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RESEARCH ARTICLE

Three-year-period nitrogen additions did not alter soil organic carbon content and lability in soil aggregates in a tropical forest

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Abstract

Soil immobilizes a considerable proportion of carbon (C) as organic matter in terrestrial ecosystems and is thus critical to stabilize the global climate system. Atmospheric nitrogen (N) deposition could influence soil C storage and stabilization, but how N deposition changes soil organic C (SOC) fractions and lability remains elusive. We investigated the effects of 3-year-period N inputs on SOC fractions and lability along three soil depths (0–10, 10–20, and 20–40 cm) in a tropical forest of southern China. Results showed that N additions did not significantly change contents of SOC fractions and the C lability, either in bulk or aggregate-based soils at any of the three depths, and it showed no significant interaction with soil aggregate or soil depth. The SOC content was 43.7 ± 1.5, 18.2 ± 1.0, and 10.7 ± 0.4 mg g^{-1} at the three soil layers downwards, with the non-readily oxidizable SOC (NROC) contributing over 70% while the remaining SOC consisting of readily oxidizable SOC at each soil layer. Moreover, contents of SOC and NROC were consistently higher in small soil aggregates, but the C decrement with increasing size of soil aggregates declined along soil profile downwards. This scenario suggests that physical protection of the small soil aggregate is limited, but its greater specific surface area could obviously contribute to the SOC pattern among soil aggregates. These results indicate that the highly developed forests could be resistant to short-term N deposition, even with a high load, to maintain its SOC stabilization.

Keywords Atmospheric N deposition · Ecosystem resistance · Global changes · Soil C storage · SOC fractions · SOC stabilization

Introduction

Atmospheric nitrogen (N) deposition as a debatable environmental change issue has received wide attention, since it may exert positive (e.g., fertilizing effect) and negative (e.g., acidifying effect) effects on ecosystem structure and functions (Lu et al. 2010; Reay et al. 2008; Song et al. 2020). At global scale, N deposition is projected to be 105 Tg N yr^{-1} based on current global N emission and its increasing trend due to rapidly developing agriculture and industries, and the spatial distribution of N deposition is uneven (Galloway et al. 2008; Schwede et al. 2018). China, as a rapidly developing and huge economic entity, has emitted a great amount of active N by anthropogenic activities such as intensive use of N fertilizers and vehicles, and therefore a large percentage of the territory (especially the central and southern China) are facing severe atmospheric N deposition (Liu et al. 2013; Schwede et al. 2018; Yu et al. 2019a). Across regions, there exist extremely high N deposition loads that have exceeded critical thresholds of local terrestrial ecosystems (Fang et al. 2008; Williams and Tonnessen 2000; Zhao et al. 2017). Atmospheric N deposition on the surface of the earth could result in many consequences in ecosystem traits, e.g., to affect the plant growth and community diversity (Lu et al. 2010; Schulte-Uebbing and de Vries 2018), to induce phosphorus limitation, and to shift the soil microbial communities in terrestrial ecosystems (Deng et al. 2017; Tian et al. 2019; Zhang et al. 2018).

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Regarding that global N deposition is changing, it remains urgently needed to further clarify its ecological consequences in diverse ecosystems.

The terrestrial ecosystem contains three huge pools of organic carbon (C), i.e., soil, vegetational, and atmospheric C pools, and the soil C pool is of the greatest size (~1500 Pg C at 1 m depth) that is comparable with that of the combined vegetational and atmospheric C pools (Batjes 2016; Lal 2018). Therefore, soil is regarded as an important regulator in stabilizing the global climate system (Melillo et al. 2017; Zhu et al. 2019). Previous studies suggest that N deposition promotes global C sinks, associated with increases in soil C sequestration in several ecosystems (De Vries et al. 2006; Yue et al. 2016), and therefore sequester an additional proportion of anthropogenic C emissions (e.g., 10%; Reay et al. 2008). This could be attributable to effects of N deposition on soil C inputs and outputs, e.g., by increasing plant derived C production (Chen et al. 2015; Liu and Greaver 2010) or/and declining decomposition of soil organic C (SOC) by soil microbial communities (Chen et al. 2018b; Tian et al. 2019).

Although the total SOC content may increase under N deposition, how the compositions of SOC could respond to N deposition has been less studied and inconclusive. Previous studies demonstrate that N additions can alter litter chemistry, such as aromaticity, C/N ratio, and saturated and carbonyl C contents of above- and below-ground biomass (Gallo et al. 2005; Li et al. 2015), and litter decomposition (Hobbie et al. 2012), consequently changing soil C inputs. Moreover, N enrichment may regulate the composition and decrease the activity of soil microbial communities, resultantly reducing soil C emissions (Chen et al. 2015; Liu and Greaver 2010; Tian et al. 2019). Such changes in litter C chemistry and decomposer communities could influence SOC components and then modify its stability, as observed in a recent study that shows contrasting responses of SOC fractions (particulate OC versus mineral-associated OC) to N additions in a subtropical forest (Chen et al. 2020b). Moreover, Oladele and Adetunji (2021) reported that N addition (as N fertilizer) could significantly increase the contents of the total and labile SOC fractions such as soil easily oxidizable OC, dissolved OC, and microbial biomass C in a recent study conducted in southwest Nigeria. Nevertheless, while N enrichment increases both of the labile and non-labile SOC contents at global scale, discrepant observations still exist among studies in various ecosystems (Chen et al. 2018a) and even among different investigation years in the same study (Oladele and Adetunji 2021). This could be associated with variations in N addition rate, form, or/and duration among studies (Liu and Greaver 2010; Tan et al. 2018) but less related with the climatic and edaphic conditions (Chen et al. 2018a). Moreover, a recent field investigation shows that long-term N deposition significantly increased the contents of SOC and its fractions (including particulate, humus, and readily oxidizable SOC) in a broadleaved forest, but the same N additions did not change the SOC contents in a neighboring coniferous forest (Yu et al. 2020), suggesting that N effects on SOC may be highly variable among ecosystems. Furthermore, although contents of SOC fractions could be increased under N additions, proportions of labile to the total SOC could be unaltered or even reduced (Oladele and Adetunji 2021). Regarding the divergent responses and ecosystem dependence of SOC fractions in response to N deposition, more studies are needed to improve our understanding on the topic.

Diverse methodologies, including physical, chemical, and biological techniques, can be used to fractionate SOC into labile vs. non-labile groups. It has been well established that proportion of soil aggregates of different sizes determines the SOC stability to a great extent, which could be attributed to multiple mechanisms produced by soil aggregates (Han et al. 2016; Six et al. 2002; von Lützow et al. 2007). Among them, physical protection considering accessibility of SOC to soil microorganisms is unneglectable when predicting SOC dynamics under global change scenarios (Luo et al. 2017; Qin et al. 2019). Therefore, a physical method based on soil aggregate size has been widely accepted to fractionate SOC, with a general view that the occluded SOC in smaller soil aggregates could be higher stable than that of the larger ones (Qin et al. 2019; Six et al. 2004). This could be due to inaccessibility of the small aggregated SOC to soil microorganisms and its environmental depressions on soil microbial community size and activity (Han et al. 2016; Six et al. 2004; Zhong et al. 2017). The SOC fractions occluded in different soil aggregates may respond to N deposition differently, with the highly physically protected SOC compounds being less affected (Chen et al. 2018a). Contrastingly, a recent study shows that N additions could decline the mineral-associated SOC content, due to decreases in the mineral sorption of microbial derived OC to the soils (Chen et al. 2020b). These existing empirical experiments show inconsistent effects of N additions on the aggregated SOC fractions; the SOC fractions in different soil aggregates could be highly resistant to N additions in several ecosystems (Hagedorn et al. 2003; Riggs et al. 2015; Zhong et al. 2017), whereas they may be vulnerable in some others likely depending on the applied N form or load (Fang et al. 2014).

Moreover, SOC lability, indicated by the ratio of chemically labile and non-labile SOC fractions, can reflect the easiness of SOC oxidization and therefore SOC stabilization (Blair et al. 1995). Our previous studies showed that the SOC lability could be sensitive to environmental changes in farmlands but not in urban forests (Wei et al. 2018; Zhang et al. 2020). Moreover, a recent case study showed that N enrichment could increase or decrease the lability index of SOC in a farmland of southwest Nigeria (Oladele and Adetunji 2021). On global scale, Chen et al. (2018a) reported that N additions could increase the contents of both chemically labile and non-labile SOC fractions, while the N-induced increment of labile SOC was greater than that of non-labile SOC (please see OXC vs. UOXC in Fig. 2 of Chen et al. 2018a). Both studies indicate a potentially high sensitivity of the SOC lability to N enrichment. Under N deposition, however, the SOC lability in the soil aggregates has been rarely studied. With the atmospheric N deposition scenario, combining the physical fractionation based on the size of soil aggregates with a chemical oxidization method could help further reveal changes in the SOC lability of soil aggregates.

The northern land contributes significantly to the global land C sink and the low-latitude forests constitute a considerable proportion of the global C stock (Ciais et al. 2019; Scharlemann et al. 2014). It remains important to further clarify the SOC storage and its changes under global change scenarios including N deposition (Ciais et al. 2019). The tropical natural forests in China, with higher C sequestration rates than planted ones (Yu et al. 2019b), are functioning as obvious C sinks (Zhou et al. 2006). Several pioneering studies in the studied forest show that N deposition could increase the particulate and humus SOC concentration (Chen et al. 2012a; Yu et al. 2020), which may contribute to the SOC increment at the surface soil of the forest as observed previously (Zhou et al. 2006). Alternatively, the N effects may be neutral in forests (e.g., young coniferous and broadleaved plantations) in this or other regions across the globe (Chen et al. 2018a; Yu et al. 2020; Zhong et al. 2017). This study was conducted to investigate how N additions would influence the SOC content and lability in different soil aggregates along a soil profile of a monsoon evergreen broadleaved forest of tropical China. We expected that the SOC content and lability would decrease with soil depth and that the SOC content would decrease, but its lability would increase as size of the soil aggregates increased, considering the SOC processing along soil depths and soil aggregates. Furthermore, we hypothesized that N additions would increase the SOC content and its lability in large soil aggregates while reduce them in small soil aggregates, due to potential effects of mineral N on formations of organic matter and organo-mineral complexes (Chen et al. 2018a; Kleber et al. 2007; Moran et al. 2005).

Materials and methods

Site description

This study was conducted in a tropical forest located in Dinghushan Biosphere Reserve (112°30′39″–112°33′41″ E, 23°09′21″–23°11′30″ N) in Guangdong Province of Southern China. This region experiences typical monsoon climate, which is characterized by high air temperature associating with high rainfall amount in the warm-wet season of each year (ranging from April to September). Annual air temperature is 22.3 °C, while precipitation is 1678 mm in the study site, with most (~80%) occurring in the warm-wet season (Wei et al. 2015). The studied forest is an evergreen broadleaved forest, with few anthropogenic disturbances for more than a hundred of years under protections. Dominant tree species in the forest include *Castanopsis chinensis*, *Schima superba*, *Cryptocarya chinensis*, *Machilus chinensis*, and *Syzygium rehderianum*, and the soil is Oxisol according to the USDA soil taxonomy (Tang et al. 2006). For the surface soil at 10 cm depth, the soil pH (water extracted at a ratio of soil/water being 1:2.5) was 3.8 ± 0.05 before the experiment started. The total contents of soil C, N, and phosphorus (P) were 40.2 ± 2.1, 2.4 ± 0.01, and 0.2 ± 0.001 mg g^{-1}, respectively, which were quantified by external heating method with potassium dichromate, semi-micro Kjeldahl digestion, and molybdenum antimony colorimetric methods, respectively.

Experimental design and sample collections

Experimental treatments followed randomized block design, and three blocks were established as experimental replicates. In October of year 2013, we established three experimental blocks, and four quadrats at the size of 15 × 15 m^2 were set up for experimental treatments in each block, with a buffer distance > 10 m being reserved between each two quadrats. Finally, a total of 12 quadrats were established in this study. In each block, four quadrats were randomly assigned to conduct N addition or control treatments, and experimental duration was 3 years from September of 2014 to September of 2017.

Three levels of extra N addition (35, 70, and 105 kg N ha^{-1} yr^{-1}) were sprayed on the surface of the assigned plots in the form of NH_4NO_3 solution, and the control plots did not receive extra N addition, producing four levels of N treatment (i.e., low N treatment [LN], medium N treatment [MN], high N treatment [HN], and control, respectively). The chemical NH_4NO_3 was used to simulate deposited N from the atmosphere, because ammonia and nitrate were the dominating components and accounted for most of the atmospheric N deposition load over China or elsewhere (Liu et al. 2013; Schwede et al. 2018). The three levels of N addition were determined on basis of load of inorganic N deposition occurring in this region (34.1 kg N ha^{-1} yr^{-1}; Fang et al. 2011), which simulated double, triple, and quadruple of the ambient N deposition load. Throughout the experimental period, N additions were conducted by manually spraying 30 L of NH_4NO_3 solution with the corresponding amount of N onto the surface of the treated quadrats at the end of each month, while the same amount of water (30 L) was homogenously sprayed as the same but no extra N in the control quadrats. The volume of water addition corresponded to increase 1.6 mm of precipitation in each quadrat each year, which could be neglectable in such a rainfall-rich ecosystem. Exogenous N additions significantly decreased the soil pH and shifted the relative abundance and composition of soil N functional microbial groups (Han et al. 2018), which were associated with the changed acidity and N availability in the soil (Nie et al. 2019; Nie et al. 2018). At the end of the experiment after the 3-year-period treatments, we collected composite soil samples in each quadrat for analyses at three soil layers, i.e., 0–10, 10–20, and 20–40 cm, by using a 5-cm soil auger.

Sample preparation and analyses

After being transferred into laboratory, the soil samples were sieved to pass a 2-mm soil sieve for removal of visible rocks and plant residues and then frozen-dried. Further, the bulk soil samples were categorized into three classes of soil aggregates by passing two soil sieves (250 μm and 53 μm) in a mechanical shocker for 30 min. The three sizes of soil aggregates were defined as coarse (250–2000 μm), fine (53–250 μm), and micro (< 53 μm) intra-aggregates particulate organic matter (iPOM) (Six et al. 2004). Our preliminary experiment showed that the duration of shock (30 min) was enough to separate the soil aggregates, and total recovery percentage was greater than 95% for all samples. After categorizing the soil aggregates, we further ground the coarse and fine iPOMs to pass a 150-μm soil sieve for analyses on the total and fractionated SOC contents.

For the bulk and aggregated soils, we analyzed the SOC content using a Vario TOC element analyzer (Elementar, Hanau, Germany). Content of the readily oxidizable organic carbon (ROC) was analyzed following the description of Chen et al. (2012b). In brief, each sample that contained ~20 mg of SOC was weighed in a 100-mL centrifuge cube to be oxidized using the oxidizing agent of 333 mM of $KMnO_4$ solution. The organic materials consisting of ROC could be completely oxidized in this reaction system, and the remaining $KMnO_4$ was quantified by the colorimetry method in an ultraviolet spectrophotometer (UV-1750, Shamadzu Corporation, Kyoto, Japan) at 565 nm. The ROC content of each sample was calculated on basis of decrements in the $KMnO_4$ content, given that 1 mM of MnO_4^- could consume 9 mg C (Blair et al. 1995). For each sample, the difference in total SOC and ROC contents was regarded as the non-readily oxidizable organic carbon (NROC) content, and the ratio of ROC to NROC in each sample was used to indicate SOC lability (Blair et al. 1995).

Statistics

General linear model was used to detect significant effects of treatments on the SOC, ROC, or NROC content, as well as the SOC lability. For the bulk soils, soil depth, N treatment and experimental block were considered three fixed factors, while soil depth, aggregate size, N treatment, and experimental block were fixed factors for the separated soil samples in the general linear models. Furthermore, one-way analysis of variances (ANOVA) was employed to calculate the significance level of the N addition effects on the SOC content and lability, as well as the labile and non-labile SOC contents, in the bulk soils or each size of soil aggregate at a given soil depth. Normality and homoscedasticity of data were tested in prior to analyses, and logarithm transformation was conducted given that the assumption of normality was violated. These statistics were performed in IBM SPSS software (version 22, IBM Corp., New York, USA), and figures were drawn in SigmaPlot 10.0 (Systat Software Inc., California, USA). Data were presented as mean ± standard error unless specifically clarified. The significance level was set at $p < 0.05$ for all the statistics.

Results

Effects on the SOC content of bulk soil and soil aggregates

Regardless of bulk soils or soil aggregates, N additions did not exert any significant effect on the SOC content, and no significant interactive effects between N addition and soil aggregate or depth existed for the SOC content ($p > 0.05$, Table 1). Across all the N treatments, average SOC content of the bulk soil was 43.7 ± 1.5, 18.2 ± 1.0, and 10.7 ± 0.4 mg g^{-1} at the soil layers of 0–10, 10–20, and 20–40 cm, respectively, which was significantly different among the soil profiles ($p < 0.05$). When separating bulk soils into different size of soil aggregates, we observed that the SOC content was significantly different among soil aggregate size and soil depth ($p < 0.001$, Table 1), while their interactive effect was also significant ($p < 0.001$, Table 1). At the surface and subsurface soil layers, the SOC content was significantly higher in the micro than in the coarse and fine iPOMs ($p < 0.05$, Fig. 1 a and b). At the soil layer of 20–40 cm depth, however, the SOC content was significantly lowest in the fine iPOM relative to the other aggregates ($p < 0.05$, Fig. 1c).

Effects on the ROC content of bulk soil and soil aggregates

Likewise, the N treatments, independently or interactively with other factors, did not significantly alter the ROC content and its portion to SOC in the bulk soil or any soil aggregate ($p > 0.05$, Table 1). Regarding the bulk soils, the average ROC content across all the N treatments was 11.3 ± 0.8, 3.8 ± 0.3, and 1.8 ± 0.1 mg g^{-1} for the soils at the three depths from the surface downwards ($p < 0.001$). The values corresponded to 26.1 ± 2.1, 20.7 ± 1.0, and 16.9 ± 0.6% of the total SOC content and were significantly different among soil layers

Table 1 Statistical *F* and determination coefficients calculated by general linear model with soil aggregate size (A), soil depth (B), N treatment (C), and experimental block as the four fixed factors

	SOC	ROC	NROC	SOC lability
Aggregate (A)	30.094 ***	14.848***	29.140 ***	8.361 **
Depth (B)	626.166***	515.391 ***	527.872 ***	99.347 ***
Treatment (C)	1.605	0.547	1.987	0.208
Block	1.437	0.769	1.577	0.402
A × B	5.840 ***	27.304 ***	11.733 ***	72.736 ***
A × C	0.352	0.554	0.438	1.352
B × C	0.843	1.099	0.685	1.228
A × B × C	0.184	0.227	0.393	1.001
Corrected model	36.566 ***	32.041 ***	31.933***	14.467 ***
R^2 (adjusted R^2)	0.951 (0.925)	0.944 (0.915)	0.944 (0.915)	0.884 (0.823)

In the table, SOC is soil organic carbon, ROC is readily oxidizable SOC, and NROC is non-readily oxidizable SOC. Significance levels at $p < 0.05$, 0.01, or 0.001 are indicated by *, **, or ***, respectively. Data were logarithmically transformed with the natural base before the analysis

($p < 0.001$). The size of soil aggregates significantly affected the ROC content, and interactive effect between soil aggregate and depth was also significant ($p < 0.001$, Table 1). In the upper two soil layers, the ROC content was higher in the micro iPOM than in the coarse and fine iPOMs (Fig. 2 a and b). The ROC pattern among soil aggregates, however, reversed in the 20–40 cm soil depth, with significantly lower ROC content in the micro iPOM than in the coarse and fine iPOMs ($p < 0.05$, Fig. 2c).

Effects on the NROC content of bulk soil and soil aggregates

The non-labile NROC fraction contributed most of the SOC content in the bulk soil, with the proportion being 73.9 ± 2.1, 79.3 ± 1.0, and 83.1 ± 0.6% for the soils of 0–10, 10–20, and 20–40 cm, respectively. The NROC content and proportion were significantly different with soil depth ($p < 0.001$). Moreover, the NROC content was significantly different among soil aggregates, and there was significantly interactive effect between soil aggregate and depth ($p < 0.001$, Table 1 and Fig. 3a–c). The NROC content was significantly higher in the micro iPOM than in the coarser soil aggregates at the surface soil layer of 0–10 cm depth ($p < 0.001$, Fig. 3a), but it was not significantly different at the subsurface soil layer ($p > 0.05$, Fig. 3b). We observed the lowest NROC content in the fine iPOM at the soil depth of 20–40 cm ($p < 0.05$, Fig. 3c). However, the N treatments did not significantly change the NROC content among the soil aggregates with soil depth ($p < 0.001$, Table 1).

Effects on the SOC lability of bulk soil and soil aggregates

In the bulk soil, the SOC lability significantly decreased downwards with soil depth ($p < 0.001$), with the highest value

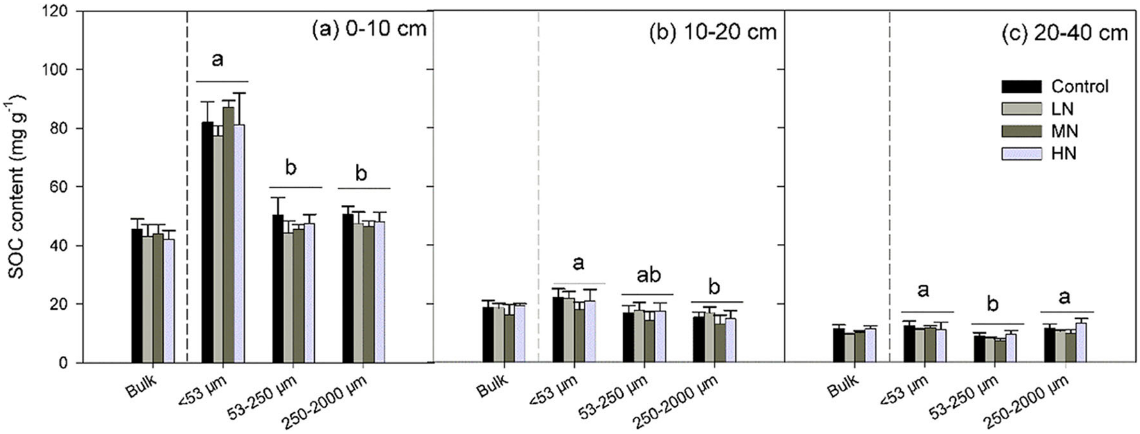

Fig. 1 Soil organic carbon (SOC) content in the bulk soils and different size of soil aggregates at the three soil layers (**a**, 0-10 cm; **b**, 10-20 cm; **c**, 20-40 cm) under nitrogen additions. The bars are average values with the error bars indicating standard errors ($n = 3$). In each panel, different lowercase letters indicate that SOC content was significantly different at $p < 0.05$ among the three groups of soil aggregates. LN, MN, and HN stand for low, medium, and high nitrogen addition, respectively. The three sizes of soil aggregates were defined as coarse (250–2000 μm), fine (53–250 μm), and micro (< 53 μm) intra-aggregates particulate organic matter (iPOM) in the present study

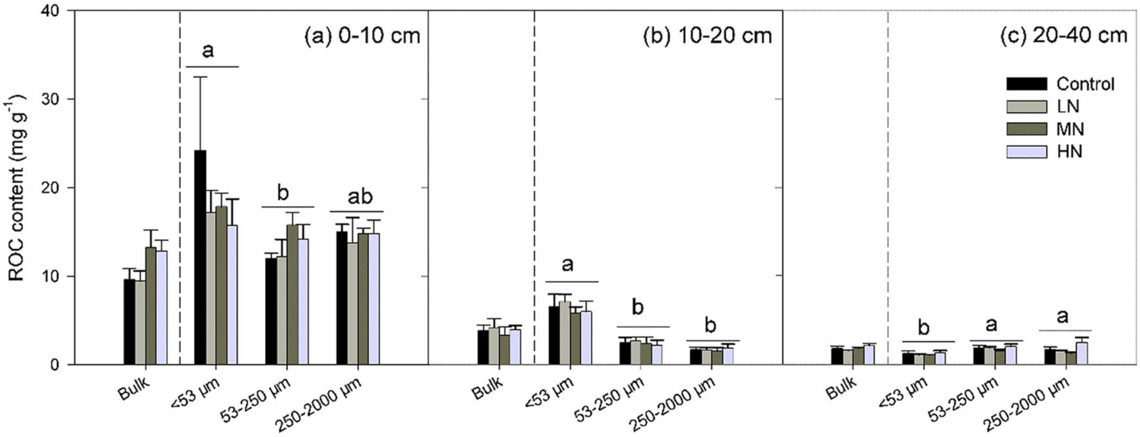

Fig. 2 Soil readily oxidizable organic carbon (ROC) content in the bulk soils and different size of soil aggregates at the three soil layers (**a**, 0-10 cm; **b**, 10-20 cm; **c**, 20-40 cm) under nitrogen additions. The bars are average values with the error bars indicating standard errors ($n = 3$). In each panel, different lowercase letters indicate that ROC content was significantly different at $p < 0.05$ among the three groups of soil aggregates. The three sizes of soil aggregates were defined as coarse (250–2000 μm), fine (53–250 μm), and micro (< 53 μm) intra-aggregates particulate organic matter (iPOM) in the present study

in the surface soil of 0–10 cm (0.37 ± 0.04), followed in the soil at 10–20 cm (0.26 ± 0.01) and then at 20–40 cm (0.20 ± 0.01). The N additions did not significantly modify the SOC lability of the bulk soils at any soil depth ($p > 0.05$). Moreover, the SOC lability was significantly lower in the micro iPOM than in the relatively coarser soil aggregates of the size > 53 μm at the surface layer and at the lower soil layer of 20–40 cm depth ($p < 0.05$, Fig. 4a and c). At the subsurface soil layer of 10–20 cm, however, the SOC lability in the finest soil microaggregate was the highest, followed by the fine iPOM and then by the coarse iPOM ($p < 0.05$, Fig. 4b). Nevertheless, the N treatments did not result in any significant variation in the SOC lability, regardless of soil depth or aggregate size class ($p > 0.05$, Fig. 4).

Discussion

In the present study, the total and chemically labile and non-labile SOC contents significantly decreased downwards with soil depth (Table 1 and Figs. 1, 2, and 3). These observations follow our expectations and are in line with the well-established knowledge that the SOC content decreases with soil depth (Jobbágy and Jackson 2000; Kramer and Gleixner 2008). The pattern has been frequently observed in natural ecosystems with few disturbances, due to higher inputs of organic materials from the above- and below-ground components of plants to the surface of the earth and then processed to move downwards by the soil microbial activities and physical processes such as leaching (Chen et al. 2018c; Schenk and

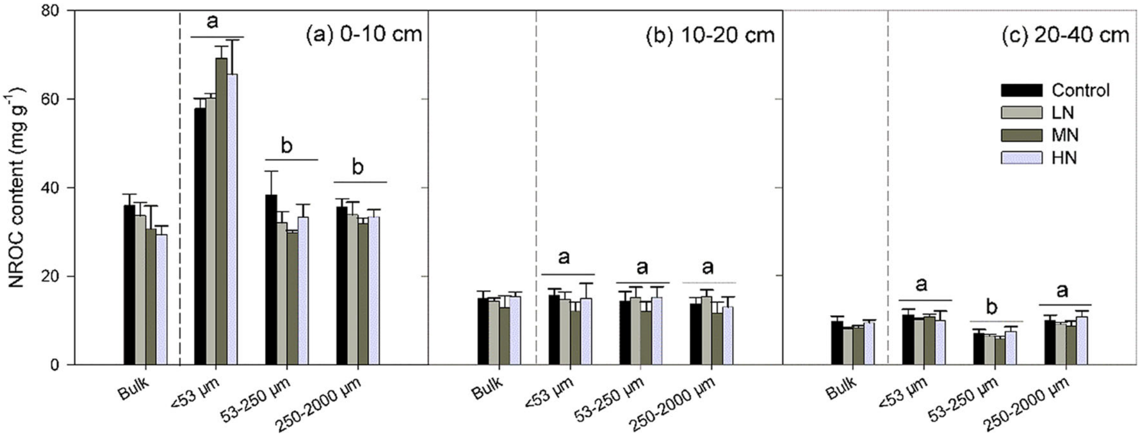

Fig. 3 Soil non-readily oxidizable organic carbon (NROC) content in the bulk soils and different size of soil aggregates at the three soil layers (**a**, 0-10 cm; **b**, 10-20 cm; **c**, 20-40 cm) under nitrogen additions. The bars are average values with the error bars indicating standard errors ($n = 3$). In each panel, different lowercase letters indicate that NROC content was significantly different at $p < 0.05$ among the three groups of soil aggregates. The three sizes of soil aggregates were defined as coarse (250–2000 μm), fine (53–250 μm), and micro (< 53 μm) intra-aggregates particulate organic matter (iPOM) in the present study

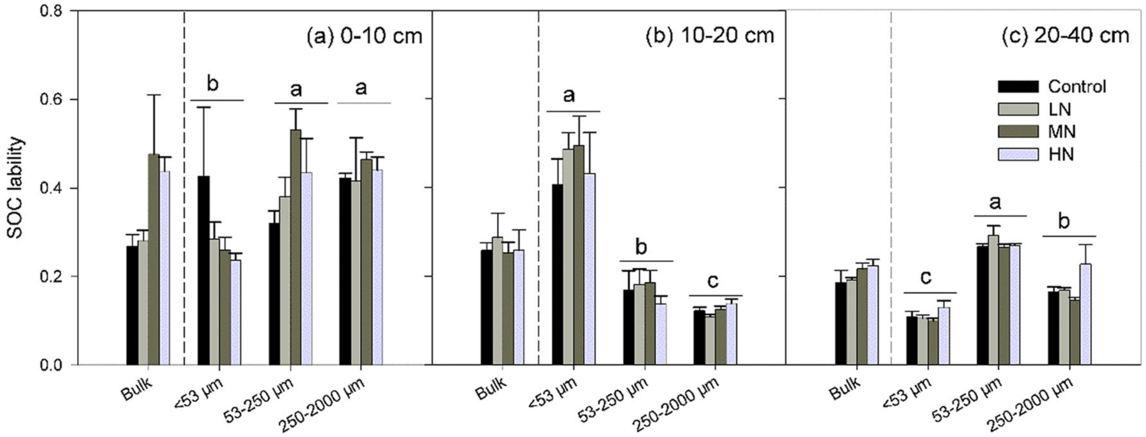

Fig. 4 Lability of soil organic carbon (SOC) in the bulk soils and different size of soil aggregates at the three soil layers (**a**, 0-10 cm; **b**, 10-20 cm; **c**, 20-40 cm) under nitrogen additions. The bars are average values with the error bars indicating standard errors ($n = 3$). In each panel, different lowercase letters indicate that NROC content was significantly different at $p < 0.05$ among the three groups of soil aggregates. The three sizes of soil aggregates were defined as coarse (250–2000 μm), fine (53–250 μm), and micro (< 53 μm) intra-aggregates particulate organic matter (iPOM) in the present study

Jackson 2002). In several ecosystems, however, vertical distribution of the SOC and its fractions may be influenced by interventions of anthropogenic activities or natural processes (Dolan et al. 2006; Hobley et al. 2016). Furthermore, the chemically non-labile fractions (i.e., NROC) contributed most of the total SOC content in this forest (> 70% at all the soil depths), and the SOC lability was observed to decrease as soil depth increased (Table 1 and Fig. 4), suggesting that organic materials are further processed by soil microbes to produce high chemical recalcitrance with soil depth (Kramer and Gleixner 2008; Miltner et al. 2011; Yao and Shi 2010).

Among the soil aggregates of different sizes, the SOC content was significantly higher in the smaller soil aggregates (Table 1 and Fig. 1). Such a pattern has been reported in previous studies (Jiménez et al. 2011), whereas contrasting patterns are also observed in some others (Huang et al. 2017; Wei et al. 2013). It is not immediately apparent which mechanisms are underlying the discrepancy among studies, because the distributions of SOC among soil aggregates could be influenced by multiple factors including vegetational and edaphic properties (Six et al. 2004; Tamura et al. 2017). However, we found that the differences in SOC content among the soil aggregates decreased as soil depth increased; for example, difference in the C content between the micro (< 53 μm) and coarse (250–2000 μm) iPOMs was 33.7 (41%), 5.7 (27%), and 0.3 (2%) mg g^{-1} at the three soil depths downwards (Fig. 1). This scenario suggests that a significantly higher SOC content in the small soil aggregate may be attributable to its high specific surface area to provide more space for adsorption of the SOC (Tamura et al. 2017), given excess C inputs at the surface soil. When external C inputs decrease, e.g., with soil depth, the SOC content tends to be comparable among different soil aggregates (Fig. 1), indicating that the physical protection of soil aggregates may have an upper threshold for any given soil. Again, the SOC pattern mainly was derived from the distribution of the chemically non-labile fraction among soil aggregates, basing on the observation of the high proportion of NROC to total SOC (comparing the C content in Fig. 3 with that in Fig. 1). The SOC lability decreased downwards with soil depth, but did not change among the soil aggregates, which is consistent at the three soil layers (Fig. 3). Contrary to our expectation, the SOC lability decreased as the size of soil aggregates increased at 10–20 cm depth (Fig. 4b). This may be associated with the relatively higher productivity and turnover rate of fine roots at the subsurface soil in this forest (Wang et al. 2019), therefore producing a considerable amount of labile SOC that are absorbed in soil aggregates (Tamura et al. 2017).

Opposite to our hypothesis, however, N additions did not significantly affect the contents of the SOC fractions and the SOC lability in the bulk or aggregate-based soils (Table 1 and Figs. 1, 2, 3, and 4), despite that the treatments had significantly altered soil N availability and relative abundances of the N functional genes such as *amo*A and *nir*K in this forest (Han et al. 2018; Nie et al. 2019). This could be in parts contributable to relatively constant litterfall production and quality (Wei et al. unpublished data; Li et al. 2018) and soil microbial community composition under N additions in the studied forest (He et al. unpublished data; Nie et al. 2018), and short-term N additions may not change the decomposition efficiency of soil microbial community (Wei et al. 2019). As shown in a recent study, soil microbial residues may contribute greatly to the SOC (especially the mineral-associated fractions) changes (Chen et al. 2020a), and therefore, the neutral N effects as observed in the present study are expectable when the C source by litterfall productions and the soil microbial community as the engine to process soil C cycling remains unaltered after the 3-year-period N additions in our study.

However, it is very likely that contents of the SOC and its fractions such as the ROC and particulate organic C will be influenced by longer-term N enrichment (Chen et al. 2012a; Yu et al. 2020), because N additions could regulate decomposer community composition and functions, and experimental duration is very likely to amplify N effects on soil microbial communities (Chen et al. 2018b; Han et al. 2018; Zhang et al. 2018). Although a global meta-analysis suggests that N additions could promote SOC content in the bulk soil and soil macro- and mineral-associated aggregates, discrepant observations still exist across studies in various ecosystems (Chen et al. 2018a). The studied forest is the regional climax vegetation which could be resistant to external environmental changes to a certain extent. Moreover, N deposition may result in significant changes in the SOC content (especially the labile fractions such as dissolved SOC and microbial biomass C) in several young and developing forests and may interact with other global change aspects such as CO_2 enrichment in the atmosphere (Chen et al. 2012b; Zhong et al. 2017), and in a previous study, we observed that N additions decreased the temperature sensitivity of soil microbial respiration although the soil microbial community composition were not altered (Wei et al. 2017). These observations highlight the nature of context dependence and complexity of N deposition effects in the scenario of multiple global changes (Hines et al. 2014; Ni et al. 2017).

Regarding the perspective of soil C content, the forest is relatively highly resistant to short-term N additions based on our observations, despite soil N availability and N functional genes had been changed (Han et al. 2018; Nie et al. 2019). However, a recent study shows that up to 13 years N addition (150 kg ha^{-1} yr^{-1}) could increase the SOC content due to increased humus SOC and slightly increased particulate SOC contents in this forest (Yu et al. 2020). Moreover, 6-year-period N additions led to increases in particulate SOC but decreased the mineral-associated SOC content in a subtropical forest (Chen et al. 2020b). These results suggest that longer-term atmospheric N deposition, as well as interactive effects of N enrichment and other aspects of global change, may change the SOC content in this forest (Chen et al. 2012a; Chen et al. 2012b). Moreover, we investigated the SOC fractions in a combination of physical and chemical methods, although continuity nature of the SOC has been recognized (Lehmann and Kleber 2015). In spite of robust simplicity and several redundancies in the separated SOC fractions, combining multiple methods of the SOC fractionation could help us to better understand changes of the SOC under global changes (Chen et al. 2018a; Olk and Gregorich 2006; Qin et al. 2019).

Conclusions

This study was conducted in a tropical forest to investigate N-induced changes in the SOC content and lability in soil aggregates with soil depth, by combining physical and chemical methods to fractionate the SOC. Results showed that the 3-year-period N additions, even up to three times of current load of atmospheric N deposition in this region, did not significantly influence the SOC content and its distributions in soil aggregates of different sizes or between the chemically labile and non-labile fractions among the soil profile. This scenario suggests that the forest is to a certain extent resistant to atmospheric N deposition from the perspective of soil C content, possibly due to high development and stabilization of the ecosystem with a relatively stable soil decomposer community. Furthermore, we observed that N additions did not significantly change SOC lability. These results suggest that in spite of uncertainty in effects of experimental duration, relatively short-term atmospheric N deposition may not affect SOC content and stabilization in tropical forests.

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Author contribution WS and HW conceived this experiment; HW conducted experimental treatments; XC, HW, and MK finished sample collection and analyses; HW and JH analyzed the data; HW wrote the original draft; all the authors contributed to result explanations and manuscript revisions.

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Declarations

Ethics approval and consent to participate Not applicable.

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