackson, 2000). As a major source and sink of atmospheric greenhouse gases (Lal, 2010; Lehmann and Kleber, 2015), even a small change of soil organic C (SOC) pool could cause large impact on global climate change (Lal, 2010). In the past 200 years, a global C debt due to land use change (i.e., converting to agricultural lands) was 133 Pg C for the top 2 m of soil, and about 25% of this loss is attributed to SOC mineralization (Houghton, 2012; Sanderman and Berhe, 2017). Hence, the loss of SOC in agroecosystems has received increasing attention in recent years.

Crop residues as the byproducts of agriculture are the main C source for agricultural soils; returning these byproducts to soil is generally recommended to increase SOC stock (Jin et al., 2020; Liu et al., 2014). These fresh organic matters (FOMs) as an energy and nutrient source for soil microbes may influence their catabolic and anabolic processes, and consequently influence SOC turnover, known as priming effect (Kuzyakov et al., 2000); the increase of SOC mineralization (compared to that without FOM addition) represents positive priming effect, while the decrease of SOC mineralization represents negative priming effect. As one of the most important interactions between C input and output in soils, priming effect induced by FOM addition has been studied intensively. Most of the previous studies, however, focused on topsoil (Abolat and Ekinci, 2017; Han et al., 2018; Wang et al., 2015a), and our understanding of the occurrence and the importance of the priming effect in subsoil is limited, despite more than 50% of C is stored in subsoil (Batjes, 2014).

Subsoil C is generally characterized by high mean residence time (Rethemeyer et al., 2005) and assumed to be relatively stable and unsusceptible to FOM addition (Salomé et al., 2010; Wordell-Dietrich et al., 2017) compared to C in topsoil. Previous studies suggested that development of deep rooting plants or mechanical input of FOM to subsoil may have the potential to hold the added FOM for a long term, therefore reducing CO_2 emission (Kell, 2011; Lorenz et al., 2011; Lynch and Wojciechowski, 2015; Torres-Sallan et al., 2017). However, recent studies found that the subsoil C could be destabilized and subjected to a large amount of loss through priming effect when energy rich FOM is added and available to microbes in subsoil (Fontaine et al., 2007). It seems that the FOM with relatively high energy may result in higher SOC mineralization. However, there are only a few studies that have examined SOC loss through priming effect in subsoil with FOM addition, the findings

of which are contradictory (Fontaine et al., 2007; Salomé et al., 2010) and we have little knowledge on the effect of FOM quality on priming effect in subsoil.

Residue quality is one of the most important factors for the direction and intensity of priming effect in topsoil. The residue quality is generally defined by chemical composition (e.g., lignin content) and stoichiometry (C:N ratio) and varies among plant organs (e.g., shoot and root); root usually contains more recalcitrant compounds and has higher C:N ratio than shoot and represents relatively low quality (Barel et al., 2019; Freschet et al., 2013). Thus, previous studies used root and shoot residues to represent different plant residue quality for understanding the effects of plant residue quality on SOC dynamics (Clemente et al., 2013; Mwafulirwa et al., 2019). The addition of relatively high quality maize shoot residue (indicated by low C:N ratio) has been found to lead to a greater positive priming effect than low quality maize root residue (Mwafulirwa et al., 2019). On the other hand, Shahbaz et al (2017) found that maize root residue induced the maximum positive priming effect among maize leaf, stalk and root treatments. This inconsistency may be explained by the different dominance of mechanisms of positive priming effect (microbial stoichiometric decomposition, microbial N mining and co-metabolism) controlled by FOM quality and soil nutrient status (Chen et al., 2014; Fang et al., 2020).

The microbial N mining hypothesis assumes that limited N availability for supporting microbial growth in C-rich soils facilitates the microbial activity on SOM decomposition to acquire N. Alternatively, microbial stoichiometry decomposition assumes that microbial stoichiometry of FOM is a driving force on SOM mineralization, meaning that the microbial activity and SOM mineralization could be stimulated when N limitation is alleviated (Craine et al., 2007; Chen et al., 2014). Meanwhile, accelerated microbial activity with the input of crop residues, may also enhance SOC mineralization as a result of co-metabolism (Blagodatskaya and Kuzyakov, 2008). However, there is a lack of knowledge on the role of the FOM quality in controlling the priming effect and the underlying dominant mechanism in subsoil and the dominant mechanism of positive priming effect in subsoil may be different from in topsoil as subsoil is more C limited.

The objective of this study is to clarify how the crop residue quality affects priming effect in agricultural subsoil and the difference of priming effect in subsoil compared to topsoil. ^{13}C-labeled maize shoot and root residues were used to represent different quality of crop residue and to trace the CO_2 derived from crop residue and native SOC to calculate priming effect and to fit a two-pool exponential decay model to simulate the mineralization of the native SOC. I hypothesized that the relatively

high-quality shoot residue will stimulate more native SOC decomposition, and therefore will lead to higher positive PE, thereby reducing relatively easily degradable organic matter in the subsoil which was limited by labile C.

4. 2 Materials and Methods

4.2.1 Soils and ^{13}C-labeled maize residue

Topsoil (0–10 cm) and subsoil (40–60 cm) samples were taken from two farmlands used for vegetable cultivation. One is located in Kyoto prefecture, Japan (35°3′N, 135°48′E); the soil is classified as Entisol (ENT) (Soil Survey Staff, 2014). The other is located in Nagano prefecture, Japan (36°31′N, 138°21′E); the soil is derived from volcanic ash and classified as Andisol (AND) (Soil Survey Staff, 2014). After air-drying, soil samples were sieved (≤ 2mm) to eliminate organic residues prior to the incubation experiment. Selected physico-chemical properties of soils are shown in Table 4.1.

Maize residues were from ^{13}C-labeled maize grown under controlled conditions (25°C, 800 μmol photons m^{-2} s^{-1}, and 12 h day/12 h night) in a biotron (NC350HC; Nippon Medical & Chemical Instruments Co. Ltd, Osaka, Japan). Briefly, maize plants cultivated in potted trays filled up with perlite and vermiculite. The pulse-labelling of maize plant 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 one month. In each pulse-labelling event, the plants were transferred to a portable labelling chamber which was airtightly sealed with silicone rubber. The chamber air was circulated by two battery operated mini-fans. Pulse of $^{13}CO_2$ was generated by injecting 120 mL of $^{13}CO_2$ (99 atom %) with a 60-mL syringe. To maximize the uptake of $^{13}CO_2$ in each pulse labelling, 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_2 concentration for maize growth. After harvest, maize shoots and roots were washed and dried at 70°C and milled to pass through a 2 mm sieve prior to incubation. The characteristics of the maize shoot and root residues are given in Table 4.1.

Table 4.1 Initial properties of topsoil and subsoil of Andisol (AND) and Entisol (ENT), and maize residue used for incubation experiment.

	Andisol topsoil (AND TOP)	Andisol subsoil (AND SUB)	Entisol topsoil (ENT TOP)	Entisol subsoil (ENT SUB)	Maize shoot residue	Maize root residue
Total C (%)	8.36	3.66	2.87	0.87	41.4	41.3
Total N (%)	0.57	0.26	0.27	0.06	3.32	1.60
C:N	14.5	13.9	10.5	13.5	12.5	25.8
^{13}C (%)	1.09	1.09	1.09	1.09	7.13	5.42
NH_4^+-N (mg kg^{-1})	20.3	23.8	25.6	15.1		
NO_3^--N (mg kg^{-1})	112.0	76.0	18.6	13.7		
EOC(g kg^{-1})					11.4	6.2
EN(g kg^{-1})					4.6	3.2
Sand (%)	22	30	60	63		
Silt (%)	50	42	25	22		
Clay (%)	28	28	15	14		
pH (H_2O)	6.5	6.3	7.2	5.8		

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

4.2.2 Experimental design and incubation settings

The experiment was conducted with 275 mL glass jars (Tokyo Glass Co., Ltd., Tokyo, Japan) equipped with airtight lids. In total, seven treatments were applied for topsoil (TOP) or subsoil (SUB) of each type of soil (i.e., AND and ENT): non-amended; amended with shoot residue (SR); amended with root residue (RR); amended with shoot residue and either NH_4^+-N (SR-NH_4) or NO_3^--N (SR-NO_3); amended with root residue and either NH_4^+-N (RR-NH_4) or NO_3^--N (RR-NO_3). Each treatment contained six replicates to allow for two times (three replicates each time) of destructive soil sampling at day 7 and day 110 of incubation.

Before the treatments were initiated, soils were pre-incubated at 55% of soil water holding capacity for 7 days to avoid microbial respiration flush induced by soil rewetting (Shi and Marschner, 2017). Forty-five grams of air-dried soil was added to each glass jar and moistened to 55% of their water holding capacity. The jars were maintained under a constant temperature (25°C) using an incubator (LTI-1200, Eyela,

Tokyo, Japan) throughout the experiment. After pre-incubation, milled powder of ^{13}C labeled maize residue (i.e., shoot or root residue less than 2 mm) was added to each soil type (i.e., AND and ENT) at 3.0 g C kg^{-1} soil and then 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 and thoroughly mixed. NO_3^--N and NH_4^+-N were applied at 60 mg N kg^{-1} soil as KNO_3 and $(NH_4)_2SO_4$, respectively. Each glass jar including a plastic bottle containing 10 mL of 1 M NaOH solution to trap CO_2 derived from mixed soil and a glass vial containing 5 mL of 5 mM HCl to keep soil moisture. Five glass jars containing only plastic bottle and glass vial (i.e., without soil) were treated as blank.

4.2.3 CO_2 sampling and analysis

The mineralized C (CO_2) derived from maize residue and soil was trapped in 10 mL of 1 M NaOH in a plastic bottle placed inside each jar. The trap solution was replaced at day 2, 4, 7, 14, 21, 28, 48, 68, and 90 of incubation. Following each sampling, half of the trapped NaOH solution (5 mL) was titrated 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 carbonate amount. To correct any CO_2 contamination from air 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 thus 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 $SrCl_2$. The NaOH solution containing $SrCO_3$ was repeatedly centrifuged (2000 rpm, 5 min) and washed in between with deionized water until NaOH was removed and the pH reached 7 (Blagodatskaya et al., 2011). The $SrCO_3$ precipitate was dried at 70°C, and ^{13}C abundance was determined using an elemental analyzer connected to an isotope ratio mass spectrometer (EA-IRMS) (Delta V advantage, Thermo Fisher Scientific, MA, USA).

4.2.4 Soil analysis

Air dried and sieved soils were analyzed for selected physico-chemical properties including soil texture, soil pH, total C (TC) and its ^{13}C abundance, total N (TN) and mineral N (NH_4^+-N and NO_3^--N) (Table 4.1). For the soil texture analysis, firstly the organic matters in soil samples were removed by H_2O_2, then adjusted the

pH to 9 to 10, and then ultrasonicated. The sand fraction (0.05–2 mm) were determined by using the sieving method. The silt fraction (2–50 μm) were determined by sieving with pipette method. The clay fraction (< 2 μm) contents were determined by the pipette method (Gee and Or, 2002). Soil pH was measured at a soil to water ratio 1:5 by using a pre-calibrated pH electrode (Benchtop pH meter F-70 Series, Horiba, Kyoto, Japan). For TC, TN, and ^{13}C abundance analysis, soils were dried at 100 °C, fine-ground and analyzed by the EA-IRMS (Delta V advantage, Thermo Fisher Scientific). For mineral N measurement, 5g soil 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 filter paper (No. 6, Advantec), NH_4^+ and NO_3^- in the obtained extracts were determined by colorimetric analysis using an automated flow injection analyzer (AQLA-700 Flow Injection Analyzer, Aqualab, Tokyo, Japan).

Destructive soil samplings were conducted at day 7 and day 110 of incubation for measurement of soil microbial biomass C (MBC). The fumigation extraction method was used to measure MBC, as described by (Vance et al., 1987). Briefly, 16 g of sampled soil was equally divided into two subsamples, and one subsample was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. 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 filter paper (No. 6, Advantec, Tokyo, Japan). The obtained extracts were analyzed for total C content using a TOC analyzer (Shimadzu TOC-V_{CSH}, Shimadzu, Kyoto, Japan). MBC was calculated as EC/k_{EC}, where EC = (organic C from fumigated soils) – (organic C from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990).

4.2.5 Calculations

The proportion of maize residue derived C (*P*res) in various pools was calculated based on a two source mixing model, using Eq. (1) (Shahbaz et al., 2017a):

$$Pres = (Attr - Atc) / (Atr - Atc) \quad (1)$$

where *Attr* represents At%^{13}C values of CO_2-C trapped in NaOH; *Atr* represents At%^{13}C values of initially incorporated maize residues, and *Atc* represents At%^{13}C values coming from the unamended control soil sample.

Thus, the amount of residue derived C ($C_{res\text{-}derived}$) in various pools was calculated using Eq. (2) (Poirier et al., 2013).

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

Where [C] represents total respired CO_2 (mg C kg^{-1}) measured by titration method.

The amount of priming effect (PE, mg C kg^{-1}) was calculated according to the following Eq. (3) (Blagodatskaya et al., 2011).

$PE = (CO_{2\ total} - CO_{2\ res\text{-}derived}) - CO_{2\ control}$ (3)

where $CO_{2\ total}$, $CO_{2\ res\text{-}derived}$, and $CO_{2\ control}$ represent CO_2 amounts (mg CO_2-C kg^{-1} soil) coming from residue amended soil, maize residue and unamended control soil, respectively.

A two-pool exponential decay model was adopted to simulate the mineralization of the native SOC Eq. (4) (Meng et al., 2017):

$$Cres = 1 - pL \times e^{-kL \times t} - (1 - pL) \times e^{-kS \times t} \quad (4)$$

where Cres is the proportion (%) of the SOC mineralized after t days of incubation; pL and (1 − pL) is the proportion of the labile and stable pool of SOC, kL and kS are the decomposition constant of the labile and stable pool of SOC; t is the incubation days.

To address the different SOC content in topsoil and subsoil, specific respiration were obtained by dividing CO_2-production through the initial SOC content (g) (Wordell-Dietrich et al., 2017). Relative priming effect was calculated by cumulative primed soil CO_2-C divided by released CO_2-C in control at the corresponding times.

4.2.6 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 soil layers (topsoil and subsoil), residue type (maize shoot and root), and their interactions on cumulative specific soil mineralization, cumulative maize residue decomposition, priming effect, relative priming effect, parameters in the two-pool exponential decay model and MBC at day 7 and the end of the incubation. For cumulative specific soil mineralization and parameters in the two-pool exponential decay model, the effect of residue type contains three patterns (without residue addition, maize shoot addition, and maize root addition). Two-way ANOVA was also 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 priming effect in the early phase (0–28 day) and at the end of the incubation. Following each *F*-value, multiple comparisons of means with a Turkey test was conducted. Statistically significant difference was

identified at the 0.05 level. Statistical analysis was conducted with SPSS Statistics (version 20.0, SPSS Inc., Chicago, IL, USA). Figures were generated using SigmaPlot 12.5 (SYSTAT Software, CA, USA.).

4. 3 Results

4.3.1 Maize residue decomposition

Cumulative residue decomposition of added residue is shown in Fig. 4.1 and it was significantly ($p < 0.01$) affected by residue type, soil layer and their interaction after 110-day of incubation (Table 4.2). The decomposition of added maize shoot residue was significantly higher ($p < 0.01$) than maize root residue in both Andisol and Entisol. Meanwhile, maize residue decomposition was significantly higher ($p < 0.01$) in topsoil than in subsoil of both Andisol and Entisol. Cumulative decomposition of added shoot and root residue (mg C g^{-1} C $_{residue}$) were in the order of SR TOP (482) > RR TOP (412) ≈ SR SUB (402) > RR SUB (285) in Andisol and SR TOP (478) > RR TOP (450) > SR SUB (391) > RR SUB (270) in Entisol. The decomposition rate of maize residue have two distinct phase: early intensive decomposition phase (day 0-28) and later slow decomposition phase (day 29-110).

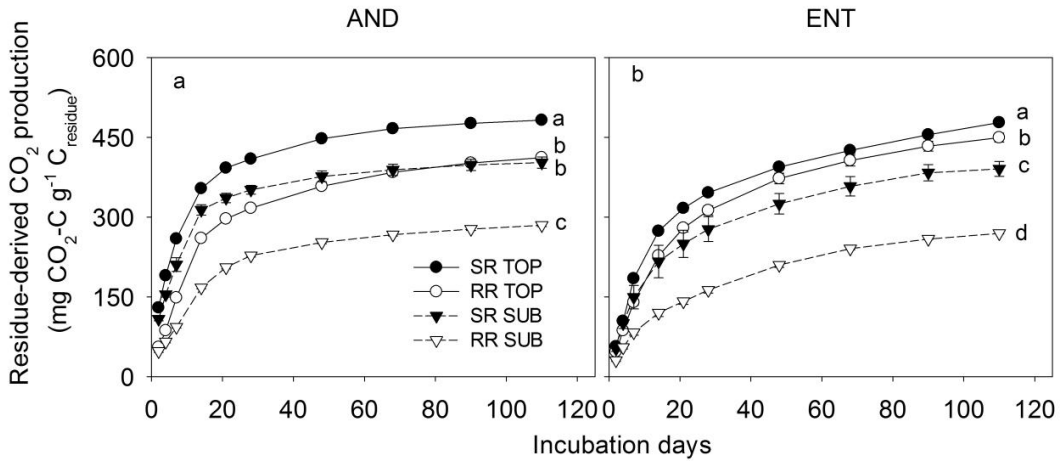

Fig. 4.1 Cumulative residue decomposition of added residue in shoot- and root-residue amended topsoil (SR TOP and RR TOP) and subsoils (SR SUB and RR SUB) of Andisol (AND) and Entisol (ENT) during 110 days of incubation. Values are expressed as mg CO_2-C g^{-1} $C_{residue}$. Error bar represents standard error of the mean (n = 3). Different letters at the end of the line indicate significant differences (P < 0.05) among the treatments after 110 days of incubation.

Table 4.2 *F* values from results of ANOVA showing the effects of soil layer (topsoil and subsoil), maize residue (maize shoot and root residue), and their interactions on cumulative CO_2-C respired from soil (CO_2-C_{soil}), specific soil mineralizatiion (CO_2-$C_{spesoil}$), maize residue decomposition of added residue (CO_2-C_{res}), priming effect (CO_2-C_{primed}), relative priming effect (CO_2-C_{relpe}) and MBC derived from residue (MBC_{res}) in day 7 and day 110.

Soil type		CO_2-$C_{spesoil}$ (mg C kg^{-1} SOC)	CO_2-C_{res} (mg C g^{-1} $C_{residue}$)	CO_2-C_{primed} (mg C kg^{-1} soil)	CO_2-C_{relpe} (mg C kg^{-1} SOC_{min})	MBC day7	MBC Day110
Andisol	Layer	1706.5***	888.8***	5.9*	4656.7***	220.2***	109.9***
	Residue	1278.5***	734.5***	19.8***	75.0***	3026.2***	1986.9***
	Layer × residue	513.6***	46.7***	7.1**	376.9***	31.9***	4.2*
Entisol	Layer	208.4***	728.6***	515.4***	17754.1***	404.3***	117.6***
	Residue	3679.0***	227.9***	10.4*	44.1***	1553.2***	1492.1***
	Layer × residue	1727.7***	88.1*	238.2***	427.7***	24.7***	10.46**

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

4.3.2 Soil organic carbon mineralization

The specific SOC mineralization (cumulative SOC mineralization divided by SOC content) during the 110-day of incubation was shown in Fig. 4.2, and it was significantly ($p < 0.01$) affected by residue quality, soil layer and their interaction after 110-day incubation (Table 4.2). In Andisol, the specific SOC mineralization without maize residue addition were similar ($p > 0.05$) between topsoil and subsoil (10.2 vs. 10.2 mg C kg^{-1} SOC) after 110-day incubation. The specific SOC mineralization without maize residue addition was significantly higher ($p < 0.05$) in topsoil than subsoil of Entisol (22.6 vs. 10.2 mg C kg^{-1} SOC). Residue addition significantly increased ($p < 0.01$) the specific SOC mineralization, and it was significantly higher ($p < 0.01$) in subsoil than topsoil of both Andisol and Entisol, i.e., increased by 75% and 93% in subsoil, and 16% and 28% in topsoil of Andisol for root

and shoot residues, respectively; 267% and 321% in subsoil, and 16% and 46% in topsoil of Entisol for root and shoot residues, respectively.

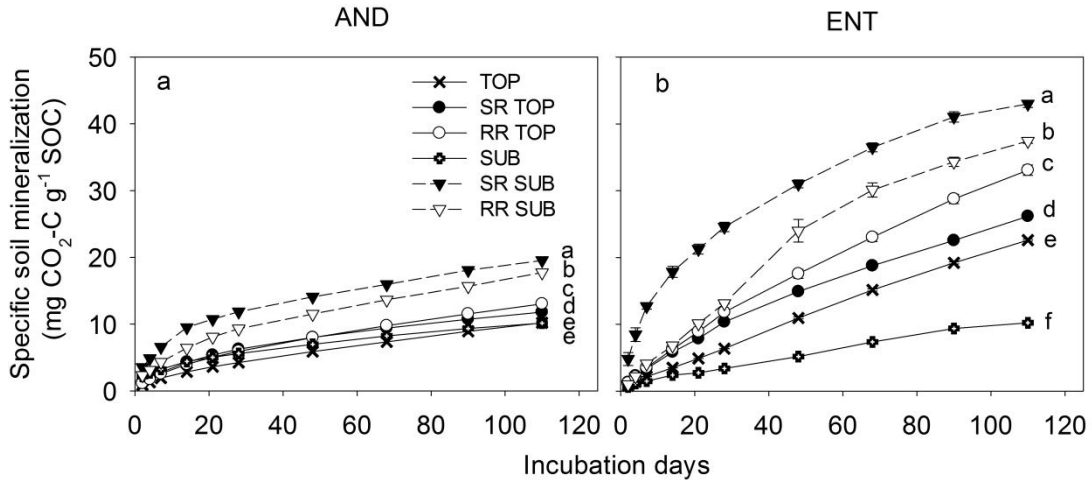

Fig. 4.2 Specific soil mineralization (cumulative soil mineralization of SOC) for topsoil (TOP), subsoil (SUB), shoot- and root-residue amended topsoil (SR TOP and RR TOP) and subsoil (SR SUB and RR SUB) in Andisol (AND) and Entisol (ENT) during 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters at the end of the line indicate significant differences ($P < 0.05$) among the treatments after 110 days of incubation.

SOC mineralization was simulated by the two-pool model ($R^2 > 0.99$, Table 5.3). The proportion of the labile C pool significantly increased ($p < 0.01$) after maize residue addition and this increased proportion of labile C pool was significantly higher ($p < 0.01$) in subsoil than topsoil of both Andisol and Entisol. Meanwhile, the decomposition constant of the stable pool of SOC (kS) was significantly higher ($p < 0.01$) in subsoil compared to topsoil of both Andisol and Entisol.

Table 4.3 Soil organic carbon decomposition in no-amended, shoot-, and root-residue amended topsoil (TOP, SR TOP and RR TOP) and subsoil (SUB, SR SUB and RR SUB) of Andisol (AND) and Entisol (ENT)

Soil type	Treatment	Proportion of SOC mineralization at the end of incubation	$Cres = 1 - pL \times e^{-kL \times t} - (1 - pL) \times e^{-kS \times t}$			
			pL	*kL*	1-*pL*	*kS*
Andisol (AND)	TOP	0.0102	0.0023e	0.1418	0.9977	0.000074d
	SR TOP	0.0118	0.0048c	0.0944	0.9952	0.000066e
	RR TOP	0.0130	0.0039d	0.1007	0.9961	0.000085c
	SUB	0.0101	0.0038d	0.2675	0.9962	0.000061e
	SR SUB	0.0195	0.0092a	0.1663	0.9908	0.000098b
	RR SUB	0.0177	0.0064b	0.1271	0.9936	0.000105a
ANOVA *p* value						
	Layer	<0.001	<0.001	<0.001	<0.001	<0.001
	Residue	<0.001	<0.001	<0.001	<0.001	<0.001
	Layer×residue	<0.001	<0.001	<0.001	<0.001	<0.001
Entisol (ENT)	TOP	0.0226	0.0038e	0.2675	0.9962	0.000061e
	SR TOP	0.0262	0.0072d	0.0482	0.9928	0.000175c
	RR TOP	0.0331	0.0083c	0.0341	0.9917	0.000234b
	SUB	0.0102	0.0010f	0.3152	0.9990	0.000088d
	SR SUB	0.0430	0.0198a	0.1062	0.9802	0.000232b
	RR SUB	0.0374	0.0103b	0.0500	0.9897	0.000268a
ANOVA *p* value						
	Layer	<0.001	<0.001	<0.001	<0.001	<0.001
	Residue	<0.001	<0.001	<0.001	<0.001	<0.001
	Layer×residue	<0.001	<0.001	<0.001	<0.001	<0.001

Cres represents the proportion of SOC mineralization at time t (days), pL and (1 − pL) are the proportion of the labile and stable SOC pools, respectively; kL and kS are the decomposition constants for the labile and stable pools of SOC, respectively. R^2 of the model fitting for all treatments were higher than 0.99 and not presented in the table. Different letters indicate the significant differences ($p < 0.05$) among the treatments.

4.3.3 Soil organic carbon priming

The priming effect induced by maize shoot and root residue addition is shown in Fig. 4.3, and it was significantly ($P < 0.01$) affected by residue quality, soil layer and their interaction in both Andisol and Entisol after 110-day incubation (Table 4.2). The intensity pf priming effect was higher in root than shoot amended topsoils of Andisol (237 vs. 135 mg C kg^{-1} soil) and Entisol (301 vs 103 mg C kg^{-1} soil), while the intensity of priming effect was higher in shoot than root residue amended subsoils of Andisol (344 vs. 277 mg C kg^{-1} soil) and Entisol (285 vs. 236 mg C kg^{-1} soil). The intensity of positive priming effect was significantly higher ($p < 0.05$) in subsoil than topsoil under same residue treatment in both Andisol and Entisol, except root residue treatment in ENT.

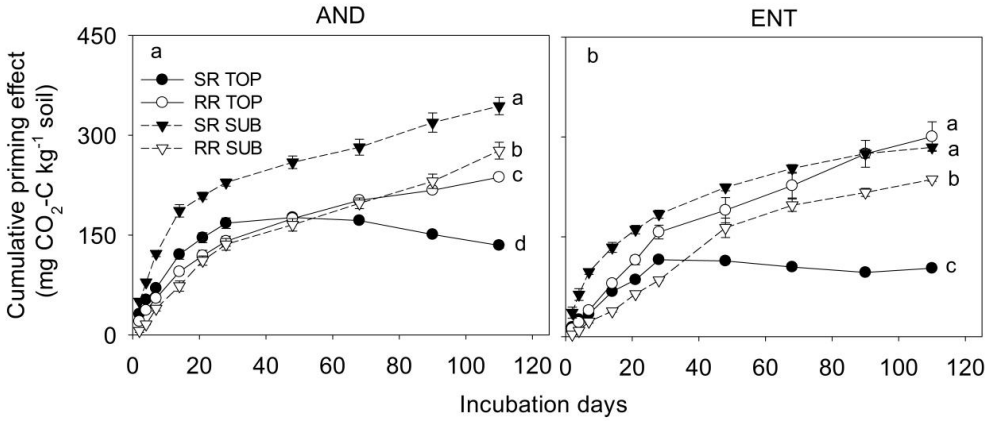

Fig. 4.3 Cumulative priming effect in shoot- and root-residue amended topsoil (SR TOP and RR TOP) and subsoil (SR SUB and RR SUB) of Andisol (AND) and Entisol (ENT) during 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters at the end of the line indicate significant differences ($P < 0.05$) among the treatments after 110 days of incubation.

The relative priming effect (cumulative primed soil CO_2-C divided by mineralized SOC in control) at the end of the incubation was shown in Fig. 4.4, and it was significantly ($p < 0.01$) affected by residue type, soil layer and their interaction (Table 4.2). The relative priming effect of residue treatment was greater for subsoil

than for topsoil of both Andisol (93% vs. 16% in shoot amended soils and 75% vs. 28% in root amended soils) and Entisol (350% vs. 16% in shoot amended soils and 291% vs. 46% in root amended soils).

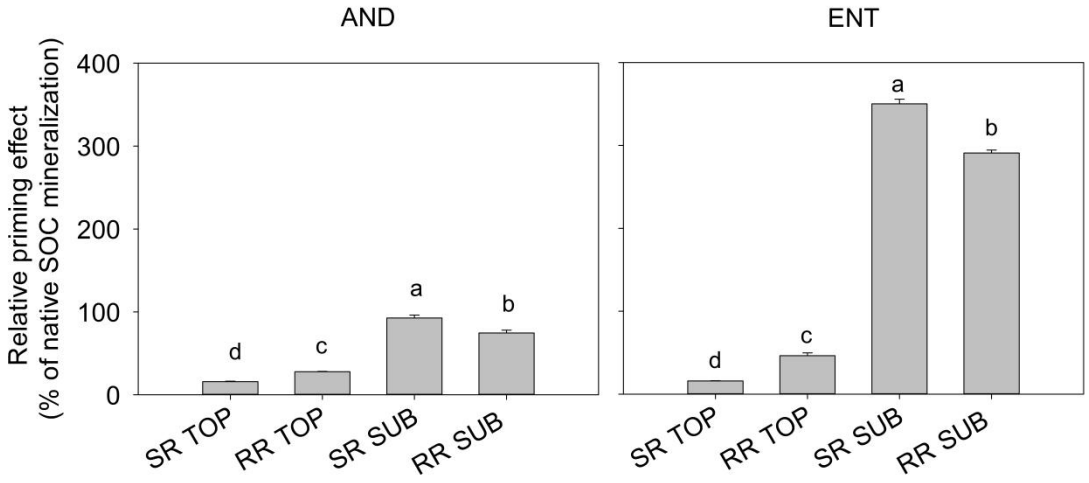

Fig. 4.4 Relative priming effect (% of native SOC mineralization) for shoot- and root-residue amended topsoil (SR TOP and RR TOP) and subsoil (SR SUB and RR SUB) of Andisol (AND) and Entisol (ENT) after 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters above the bars indicate significant differences among the treatments ($P < 0.05$).

The priming effect was interactively affected ($p < 0.05$) by residue type and N fertilization after 110-day incubation in both topsoil and subsoil of Andisol and Entisol (Fig. 4.5). In topsoil, N fertilization did not affect ($p > 0.05$) the intensity of priming effect induced by shoot residue addition but reduced ($p < 0.01$) the intensity of priming effect by root residue addition both in Andisol and Entisol. In subsoils, N addition did not affect ($p > 0.05$) the intensity of priming effect with root residue in both Andisol and Entisol, except NH_4^+-N combined with root residue in Entisol. Meanwhile, N addition did not affect ($p > 0.05$) or increased ($p < 0.01$) the intensity of priming effect with shoot residue in Andisol or Entisol, respectively, except NH_4^+-N combined with shoot residue in Andisol. In the early intensive phase of maize residue decomposition (0–28 day), the cumulative priming effect was significantly higher ($p < 0.01$) in shoot than root residue alone treatment in the topsoil of Andisol and the addition of NO_3^--N did not affect the intensity of priming effect induced by shoot or root residue ($p > 0.05$). In the topsoil of Entisol, the cumulative priming effect was significantly higher ($p < 0.01$) in maize root than shoot residue alone treatment and 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 of root residue plus NH_4^+-N. In subsoil, cumulative priming

effect was significantly higher ($p < 0.01$) in shoot than root residue treatment and N addition did not affect ($p > 0.05$) the priming effect in both Andisol and Entisol.

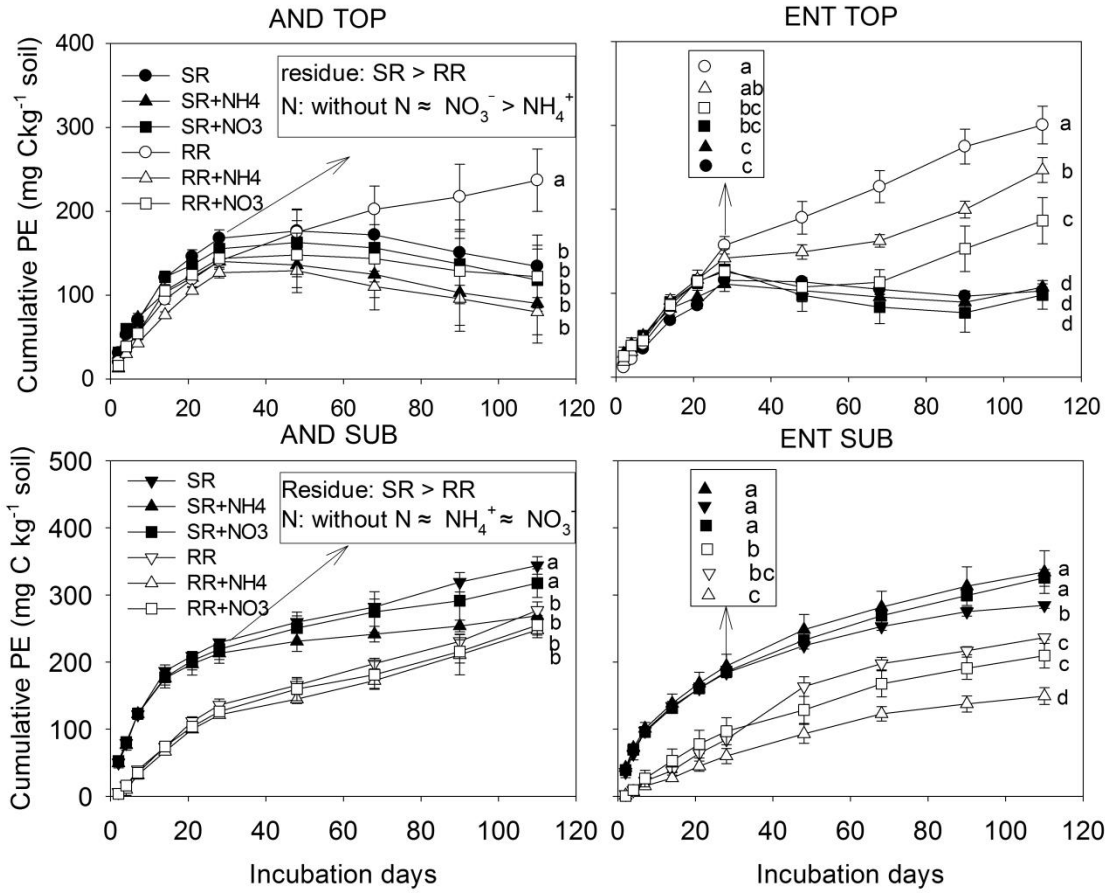

Fig. 4.5 Cumulative priming effect (PE) under different treatments in Andisol topsoil (AND TOP), Andisol subsoil (AND SUB), Entisol topsoil (ENT TOP) and Entisol subsoil (ENT SUB) during 110 days of incubation. Error bar represents standard error of the mean ($n = 3$). Different letters in the box and 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

4.3.4 Soil microbial biomass carbon

MBC amount of each treatment on day 7 and day 110 is shown in Fig. 5.6. Maize residue addition significantly increased ($P < 0.05$) MBC compared with control both on day 7 and day 110 of incubation across topsoils and subsoils of Andisol and Entisol. In Andisol, the amount of MBC were 216 and 25 mg C kg^{-1} soil in topsoils and subsoils at day 7, respectively and MBC increased by 69.4% and 37.5% with shoot and root addition, respectively. The corresponding values in subsoils were 267.9% and 153.8%. Similarly in Entisol, the amount of MBC were 156 and 22 mg C kg^{-1} soil in topsoils and subsoils after 7 days of incubation, respectively and MBC increased by 136.4% and 44.8% with shoot and root residue addition in topsoils,

respectively. The corresponding increased values in subsoils were 566.5% and 238.2%. Although MBC decreased from day 7 to day 110, the MBC amount still significantly ($P < 0.05$) higher in maize residue treated soils than relative control.

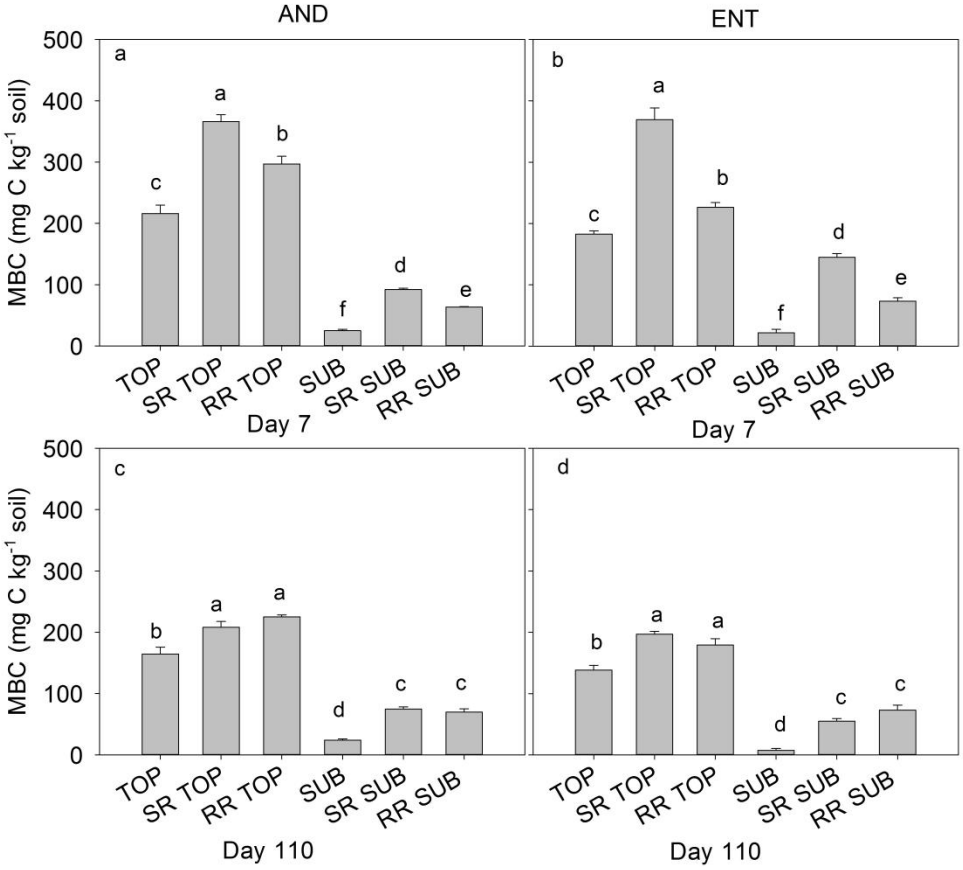

Fig. 4.6 Microbial biomass C (MBC) in the topsoil (TOP), subsoil (SUB), maize shoot- and root-residue amended topsoil (SR TOP and RR TOP) and subsoil (SR SUB and RR SUB) in Andisol (AND) and Entisol (ENT) after 7 and 110 days of incubation. Different letters above the bars indicate significant differences among the treatments ($P < 0.05$).

4.4 Discussion

4.4.1 Effects of maize residue type on priming effect in topsoil and subsoil

Affirming our hypothesis, shoot residue resulted in a higher positive priming effect than root residue in subsoil probably because the co-metabolism plays a more important role on this higher priming effect. I found that the higher intensity of positive priming effect occurred in maize shoot than the root residue treatment in subsoil of both Andisol and Entisol after 110-day incubation and this difference mainly came from the intensive phase of maize residue decomposition (Fig. 4.3) that

N addition did not affect the intensity of priming effect induced by maize residue addition (Fig. 4.5). Therefore, microbial activity on SOM decomposition in subsoil would be more controlled by C availability rather than N limitation. Our result was different from previous studies that the high positive priming effect in subsoil usually attributed to high microbial N mining, due to the low N availability in subsoil (Shahzad et al., 2019; Wang et al., 2015b), but was in line with Jones et al. (2005 and 2018) who also found that the subsoil is more limited by C source rather than N. In the intensive phase of maize residue decomposition, the higher shoot residue decomposition compared with root residue (Fig. 4.1) and the higher amount of MBC in shoot- than root-amended subsoil occurred after 7 days of incubation (Fig. 4.6) confirms that shoot residue more supported microbial growth, which would produce more extracellular enzymes (Kuzyakov et al., 2000) capable of degrading native SOM and result in greater priming effect. Thus, the greater intensity of positive priming effect in shoot than root residue treatments in subsoil could be attributed to the more co-metabolism.

Contrary to the subsoil, root residue induced higher positive priming effect than shoot residue in topsoil probably because microbial N mining dominate the contribution of this higher positive priming effect. In topsoil, the higher intensity of positive priming effect occurred in the maize root than shoot residue treatment in both Andisol and Entisol after 110-day incubation and this difference was mainly attributed to the slow phase of maize residue decomposition (Fig. 4.3). In addition, N addition significantly reduced the priming effect in topsoil with root residue but not shoot residue after 110-day incubation (Fig. 4.5). These results suggested that microbial activity on SOM decomposition in topsoil was more driven by N limitation. The low-quality root residue containing less N compared to shoot residue (Table 4.1) would not supply enough N for the demand of microbial growth (Barel et al., 2019), especially in the slow phase of root residue decomposition, which would enhance the microbial N mining from SOM and result in higher SOM mineralization (Dimassi et al., 2014; Nguyen and Marschner, 2016). Thus, the greater intensity of positive priming effect in the root than shoot residue treatment in topsoil could be attributed to the more microbial N mining.

4.4.2 Occurrence and significance of positive priming effect in subsoil

The stability of SOC in subsoil can be strongly decreased with FOM addition (i.e., positive priming effect occurred with FOM addition in subsoil). Maize residue addition resulted in positive priming effect in subsoil of both Andisol and Entisol after

110-day incubation, particularly with shoot residue addition (Fig. 4.3). Our results are different from previous studies which reported that no positive priming effect was found with FOM addition in subsoil (Salomé et al., 2010; Wordell-Dietrich et al., 2017) due to a lower SOC content in the subsoil which represents a larger spatial segregation between SOC and microorganisms, thereby protecting the SOC from microbial attack (Salomé et al., 2010), but support that the stability of SOC in subsoil is controlled by FOM addition (Fontaine et al., 2007). In our study, microbial community quickly adapted to FOM addition and the biomass increased, especially with shoot residue addition (Fig. 4.6); this, in turn, may access the spatially separated SOC by their hyphae (Fontaine et al., 2011; Shahzad et al., 2015). Jones et al. (2018) also found that the abundance of microbes in the subsoil was C limited and readily stimulated upon FOM addition which enhanced SOC mineralization. This directly challenges the assumption that subsoil C was stable and unsusceptible to FOM addition (Salomé et al., 2010; Wordell-Dietrich et al., 2017). Therefore, the prompted proposals by previous studies (Kell, 2011; Lorenz et al., 2011) that incorporating FOM mechanically to deep soils or developing deeper rooting crops for long term C sequestration and reduce CO_2 emission should be carried out with careful considering because the large positive priming effect would occur after FOM addition in agricultural soils, especially when FOM with high quality.

In contrary to our second hypothesis, subsoil is likely more vulnerable to SOC loss through priming effect compared to topsoil, which can be attributed to the relatively higher microbial growth and activity, higher proportion of labile C and higher decomposition constant of stable C pool in subsoil than in topsoil after FOM addition. Our results showed that the relative priming effect in response to maize residue addition was 2.7-22 times higher in the subsoil than topsoil (Fig. 4.3), suggesting a higher vulnerability to SOC loss through priming effect in the subsoil. The increase of MBC in response to maize residue addition in the early phase of incubation (day 7) in the subsoil was larger relative to those in the topsoil (~200% vs. ~50% in Andisol and ~400% vs. ~100% in Entisol) (Fig. 4.6). Such a surge in microbial biomass in subsoil could have been responsible for the greater mineralization of native SOC (Jia et al., 2017; Jones et al., 2018; Paterson and Sim, 2013). In addition, microbes in subsoil is more C limited than topsoil (Fontaine et al., 2007). What is more, the proportion of labile C pool and the decomposition constant of stable C pool was significantly higher in subsoil than topsoil after maize residue addition (Table 4.3), indicating the subsoil C is more prone to be decomposed after FOM addition.

It is worthy to note that in order to maintain the same treatments for both topsoil and subsoil, I added the same amount of C to both soil layers. Consequently, the ratio of C addition rate to soil initial MBC was much higher in subsoil (119 for Andisol and 137 for Entisol) than those in topsoil (14 for Andisol and 18 for Entisol), indicating that more C would have been available to microorganisms in the subsoil. This would draw certain caution to our interpretation on the susceptibility of subsoil to SOC loss as compared to topsoil. Nevertheless, the response of microbes to priming effect induced by maize residue addition in subsoil is relative more intense compared to topsoil due to microbial activation (Wang et al., 2016b).

4.5 Conclusions

Our study demonstrated that maize residue addition induced positive priming effect in both topsoil and subsoil of Andisol and Entisol, and the intensity of positive priming effect was higher in shoot- than root-residue treatment in subsoil, which was contrast to that in topsoil. Because the co-metabolism played a more important role on higher positive priming effect in subsoil due to the microbes are more limited by C in subsoil, while microbial N mining contribute more in topsoil due to the SOM mineralization was more controlled by N limitation. The higher relative priming effect (% of native SOC mineralization) in subsoil than in topsoil reveals that subsoil C would be more prone to be decomposed in response to FOM addition compared to topsoil, especially when high-quality FOM (e.g., shoot residue) is added. Our study highlights the subsoil C is not as stable as previously perceived, and could be even more susceptible to priming effect than that in the topsoil, especially when FOM with high quality, and warrant future in situ research on the effects of FOM addition in terms of FOM quality on subsoil C budgets before the suggestion of FOM input to subsoil to reduce CO_2 emission was executed. In addition, the priming effect differently induced by high- and low-quality residues between topsoil and subsoil should be considered for more efficient residue management in agroecosystems.

Chapter 5

General discussion

5.1 Integrated assessment on the response of priming of SOC mineralization to crop residue return and N fertilization

The data integration shows a holistic view of the priming effect, which was affected by crop residue quality, N fertilization, and soil layer (Fig. 5.1). In addition, the data from relatively short- (0–28 days; intensive phase of maize residue decomposition) and long-term incubation (0–110 days; including slow decomposition phase of maize residue) provides a better insight into the effect of residue decomposition stage on priming effect, which could be biased if the researches are conducted only in a short-term interval (e.g., intensive decomposition phase of residue), which subject to prevent a full examination of the potential effect of crop residue return in altering SOC mineralization. The comparison of priming effect between topsoil and subsoil enlightens the importance of priming effect in the subsoil. The data from two different types of soil (Andisol and Entisol) provided a better insight into the general pattern of these effects.

5.2 Different effects of crop residue quality on priming effect in the topsoil in the early phase of maize residue decomposition

A higher positive priming effect occurred with high-quality crop residue attributing to co-metabolism, while a higher positive priming effect occurred with low-quality crop residue attributing to N mining in the early phase of residue decomposition. Cumulative priming effect in different treatments in the topsoil and subsoil of Andisol and Entisol after 28 days of incubation (in the early phase of maize residue decomposition, Chapters 3 and 4) were shown in Fig. 5.1a and b. A higher positive priming effect occurred with high-quality shoot residue addition in the topsoil of Andisol (Fig. 5.1a) and subsoil of both Andisol and Entisol (Fig.5.1a, b), where N addition did not affect the intensity of positive priming effect (Fig.5.1a, b). While in the topsoil of Entisol, a higher positive priming effect occurred with low-quality root residue addition, and N addition reduced the intensity of the positive priming effect (Fig. 5.1b). The different effects of crop residue quality on priming effect indicate that

a higher priming effect can occur with high-quality residue in the short-term incubation due to co-metabolism. It was in line with previous studies (Fang et al., 2020; Mwafulirwa et al., 2016). However, a higher positive priming effect could also occur with low-quality residue (i.e., root residue) under N-limited conditions due to enhanced N mining in the short-term incubation.

5.3 Different effects of crop residue quality on priming effect between soil layers in the long-term incubation

Contrasting effects of crop residue quality on the positive priming effect between agricultural topsoil and subsoil for the long-term incubation are attributed to the different dominant mechanisms of the positive priming effect. The N mining contributed more to the topsoil's higher positive priming effect, but co-metabolism contributed more to the subsoil (Chapter 4). For the long-term incubation (i.e., 110 days, including the slow decomposition stage of maize reside), the higher intensity of positive priming effect occurred with maize shoot than the root residue treatment in the subsoil. Still, the higher intensity of positive priming effect happened in the maize root than shoot residue treatment in the topsoil of both Andisol and Entisol (Fig. 5.1). These different dominant mechanisms of priming effect changed with soil layer, which may explain the inconsistent results of crop residue quality on priming effect in previous studies (Shahbaz et al. 2017; Mwafulirwa et al. 2019). It also emphasizes that SOC mineralization response to FOM addition in the topsoil and subsoil are different. It was not as previously perceived that the underlying mechanisms that control C dynamics are the same in topsoil and subsoil (e.g., Lomander et al., 1998; Jenkinson and Coleman, 2008).

The subsoil is likely more vulnerable to SOC loss through priming effect compared to topsoil in the long-term incubation. In general, our results showed that the priming effect in response to maize residue addition was significantly higher in the subsoil than topsoil, especially with high-quality maize shoot residue (i.e., shoot residue) addition (Fig. 5.1c, d and Table 5.1), suggesting a higher vulnerability to SOC loss through priming effect in the subsoil.

5.4 Comparison of priming effect in short- and long-term incubations

The effect of crop residue quality on priming effect changed with the decomposition stage of maize residue in the topsoil, but this effect was consistent in the subsoil. A higher positive priming effect occurred with high-quality shoot residue

in the early phase of maize residue decomposition when N was sufficient. However, it happened with low-quality root residue treatment in long-term incubation in topsoil (Fig.5.1). Meanwhile, the subsoil's higher priming effect always occurred with high-quality crop residue (i.e., shoot residue), no matter in the short- or long-term incubation periods (Fig. 5.1). The topsoil findings emphasize that incubation duration plays an essential role in crop residue quality on priming of SOC mineralization. It may help explain the inconsistent results of crop residue quality on the intensity of priming effect in topsoil in previous studies (Shahbaz et al., 2017; Mwafulirwa et al., 2019). The consistent outcome of a higher positive priming effect occurred with high-quality crop residue (i.e., shoot residue) in subsoil. It reveals that the low-quality crop residue (e.g., root residue) is more beneficial for reducing priming effect than high-quality crop residue (e.g., shoot residue), especially when soil with high N availability (i.e., with N fertilization).

The intensity of the positive priming effect changes with incubation time (Fig. 5.1 and Chapter 3). Considering that the crop residue can be retained for a long time in the field, relative long-term (e.g., several months) experiments should be conducted to better capture the priming effect dynamics (i.e., intensity) and the underlying mechanisms of priming effect. It was better than previous studies, which just evaluated the priming effect of crop residue quality in the short-term incubation (i.e., few hours or days) (Chen et al., 2014; Mwafulirwa et al., 2019). The higher positive priming effect with low-quality crop residue (i.e., root residue) in the relatively long-term incubation in the topsoil reveals that the relatively high-quality crop residue (e.g., shoot residue) is more recommended to return to topsoil.

N fertilization with low-quality residue reduces SOC loss through priming effect in agricultural topsoil compared with returning low-quality residue alone. The higher positive priming effect occurred with low-quality residue addition (i.e., root residue) mainly attributed to soil N limitation, which enhanced N mining from the soil. It, therefore, promoted more SOC mineralization no matter in the short- or long-term incubation (Fig. 5.1). Thus, N fertilization could offer the N source and decrease the priming of SOC mineralization when low-quality crop residue (e.g., root residue) returned to agricultural soils (Chapter 3).

The subsoil is likely more vulnerable to SOC loss through priming effect than topsoil, no matter in the short- or long-term incubation. Our results showed that the priming effect in response to maize residue addition was significantly higher in subsoil than topsoil, especially with high-quality maize shoot residue addition (Fig.

5.1 and Table 5.1), suggesting a higher vulnerability to SOC loss through priming effect in subsoil compared to topsoil. Therefore, the suggestions from previous studies, such as the development of deep-rooting plants or mechanical input of crop residue to the subsoil to reduce CO_2 emission (Kell, 2011; Lorenz et al., 2011; Lynch and Wojciechowski, 2015; Torres-Sallan et al., 2017), should be carried out with care. I warrant future field research on the effects of crop residue addition in terms of crop residue quality on subsoil C budgets before the suggestion of crop residue input to subsoil to reduce CO_2 emission is executed.

As discussed above, the increased knowledge and a better understanding of the interactive effects of crop residue quality, N fertilization, and soil layer on priming of SOC mineralization in different decomposition stages of crop residue are critical in understanding the underlying mechanisms of the priming effect. It helps to explain the inconsistent results of crop residue quality on the priming effect in previous studies (Shahbaz et al. 2017; Mwafulirwa et al. 2019). Meanwhile, it provides a full examination of the potential interactions between N fertilization and crop residue quality in altering SOC priming, which has not been evaluated in previous studies.

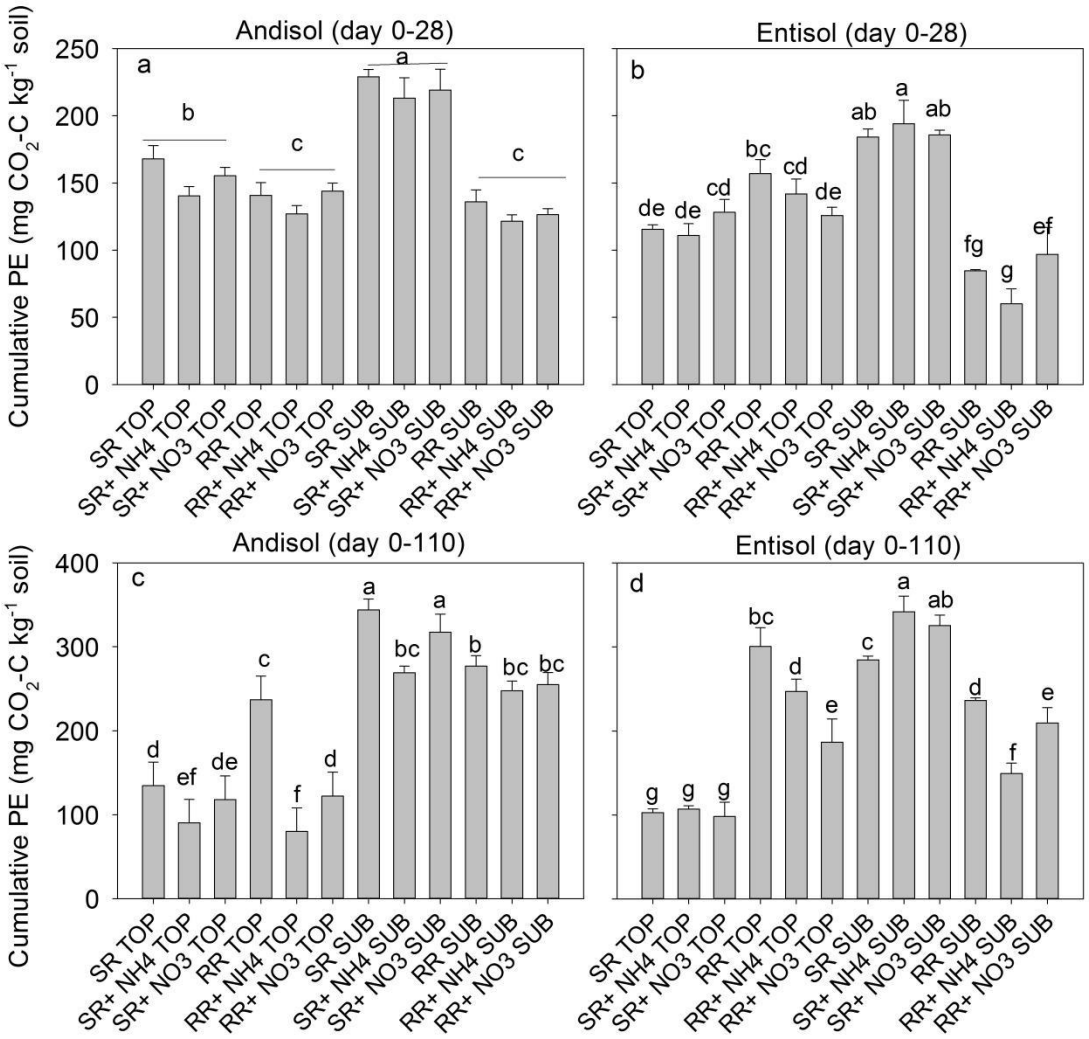

Fig. 5.1 Cumulative priming effect (PE) under different treatments in Andisol and Entisol after 28 days and 110 days of incubation. Error bar represents standard error of the mean (n = 3). Different letters above the bars indicate significant differences between treatments after 110 days of incubation, respectively ($p < 0.05$). SR, shoot residue; RR, root residue

Table 5.1 *F* values from results of ANOVA showing the effects of soil layer (topsoil and subsoil), maize residue quality (maize shoot and root residue), N fertilization (without N, NH_4^+-N and NO_3^--N addition), and their interactions on cumulative priming effect after 28 days (intensive decomposition phase of maize residue decomposition) and 110 days of incubation.

	Andisol (0–28 days)	Entisol (0–28 days)	Andisol (0–110 days)	Entisol (0–110 days)
Residue quality	334.8***	5143.3***	1.5 ns	5.0*
N fertilization	12.1***	2.4 ns	35.7***	9.4***
soil layer	89.0***	1.6 ns	434.7***	269.3***
Residue quality × N fertilization	0.7 ns	3.7 ns	3.6*	38.5***
Residue quality × soil layer	156.9***	347.3***	30.9***	647.4***
N fertilization × soil layer	1.0 ns	2.1 ns	4.2*	16.2***
Residue quality × N fertilization × soil layer	0.6 ns	10.9***	9.8***	13.5***

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns, no significant difference

Chapter 6

Concluding remarks

6.1 Summary and conclusions

With a series of incubation experiments, this thesis examined the effects of crop residue quality and N fertilization on the priming of SOC mineralization in agricultural soils.

Crop residue quality and N fertilization can interactively affect the SOC priming. The N addition decreased priming effect, which was induced by low-quality crop residue (i.e., root residue). I did not find this effect in the high-quality crop residue (i.e., shoot residue). In addition, N fertilization is beneficial for soil C sequestration when soil treated with low-quality crop residue (i.e., maize root residue) because of lowering the intensity of priming effect and crop residue decomposition by reducing N mining but has little impact on the turnover of SOC treated with high-quality crop residue (i.e., maize shoot residue) in the topsoil. Therefore, crop residue management with low-quality (e.g., root residue) combined with N fertilization would reduce priming of SOC mineralization compared with low-quality crop residue alone in the topsoil due to lowering the N mining from SOM.

The intensity of the positive priming effect in agricultural soils controlled by crop residue quality and the impact of crop residue quality is a contrast in the topsoil and subsoil. High-quality crop residue (e.g., shoot residue) induced a higher positive priming effect in subsoil because co-metabolism played a more critical role in the priming effect. On the other hand, a higher positive priming effect in topsoil occurred with low-quality crop residue (e.g., root residue) addition because N mining contributed more in topsoil. What is more, subsoil C is more vulnerable to SOC loss through priming effect compared to topsoil. Therefore, the subsoil C is not as stable as previously perceived and could be even more easily destabilized than that in the topsoil after crop residue addition. Furthermore, I should consider the different effects of the priming effect induced by high- and low-quality residues in topsoil and subsoil when crop residue return to the soil in agroecosystems. The low-quality residue or low-quality residue combined with N fertilization would reduce the SOC loss through priming effect compared to other managements (high-quality residue or high-quality residue combined with N fertilization) in the subsoil.

The priming effect induced by crop residue return is one of the most critical interactions between soil C input and SOC mineralization. N fertilization is another

critical agricultural management to ensure profitable plant growth. The improved understanding of the changes of crop residue quality and N fertilization on SOC loss through priming effect in agricultural topsoil and subsoil is beneficial for understanding the underlying mechanisms of priming effect. It is also helpful for designing effective management strategies of crop residue return and N fertilization in agricultural soils for decreasing SOC loss through priming effect. This dissertation provides some of the first evaluations, including the interactive effects of crop residue quality and N fertilization on priming of SOC mineralization, and the priming effect in response to crop residue addition in terms of crop residue quality in the subsoil, and the importance of priming effect in subsoil compared to topsoil.

6.2 Unanswered questions and future research perspective

(1) The definition and calculation of priming effect need to be further clarified. The priming effect is often known as the short-term change of the native SOC turnover after FOM addition. However, how to determine the short-term and the end-point of priming effect is still not clear. Although some researchers have pointed out that only when there is a significant difference between the priming effect and the "0" value in statistical analysis, the priming effect can be considered to exist (Kuzyakov 2010). However, in actual research, this statistical significance may be intermittent. It is not easy to find a specific sampling point as the endpoint of the priming effect, and it is very subjective and needs further discussion. Future research should also clearly indicate whether it is a relative priming effect or cumulative priming effect when discussing the priming effect due to the changing trend of these two may not be consistent.

(2) The microbial community and the enzyme production under different soil conditions and crop residue decomposition stage should be clarified. This study found different effects of crop residue quality on priming effect in topsoil and subsoil, and higher sensitivity of SOC priming in the subsoil than topsoil. Many explanations are still in the speculative stage, and the underlying contribution by microbes is still unclear. The microbial abundance and activities in the soil and the different decomposition stages of crop residue should be analyzed in the future.

(3) Field research and model application of priming effect are two important directions for future research. At present, most of the research on evaluating priming effect is mainly concentrated on laboratory experiments and stays at the theoretical level. To make the priming of SOC mineralization more accurate and meaningful, field research on the priming effect and model application is essential. However, even

if many researchers emphasized the importance of priming effect, the priming effect's model application is still tricky. It may require researchers' joint efforts in multiple disciplines, such as biologists, ecologists, and model experts, to verify the microbial changes and environmental factors that control the intensity of the priming effect.

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