



Carbon sequestration in aggregates from native and cultivated soils as affected by soil stoichiometry

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Abstract

Quantitative influence and underlying mechanisms of nutrient stoichiometry on mineralization of native Soil organic C (SOC) and straw C in different aggregate classes from cultivated and non-cultivated soils are still unclear. Soil samples (Mollisols) from a native woodlot and a farmland converted from woodlot were sieved into three aggregate classes (mega-aggregates (6.3–2 mm), macro-aggregates (2–0.25 mm), and micro-aggregates (< 0.25 mm)) and incubated (180 days) under different nutrient rates (nil, low, and high supplies of N and P) with or without ¹³C-enriched straw amendment. Significantly higher percentage of native SOC was mineralized from mega- and macro-aggregates (65.8–82.2%) compared with micro-aggregates (48.3–52.0%) in woodlot soil. Nutrient addition significantly increased aggregate-associated C in both soils with straw, and the increase was greater in farmland than in woodlot soil and in large-sized aggregates than in micro-aggregates. These results suggested that large aggregates serve as a C reservoir of labile C, while micro-aggregates that contained C was not easily mineralized even with abundant nutrients. Differences of C mineralization among aggregate size classes were significant in woodlot soil but not in farmland soil. Depletion of SOC was greater with increasing nutrient addition rates in farmland aggregates without straw, while the depletion in woodlot aggregates showed no difference among nutrient treatments, suggesting that microbial activity was nutrient-limited in farmland aggregates. The results improved our knowledge on SOC mineralization in response to residue-nutrient management in different aggregate classes from cultivated and non-cultivated soils, which have important implications for strategies to improve soil fertility or mitigate climate change via increased SOC.

Keywords Soil organic C · Aggregates · Straw incorporation · Stoichiometry · Land use · ¹³C isotope

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Introduction

Soil organic C (SOC) sequestration depends on the increase of the stable C pool of soil to ensure that the C is not easily mineralized. The importance of SOC sequestration has been highlighted in the recent decades due to its contribution to soil fertility and, particularly, the mitigation of greenhouse effect (Lal 2004). Even minor changes of SOC storage could cause a profound influence on atmospheric CO₂ concentration (Manlay et al. 2007). Studies have shown that SOC sequestration not only depended on its recalcitrance, soil type, organic inputs, climate, etc. but also was greatly affected by soil management strategies (Mack et al. 2004; Christopher and Lal 2007; Kirkby et al. 2013; Dai et al. 2018). This calls for thorough studies on strategies (land use, fertilization, residue return, etc.) to increase SOC storage in soils, especially the increase of stable SOC pool.

The assessment of C dynamics in contrasting aggregate size classes can reveal C sequestration mechanisms in

agroecosystems (Wu et al. 2012; Chen et al. 2014; Bimüller et al. 2016), because different aggregate size classes contained various concentrations of SOC with different recalcitrance and microbial accessibility, and are influenced by land use systems and soil management practices (Tisdall and Oades 1982; Six et al. 2000; Gunina and Kuzyakov 2014). Organic inputs with different recalcitrance showed higher total and native SOC mineralization in the macro- than in the micro-aggregates (Tian et al. 2016; Sarker et al. 2018). There are also studies that found greater C mineralization from micro- than macro-aggregates (Drury et al. 2004). The inconsistent results could be due to the interactions of aggregates with different soil stoichiometry, straw managements, land use systems, etc. (Bimüller et al. 2016; Sarker et al. 2018).

Among the factors suggested to affect SOC sequestration, soil nutrient stoichiometry has been drawing more and more attention in recent years (Kirkby et al. 2013; Fang et al. 2019; Piasczyk et al. 2019). Ecological stoichiometry deals with the C:N:P ratios of organisms and substrates, and it helps to gain insight into the C and nutrient cycling as well as the metabolic state of organisms (Heuck et al. 2015; de Sosa et al. 2018; Sun et al. 2019). Thus, it has important implications for SOC dynamics, microbial growth, and turnover in soil (Kirkby et al. 2013; Li et al. 2018). Kirkby et al. (2013) found that inorganic nutrient availability was critical to sequester C into stable fine fraction C pool. Butterly et al. (2018) highlighted that C mineralization was mainly determined by soil N status rather than other factors such as residue quality. Fang et al. (2019) proved that balancing the resource stoichiometry through nutrient input could enhance the mineralization of labile SOC fraction, as well as the incorporation of newly added residue C into stable C fractions in a Luvisol and a Vertisol. In addition, converting natural ecosystems to agriculture modified the nutrient dynamics and stoichiometry of C, N, and P in soil (Wang et al. 2014). However, there is a lack of knowledge on how the integrated residue-nutrient management regulates mineralization of native SOC and residue C in different classes of soil aggregates.

The conversion of natural to agricultural ecosystems has depleted the SOC pool by 60% in soils of temperate regions and 75% or more in the tropical soils (Lal 2004). Agricultural use of soil can have negative influences on soil aggregate stability and in turn SOC sequestration (Six et al. 2000; Deneff et al. 2007; Qiao et al. 2019), and there was a sharp SOC decline when forests or grasslands were converted to cropland (e.g., Poeplau et al. 2011; Wei et al. 2014). Our previous study on a clay loam soil showed that aggregates provided strong protection for SOC in the native woodlot and grassland soils, whereas this protection was not evolved in arable soil (Fan et al. 2013). In Northeast China, the large-scale deforestation has caused the remarkable depletion in C storage in black soils (Mollisol-based USDA soil taxonomy) (Liang et al. 2009; Shi et al. 2012). Residue return has

attracted great attention to improve SOC sequestration in farmland of this region (Zhang et al. 2018). However, understanding of the fate of native SOC and residue C in varied aggregate classes under different nutrient application regimes in these soils is still lacking.

Therefore, we hypothesized that large-sized aggregates would have higher mineralization of native SOC and residue C than micro-aggregates, likely due to higher labile C content and higher microbial activity (Six et al. 2002). We also hypothesized that the differences of SOC mineralization among aggregate size classes would be greater in woodlot than in farmland soil, due to negative influences of soil cultivation on aggregate structure (Six et al. 2000; Deneff et al. 2007). Furthermore, the addition of N and P to aggregates with straw could enhance the incorporation of straw C into SOC, and the responses of SOC mineralization to nutrient addition would be different in cultivated and un-cultivated soils. The objectives of this study were 1) to investigate the effects of various C-nutrient stoichiometry and aggregate classes on mineralization of native SOC and residue C in the non-cultivated and cultivated black soils; 2) to evaluate the contributions of C-nutrient stoichiometry on increase of total SOC; and 3) to identify the interactions of C mineralization, aggregate classes, and land use (soil cultivation) on SOC mineralization and sequestration.

Materials and methods

Soil sample preparation

The soil samples were collected in September 2017 from Qianjin Village (49° 02.21' N, 120° 58.50' E), Yakeshi Farm, Hulun Buir City, Inner Mongolia, China, from a long-term wheat (*Triticum aestivum* L. cv. Tonic)-rape (*Brassica campestris* L.) rotation farmland and another from adjoining native woodlot. The vegetation of the native woodlot was cold-temperate coniferous forest, which mainly included *Larix gmelinii* (Rupr.), *Pinus pumila* (Pall.), and *Quercus mongolica*. The farmland was converted from native woodlot more than 20 years ago. Field operations for the farmland included moldboard plowing to 18- to 20-cm depth in late September after rape or wheat harvest, and secondary cultivation and harrowing in the following spring before planting. Crop residues were removed from soil after harvest. Each year, N, P, and K fertilizers were applied during plowing at 90, 45, and 80 kg ha⁻¹, respectively, as starter fertilizers in the forms of ammonium nitrate (NH₄NO₃), superphosphate (CaP₂H₄O₈), and potassium sulfate (K₂SO₄), respectively. Additional 50-kg N ha⁻¹ (urea) was top dressed at the bolting stage of rape. No additional fertilizer was applied for wheat crop. The woodlot had a minimal anthropogenic disturbance with no treatment imposed. The site has a temperate

continental climate with a mean annual temperature at $-2.9\text{ }^{\circ}\text{C}$ and a mean annual precipitation at 360.8 mm. The soils were classified as Mollisols (USDA soil taxonomy). Basic soil properties ($n = 3$) are presented in Table 1. The pH (water) of soil of woodlot and farmland were 6.7 and 6.4, respectively. Five soil samples were taken in a random zigzag pattern from the top 0–10-cm layer of each of the two land use systems. First, a pit of 10-cm depth was dug to get a soil profile, then a $20 \times 20 \times 10$ -cm rectangular container was pushed into the soil and carefully dug out from the side of exposed profile to get an undisturbed soil sample. After sampling, the field-moist soils were sealed and transported to the lab in a portable refrigerator ($4\text{ }^{\circ}\text{C}$) for further analysis.

In the laboratory, the field-moist soil was gently broken to pass through a 6.3-mm sieve. Any visible stones or plant residues were removed. The soil samples were then air-dried for 2–4 days at room temperature. Soil aggregates were separated by dry sieving according to Limon-Ortega et al. (2006). Briefly, 500 g of the above air-dried soils were separated using a Ro-tap sieve shaker (W.S. Tyler Inc., Mentor, OH) by shaking through 2 sieves with different-sized openings (2.0 and 0.25 mm) for 5 min at a speed of $210\text{ cycles min}^{-1}$. Aggregates remaining in each sieve were collected. In this study, the aggregate size classes of 6.3–2 mm, 2–0.25 mm, and <0.25 mm were classified as mega-aggregates, macro-aggregates, and micro-aggregates, respectively.

Preparation of ^{13}C -labeled straw

The ^{13}C labeling of wheat plants started when the plant height reached 60 cm in a greenhouse following the method of Fang et al. (2016) with minor modifications. Briefly, a micro-plot was sealed with a portable polyvinyl chloride (PVC) chamber ($1\text{ m} \times 1\text{ m} \times 1\text{ m}$). The lower edge of the chamber was buried into soil to a depth of 10 cm to seal the chamber. The plants inside the chamber were pulse labeled with $^{13}\text{CO}_2$ (99.0 atom% ^{13}C) from 10:00 am and 4:00 pm. The ^{13}C -labeled CO_2 was injected into the sealed chamber through a flow meter (S325-15-170-F/M CO_2 , Gascon Systems, Sydney, Australia) at approximately $500\text{ cm}^3\text{ min}^{-1}$. The air in the chamber was circulated by a battery-operated mini-fan. The CO_2 concentration in the chamber was monitored using a portable CO_2 probe (Vaisala GMP 343, Helsinki, Finland).

After 3:00 am, when the air temperature increased to $40\text{ }^{\circ}\text{C}$, a portion of the chamber was opened for 30 min to allow the temperature to drop below $30\text{ }^{\circ}\text{C}$. The chamber was then resealed to capture $^{13}\text{CO}_2$ respired overnight by wheat. The chamber was kept sealed until the next morning when CO_2 inside the chamber decreased to <200 ppm. Air temperature inside the chamber varied between 20 and $40\text{ }^{\circ}\text{C}$. The labeling process was repeated for 3 times within 30 days.

After ^{13}C labeling, wheat straw (leaves and stems) was collected, oven dried at $65\text{ }^{\circ}\text{C}$, cut to 1-cm pieces, and thoroughly mixed. The wheat straw contained 465.0-g C kg^{-1} , 9.01-g N kg^{-1} , 0.60-g P kg^{-1} , and 30.21-g K kg^{-1} . The $\delta^{13}\text{C}$ value of the thoroughly mixed straw was 839.65‰ .

Experimental design and incubation

The experimental design included 36 treatments across three aggregate size classes, three nutrient rates, and two soils (woodlot, farmland) with or without wheat straw amendment. Each treatment was triplicated. Aggregate samples of 200 g (dry weight) were gently and thoroughly mixed with (or without) 3-g wheat straw, and were put in 250-mL Erlenmeyer jars fitted with gas-tight septa. All jars were pre-incubated for 3 days before treatments. Nutrients were applied to soils at three rates: no nutrient but only water (NN), a low nutrient (LN) rate at 64-mg N and 26-mg P kg^{-1} soils, and a high nutrient (HN) rate at 128-mg N and 52-mg P kg^{-1} soils in water to reach 60% water holding capacity. The nutrient solutions were prepared using ammonium nitrate and potassium dihydrogen phosphate following the method of Kirkby et al. (2013). The concentrations of nutrients were set based on the assumption that the stoichiometry of stabilized soil organic matter has a C:N:P ratio of 10,000:833:200 as suggested by Himes (1998). The availability of inorganic nutrients in the soil was ignored, since they were an order of magnitude less than those added to soil. The pH of the nutrient solutions was adjusted to 7 using a sodium hydroxide solution. The nutrient solutions were added to soil dropwise to ensure even distribution. Another set of three blank jars was used to monitor background CO_2 concentration and ^{13}C natural abundance. All 111 jars were incubated ($24\text{ }^{\circ}\text{C}$) in the dark for 180 days.

CO_2 samples were drawn from each jar daily with a 30-mL syringe on days 2, 5, 10, 16, 24, 34, 46, 58, 72, 86, 102, 118,

Table 1 Main properties of soils in native woodlot and farmland soils studied

Land use	Soil texture (%)			pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
	Sand	Silt	Clay					
Woodlot	26.84	34.67	38.49	6.7	49.43	3.02	18.55	129.8
Farmland	30.02	36.88	33.10	6.3	20.19	1.21	12.20	90.39

138, 158, and 180. Following each gas sampling, the jars were flushed with air and covered with perforated parafilm during the period between CO₂ measurements to maintain an aerobic headspace and minimize water loss. The weight of each jar was checked every other day, and deionized water was added to maintain soil water concentration. Concentrations of CO₂ and ¹³C abundance in CO₂ were determined for each sample. The ¹³C abundance in CO₂ was determined for samples collected on days 2, 16, 38, 72, 118, and 180.

Analyses

Soil microbial biomass C (MBC) and N (MBN) after incubation were determined using the fumigation extraction method (Vance et al. 1987). The total C of aggregates before and after incubation was determined using a Flash EA 1112 elemental analyzer (Thermo Finnigan, Milan, Italy). Undecomposed plant residues in soils were removed before element determination. Since the studied soils were free of carbonates, the SOC was assumed to equal the total C. Concentrations of CO₂ were measured using an Agilent N6890 gas chromatograph as described by Zhang et al. (2012). The ¹³C abundance in CO₂ and soils was analyzed using an isotope ratio mass spectrometer (IRMS) (MAT 253, Thermo Scientific, USA).

Calculations and statistical analysis

The ¹³C abundance in soils and CO₂ samples was expressed as δ¹³C values (‰):

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{V-PDB}} - 1) \times 1000 \quad (1)$$

where R_{sample} and $R_{\text{V-PDB}}$ are ¹³C/¹²C ratios of samples and the Vienna Pee Dee Belemnite (V-PDB) standard (Dawson et al. 2002), respectively.

The proportion of soil aggregate-derived CO₂-C (C_A (%)) in the total CO₂-C was determined as:

$$C_A (\%) = (\delta_T^{13}\text{C} - \delta_S^{13}\text{C}) / (\delta_A^{13}\text{C} - \delta_S^{13}\text{C}) \times 100 \quad (2)$$

where, δ_T¹³C is the δ¹³C value of the total CO₂-C released from the straw-amended aggregates, δ_A¹³C is the δ¹³C of CO₂-C released from the aggregates incubated without straw, and δ_S¹³C is the δ¹³C of the added wheat straw.

The priming effect (PE) was calculated as the difference between native SOC mineralized from the aggregates incubated with and without wheat straw. The amounts of straw C or native SOC mineralized during the incubation were determined by multiplying the proportion of straw-derived C or SOC with the total C mineralized, respectively (Fang et al. 2019). The amounts of residue C and SOC mineralized from the aggregates were then calculated as per gram of added residue C and initial aggregate-associated SOC, respectively.

The statistical comparison assumed a randomized complete block design with sub-sampling. Data not meeting the assumptions were transformed logarithmically and analyzed by using the ANOVA. A significance level of $P < 0.05$ was used unless otherwise indicated. LSD and Duncan were chosen for the multiple comparisons between different treatments. All statistical analysis was conducted using the SigmaPlot 12.5 software (Systat Software Inc., Chicago, USA).

Results

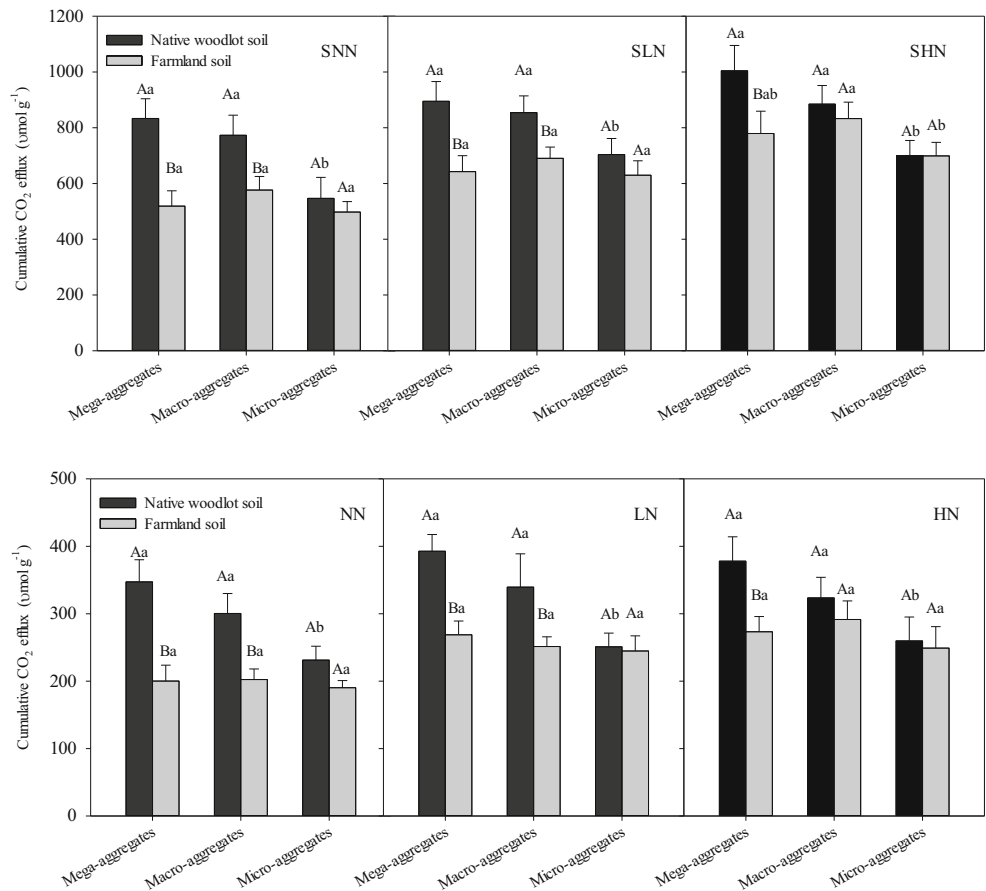
Patterns and cumulative CO₂ released from aggregates of farmland and native woodlot soils

The release pattern of total CO₂ during the 180-day incubation showed distinct variation with C-nutrient stoichiometry, aggregate size class, and land use (Fig. S1). For the farmland soil, aggregates incubated without straw showed low and relatively steady CO₂ emission rates (8.74–39.3 pmol g⁻¹ s⁻¹), which were significantly higher with higher nutrient addition rates across the aggregate size classes (Fig. S1A). CO₂ release rates were significantly increased by straw amendment, especially during the first 72 days of incubation (43.3–101.1 pmol g⁻¹ s⁻¹) (Fig. S1A). Among the three aggregate size classes of farmland soil, CO₂ release rates from macro-aggregates was generally higher than that from mega- and micro-aggregates, while the latter two classes showed no significant difference (Fig. S1A).

The CO₂ emission rates from large-sized aggregates (mega- and macro-aggregates) in woodlot soil were higher than that from farmland (Fig. S1). For mega- and macro-aggregates without straw amendment, CO₂ emission rates were significantly higher from woodlot (59.0–20.3 pmol g⁻¹ s⁻¹) than that from farmland (31.3–14.6 pmol g⁻¹ s⁻¹) soil. Mega-aggregates showed higher emission rates than macro-aggregates from woodlot soil without straw, especially during the first 34 days (Fig. S1B). Micro-aggregates had lower CO₂ release rates than the two larger classes irrespective of C-nutrient stoichiometry rates, indicating a lack of labile C in this size class in woodlot soil.

Cumulative CO₂ released from woodlot soil aggregates was higher than that from farmland soil aggregates, especially for large classes of mega- and macro-aggregates (Fig. 1). For woodlot aggregates without straw, cumulative CO₂ showed no significant difference among nutrient rates and was lower from micro-aggregates than the other larger size classes; for farmland aggregates without straw, cumulative CO₂ was significantly increased by nutrient addition and showed no difference among aggregate classes. In aggregates with straw amendment, nutrient addition induced higher CO₂, and the increase by nutrient rates was greater for farmland aggregates than for woodlot aggregates; large-sized aggregates, especially mega-aggregates from woodlot soil, produced significantly higher amount of CO₂ than micro-aggregates (Fig. 1).

Fig. 1 Cumulative amount of total CO₂ released from mega-, macro-, and micro-aggregates in farmland and native woodlot soils with different C-nutrient stoichiometry. SNN, SLN, and SHN represent incubation with straw and no, low, and high nutrient addition, respectively. Vertical bars are the standard errors (*n* = 3). Different uppercase letters indicate significant differences between the two soils in the same aggregate class with the same C-nutrient stoichiometry level; different lowercase letters indicate significant differences between aggregate classes with the same C-nutrient stoichiometry level at *P* = 0.05



Mineralization of native SOC and PE from aggregates of the two soils

The δ¹³C-based calculations revealed that land use, aggregate classes, and C-nutrient stoichiometry greatly influenced the percentage of native SOC mineralized in total CO₂-C

(Fig. 2). Significantly higher percentage of native SOC was mineralized in mega- and macro-aggregates (65.8–82.2%) compared with micro-aggregates (48.3–52.0%) in woodlot soil. By increasing nutrient addition rates, a higher percentage of native SOC was mineralized in all aggregate size classes of farmland soil. Woodlot soil aggregates also showed higher

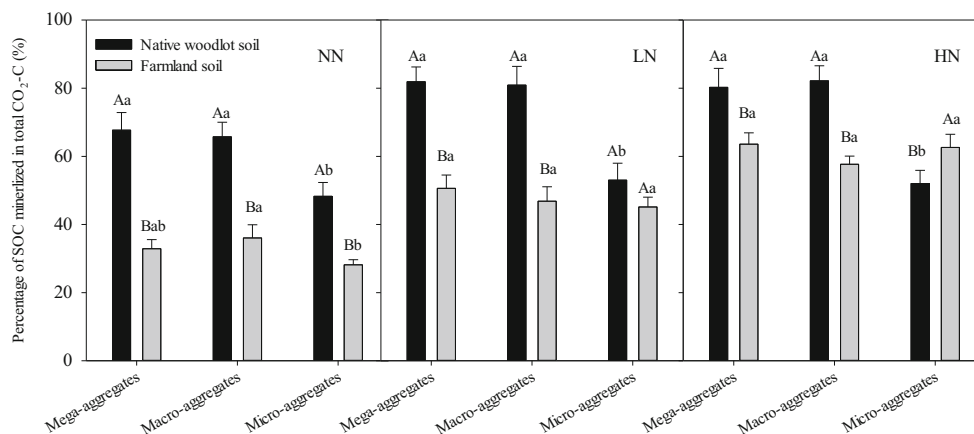


Fig. 2 Percentage of mineralized native aggregate-associated C in total CO₂-C (soil C plus straw C) derived from woodlot and farmland soils incubated with straw amendment. NN, LN, and HN represent incubation with no, low, and high nutrient levels, respectively. Vertical bars are the standard errors (*n* = 3). Different uppercase letters indicate significant

differences between the two soils in the same aggregate class with the same C-nutrient stoichiometry level; different lowercase letters indicate significant differences between aggregate classes with the same C-nutrient stoichiometry level at *P* = 0.05

percentage of mineralized native SOC when straw was applied to the LN treatment. However, the percentage did not further increase in the HN treatment with straw. This resulted in higher percentage of mineralized native SOC in micro-aggregates of farmland soil than that of woodlot soil. In the other two large size classes, the percentage was significantly higher from woodlot than farmland soil under all three C-nutrient stoichiometry rates (Fig. 2).

Land use, aggregate size class, and C-nutrient stoichiometry all significantly affected the PE of aggregates, with land use being the primary factor ($P < 0.0001$) (Table 2). Straw addition caused stronger PE in woodlot soil aggregates than in farmland aggregates (Fig. 3). The LN treatment with straw induced higher PE in large size class (mega- and macro-) aggregates of woodlot soil than the respective control, but further nutrient addition showed no effective change. The PE in farmland aggregates increased with nutrient addition rates and was significantly higher in large-sized aggregates than micro-aggregates under HN (Fig. 3).

Variation of total SOC concentrations in aggregates of farmland and native woodlot soils

Wheat straw amendment at a rate of 15 g kg^{-1} increased SOC concentrations in all aggregate classes of the two soils after the incubation period of 180 days (Fig. 4). When incubated without exogenous nutrients, the increase was significantly greater in woodlot aggregates ($1.15\text{--}1.80 \text{ g kg}^{-1}$) than in farmland aggregates ($0.77\text{--}1.26 \text{ g kg}^{-1}$). The LN with straw induced 17.2–22.1% and 61.1–120.7% higher SOC increase in aggregates of woodlot and farmland soil, respectively, compared with the respective control. The HN with straw further induced a 34.7–48.0% increase of SOC in farmland aggregates

compared with LN with straw, while no further increase was observed in woodlot aggregates. The SOC increase was generally greater in large size classes than micro-aggregates in woodlot soil, while no such difference was observed among farmland aggregates size classes (Fig. 4).

Although increase of SOC in aggregates with straw amendment was low ($0.77\text{--}2.79 \text{ g kg}^{-1}$), the decline of SOC in aggregates without straw was non-negligible ($2.28\text{--}4.71 \text{ g kg}^{-1}$). The depletion of SOC in farmland aggregates increased with nutrient addition rates and showed no difference among aggregate size classes. The depletion in woodlot aggregates, however, increased with aggregate size class and showed no response to nutrient addition rates (Fig. 4). Land use was the primary factor affecting SOC variation, followed by C-nutrient stoichiometry and aggregate size class. Aggregate size class had significant interactions with land use and C-nutrient stoichiometry for SOC variation (Table 2).

Microbial properties of aggregates

Straw incorporation effectively increased concentrations of MBC and MBN across all aggregate size classes of the two soils after the incubation (Table 3). Nutrient addition also increased these concentrations, especially in aggregates of farmland soil. Larger aggregates generally had higher MBC and MBN within the same nutrient rate in woodlot soil, while farmland aggregates showed no significant differences across size classes. Both MBC and MBN were significantly lower in aggregates of farmland than woodlot. The MBC/MBN ratio was reduced by straw incorporation in aggregates of the two soils and showed a trend of decrease with increase of nutrient addition rate and decrease of aggregate size (Table 3).

Table 2 Statistical analysis of a three-way ANOVA on the effects of land use, aggregate size class, and C-nutrient stoichiometry on priming effect and variation of SOC after incubation

	DF	Priming effect		SOC variation	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Land use	1	79.9	****	64.0	****
Aggregate size class	2	20.2	***	13.54	**
C-nutrient stoichiometry	2	38.5	****	27.1	***
Land use × aggregate size class	2	21.8	***	8.94	**
Land use × C-nutrient stoichiometry	2	0.29	ns	0.08	ns
Aggregate size class × C-nutrient stoichiometry	2	5.23	*	3.14	*
Land use × aggregate size class × C-nutrient stoichiometry	2	0.09	ns	0.28	ns

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

***Significant at the 0.001 probability level

****Significant at the 0.0001 probability level

ns, not significant at 0.05 probability level

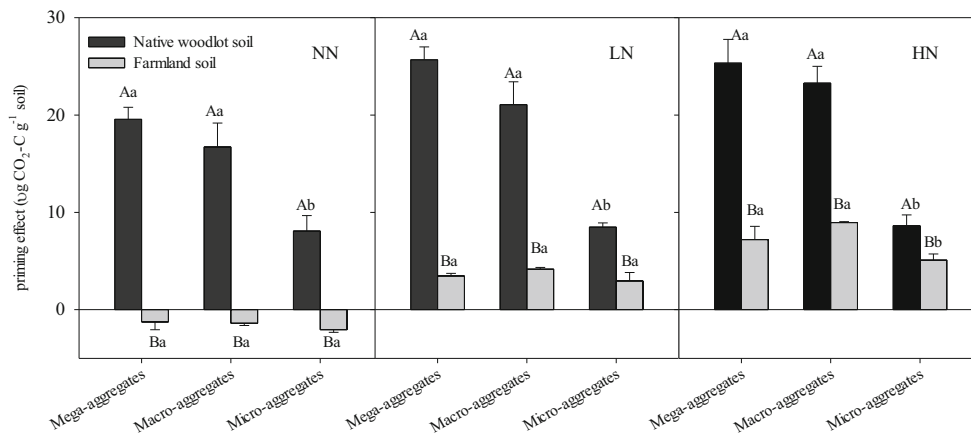


Fig. 3 The priming effect of aggregates from native woodland and farmland soils with different C-nutrient stoichiometry levels. NN, LN, and HN represent incubation with no, low, and high nutrient levels, respectively. Vertical bars are the standard errors ($n = 3$). Different

uppercase letters indicate significant differences between the two soils in the same aggregate class with the same C-nutrient stoichiometry level; different lowercase letters indicate significant differences between aggregate classes with the same C-nutrient stoichiometry level at $P = 0.05$

Discussion

Effect of C-nutrient stoichiometry and straw amendment on SOC mineralization and sequestration

Straw amendment and nutrient addition effectively increased CO_2 release from aggregates due to mineralization of straw C and native SOC, as already reported (Liu et al. 2019; Fan et al. 2020). In soils amended with straw and nutrients, Kirkby et al. (2013) found that inorganic nutrients (N, P, and S) affected C sequestration in the stable C pool of the fine fraction. This was

because these nutrients were required by soil microorganisms for transforming C-rich residues into enriched fine fraction pools of soil organic matter (Christopher and Lal 2007; Kirkby et al. 2011). There are contrasting reports with positive (de Graaff et al. 2006; Fan et al. 2019), nil (Liljeroth et al. 1994), or negative (Janssens et al. 2010; Li et al. 2018) effects of N on SOC mineralization. The two hypotheses “stoichiometric decomposition” and “microbial N mining” (Craine et al. 2007) assume opposite effects of N on SOC recalcitrance, that is, “stoichiometric decomposition” suggests that high N availability promotes SOC decomposition, while

Fig. 4 Variation of SOC concentration in aggregates of native woodland and farmland soils after incubation with and without straw. SNN, SLN, and SHN represent incubation with straw and no, low, and high nutrient levels, respectively. NN, LN, and HN represent incubation with no, low, and high nutrient addition levels, respectively. Vertical bars are the standard errors ($n = 3$). Different uppercase letters indicate significant differences between the two soils in the same aggregate class with the same C-nutrient stoichiometry level; different lowercase letters indicate significant differences between aggregate classes with the same C-nutrient stoichiometry level at $P = 0.05$

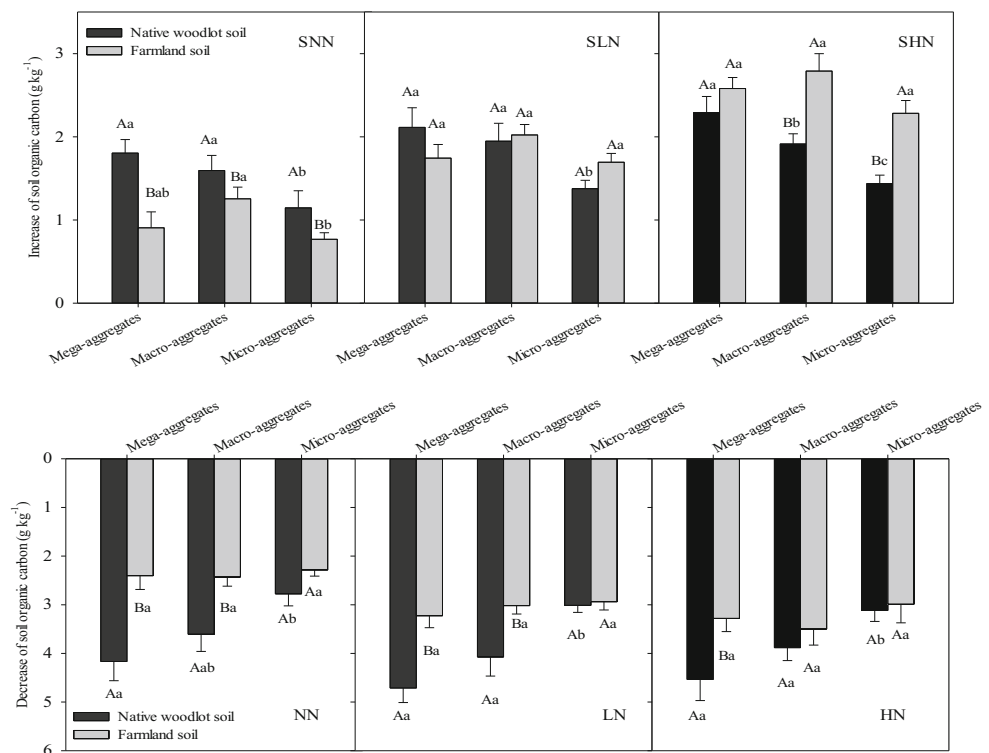


Table 3 Soil microbial biomass C and N, and MBC/MBN ratio in aggregates of the two soils amended with and without straw after a 180-day incubation

Aggregate class	Nutrient addition rate	MBC (mg kg ⁻¹)		MBN (mg kg ⁻¹)		MBC/MBN	
		Straw	No straw	Straw	No straw	Straw	No straw
Woodland soil							
Mega-aggregates	NN	137.9 ^{Ca}	90.5 ^{Ba}	26.2 ^{Ba}	13.6 ^{Ba}	5.3 ^{Aa}	6.6 ^{Aa}
	LN	208.3 ^{Bb}	107.1 ^{ABa}	41.2 ^{Aa}	18.8 ^{Aa}	5.1 ^{ABa}	5.7 ^{Bb}
	HN	254.9 ^{Aa}	115.1 ^{Aa}	57.0 ^{Aa}	22.9 ^{Aa}	4.5 ^{Bb}	5.0 ^{Bc}
Macro-aggregates	NN	155.8 ^{Ba}	83.4 ^{Ba}	30.8 ^{Ba}	12.8 ^{Ba}	5.1 ^{Aa}	6.5 ^{Aa}
	LN	220.6 ^{Aa}	130.4 ^{Aa}	40.8 ^{Aa}	21.3 ^{Aa}	5.4 ^{Aa}	6.1 ^{Aab}
	HN	248.2 ^{Aa}	128.8 ^{Aa}	47.2 ^{Ab}	22.1 ^{Aa}	5.3 ^{Aa}	5.8 ^{Ab}
Micro-aggregates	NN	84.7 ^{Bb}	42.6 ^{Bb}	13.4 ^{Bb}	4.6 ^{Bb}	6.3 ^{Aa}	9.3 ^{Aa}
	LN	169.3 ^{Ac}	92.6 ^{Ab}	30.2 ^{Ab}	14.6 ^{Ab}	5.6 ^{Ba}	6.3 ^{Bb}
	HN	185.0 ^{Ab}	98.3 ^{Ab}	30.8 ^{Ac}	15.7 ^{Ab}	6.0 ^{ABa}	6.3 ^{Bb}
Farmland soil							
Mega-aggregates	NN	87.3 ^{Ca}	54.9 ^{Ca}	10.5 ^{Cab}	4.1 ^{Ca}	8.3 ^{Aa}	13.4 ^{Aa}
	LN	159.6 ^{Ba}	75.7 ^{Ba}	25.9 ^{Ba}	10.6 ^{Ba}	6.2 ^{Ba}	7.1 ^{Ba}
	HN	188.9 ^{Aa}	93.3 ^{Aa}	36.2 ^{Aa}	14.5 ^{Aa}	5.2 ^{Ca}	6.4 ^{Ba}
Macro-aggregates	NN	95.6 ^{Ca}	60.1 ^{Ba}	13.3 ^{Ca}	6.4 ^{Ca}	7.2 ^{Ab}	9.4 ^{Ab}
	LN	150.5 ^{Ba}	74.3 ^{AB}	25.6 ^{Ba}	10.4 ^{Ba}	5.9 ^{Ba}	7.1 ^{Ba}
	HN	201.6 ^{Aa}	88.4 ^{Aa}	38.3 ^{Aa}	15.1 ^{Aa}	5.3 ^{Ba}	5.9 ^{Ca}
Micro-aggregates	NN	64.8 ^{Bb}	42.1 ^{Cb}	8.1 ^{Bb}	3.2 ^{Bb}	8.0 ^{Aa}	13.2 ^{Aa}
	LN	143.4 ^{Aa}	66.1 ^{Ba}	23.6 ^{Aa}	9.1 ^{Aa}	6.1 ^{Ba}	7.3 ^{Ba}
	HN	199.7 ^{Aa}	89.7 ^{Aa}	30.2 ^{Aa}	10.4 ^{Aa}	6.6 ^{Bb}	8.6 ^{Bb}

NN, LN, and HN represent incubation with no, low, and high nutrient input rates, respectively. Different uppercase letters in the same column within an aggregate class indicate significant difference among nutrient addition rates within the same soil; different lowercase letters in the same column within the same nutrient level indicate significant difference among aggregate classes within the same soil ($P < 0.05$)

according to the “microbial N mining” hypothesis low N availability likely facilitates SOC decomposition (Chen et al. 2014). In the present study, N and P nutrient inputs also increased total SOC concentration in farmland aggregates and large-sized woodlot aggregates after incubation with straw (Fig. 4). This confirms what was already reported that balancing nutrient stoichiometry with residue return stimulates incorporation of residue C into SOC pools (Chen et al. 2014; He and Dijkstra 2015; Fang et al. 2018, 2019; Yuan et al. 2019).

When aggregates were incubated without straw, however, nutrient addition increased CO₂ emission and decreased the SOC content, and the depletion of SOC was greater by increasing nutrient addition rates in farmland soil. This indicated the microbial use of more stable C in soil with N and P supplies, and also a change in nutrient stoichiometry (C:N:P) (Figs. 1 and 4). The depletion in woodlot aggregates, however, showed no significant difference between LN and HN, and increased with aggregate size (Fig. 4). These results suggested that microbial activity was nutrient-limited in farmland aggregates and that microorganisms were more sensitive to nutrient addition in farmland aggregates than that in woodlot soil aggregates. This was further confirmed

by the fact that the increase of SOC content was greater in woodlot than in farmland aggregates in the control treatment, while with increasing nutrient input, the difference narrowed and significantly higher amount of SOC was stored in farmland aggregates under the HN treatment. This confirmed the finding of Demoling et al. (2007) who reported that bacterial growth rates in soils with low SOC responded more to the nutrient addition than those soils with high SOC. It should be noted that SOC mineralization from farmland aggregates increased following nutrient addition without straw. This indicated that application of chemical fertilization can stimulate the depletion of SOC in farmland soil without enough crop residue return. Fortunately, increase of SOC concentration with increasing nutrient addition in straw-amended aggregates showed that with residue return, fertilization induced more newly added C into SOC, which offsets mineralization of native SOC.

Additionally, significantly higher CO₂ emission from mega- and macro-aggregates of woodlot soil was observed in the HN treatment compared with the LN treatment; however, the percentage of native SOC mineralized as well as the PE showed no difference between these two nutrient rates (Fig. S1; Figs. 1, 2, and 3). This suggested that exogenous

nutrient addition into woodlot soil mega- and macro-aggregates mainly promoted mineralization of the newly added straw C, which was confirmed by $\delta^{13}\text{C}$ -based calculations. This suggested that the SOC in woodlot aggregates was more recalcitrant than straw C (Bonanomi et al. 2013).

Effect of C-nutrient stoichiometry and straw amendment on soil microbial biomass

Increased MBC and MBN in response to nutrient addition in straw-amended treatments suggested that the presence of low nutrients such as N (Mack et al. 2004; Nordin et al. 2004) or P (Ilstedt and Singh 2005) can be the most limiting factors for microbial growth. It has been reported that adding C, N, and P together induced maximum bacterial growth rate when all nutrient limitations were removed (Demoling et al. 2007). Aggregates amended with straw in this study were characterized by significantly greater microbial biomass and lower MBC/MBN compared with the un-amended aggregates (Table 3), indicating that microbial mineralization was driven by the microbial need for C when nutrients were not limiting (de Sosa et al. 2018). When straw was added, portions are transformed into microbial biomass when soil microorganisms utilize this labile C source (Herron et al. 2009), leading to a boom of MBC and MBN. The decrease of MBC/MBN ratio by straw amendments indicated a shift in microbial community composition in soil (Li et al. 2008). The straw amendment provided substances and energy for growth and reproduction of microorganisms, which in turn consumed N and P compounds from soil and decreased mineral N and P concentration (Ros et al. 2010; Begum et al. 2014; Yuan et al. 2019). Nutrient input in this circumstance eliminated nutrient restrictions and stimulated microbial growth and activity with the increase of the decomposition of native SOC through co-metabolism (Chen et al. 2014; Tian et al. 2016). Thus, the patterns of CO_2 emission and the PE among treatments, i.e., increase with straw amendment and nutrient addition, were related to the variation patterns of MBC and MBN among treatments. Variation of MBC/MBN ratio by nutrient addition also indicated different responses of microbial groups to nutrient addition, due to the different stoichiometry of microbial groups in soils (e.g., fungi versus bacteria) (de Sosa et al. 2018). The significant lower MBC and MBN in aggregates of farmland than woodlot were consistent with the findings of Ding et al. (2020), who demonstrated that soil cultivation resulted in significant SOC depletion, which was closely associated with MBC loss.

Effect of aggregate size classes on SOC mineralization in cultivated and non-cultivated soils

Higher C mineralization rate was observed in the straw-amended large (mega and macro) aggregates than micro-aggregates in the two soils (especially woodlot soil) during incubation (Fig. S1; Figs. 1 and 3). This was consistent with

findings that SOC mineralization was higher in the coarser than the finer aggregates, probably due to low structural stability of the former (Six et al. 2000; Bimüller et al. 2016; Sarker et al. 2018). Micro-aggregates are considered to have greater physical protected SOC compared with large-sized aggregates (Six et al. 2000; Denef et al. 2001). These results also highlighted the importance of different SOC bioavailability among the aggregate size classes besides the inherent structural stability (Six et al. 2000; Tian et al. 2015). Micro-aggregates are also characterized by a lower microbial biomass due to lower available C substrate than large-sized aggregates (Balesdent et al. 2000; Six et al. 2002). This was also observed here (Table 3). Although C mineralization was greater in large-sized aggregates, the increase of SOC after incubation with straw was also greater in these aggregates (Fig. 4), indicating that more straw-derived C was sequestered in these aggregates relative to micro-aggregates. This was consistent with the above analysis that larger aggregates contained more microbial biomass with higher microbial activity and thus with microorganisms being capable of using the added straw C. The increase of straw-derived C in the larger aggregates relative to the micro-aggregates could also be explained by the microbial efficiency matrix stabilization mechanism (Cotrufo et al. 2015). If the micro-aggregate-associated C is primarily microbial derived, it may take longer to process than the direct physical stabilization. Since the incubation period of this study (180 days) is shorter than the reported time (18 months) needed to assess microbial contribution to SOC (Kallenbach et al. 2016), physical stabilization might be the reason for increase of C in micro-aggregates.

Effect of soil cultivation and interaction with aggregate size class on SOC mineralization and sequestration

The decrease of SOC content accounted for 6.0–8.1% of aggregate-associated SOC in woodlot soil but 13.5–18.9% of aggregate-associated SOC in farmland (data not shown). The C losses are expected to be greater under field conditions than under controlled laboratory conditions (Luo et al. 2017), especially for farmland aggregates with frequent soil disturbance such as tillage and fertilization. The increase of SOC content in straw-amended aggregates was greater with nutrient addition than without in both soils, and this increase was lower in woodlot aggregates than in farmland aggregates. This might be explained by the C-saturation theory that when exposed to high C inputs, soils already rich in SOC have a limit in C sequestration capacity, while soils further away from the C saturation level will stabilize more C (Hassink and Whitmore 1997; Six et al. 2002; Chung et al. 2010; Chen et al. 2017; Wang et al. 2017). The woodlot soil may be reaching SOC saturation due to related factors such as texture, mineralogy, and high organic input, while the farmland soil still has a large capacity to store C, since more than half of its initial SOC was depleted after

conversion to farmland (Table 1). More systematic research on nutrient and straw management based on long-term experiment is needed to verify this hypothesis. In addition, the change of land use type will have an impact on soil microbial community composition and their functions via diverse C:N:P ratios from exogenous organic substances, and thus might influence the PE and SOC storage (Yu et al. 2018). However, in this study, interaction between land use and C-nutrient stoichiometry and their effect on PE and SOC variation was not significant. Therefore, further research on the impacts of element stoichiometry on microbial community composition and function is needed.

Conclusions

Our incubation experiment based on ^{13}C -enriched straw revealed that nutrient input effectively promoted mineralization of native SOC and straw C in aggregates of both cultivated and un-cultivated soils. N and P addition significantly increased aggregate-associated C with straw, and the increase was greater in larger than smaller aggregates. Depletion of SOC was greater by increasing nutrient addition rates in farmland aggregates without straw amendment, suggesting that microbial activity was nutrient-limited in farmland aggregates. It can also be inferred that application of chemical fertilizer could further promote the depletion of SOC in farmland soil without crop residue return. Fortunately, the increase of SOC concentration with increasing nutrient addition suggested that with residue return, fertilization induced more newly added C into SOC, which offsets mineralization of native SOC. Results from this study improved our knowledge on mineralization of native SOC and sequestration of residue C in different size classes of aggregates in response to different C-nutrient stoichiometry, which has important implications for strategies to restore SOC in cultivated soils.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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