

**Stable isotopic signatures of carbon and nitrogen in soil aggregates following  
the conversion of natural forests to managed plantations in Eastern China**

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## 23 ABSTRACT

### 24 *Background and aims*

25 Land cover change (LCC) from natural forest (NF) to plantations (PF) has occurred worldwide  
26 over the past several decades. However, the different LCC effects on soil aggregate C and N  
27 turnover in various climatic zones remain uncertain.

### 28 *Methods*

29 Soil samples were taken from both NF and PF at five sites along an approximately 4200 km  
30 north-south transect in eastern China. We measured soil aggregate C and N concentrations, and  
31  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### 32 *Results*

33 The soil aggregate distribution is not different between NF and PF, except that the mass  
34 proportion of microaggregate is higher in PF than in NF. However, the impacts of LCC on soil C  
35 and N concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  vary among five climate zones. The changes in soil  
36 aggregate C and N concentrations and  $\delta^{15}\text{N}$  induced by LCC show nonlinear relationships with  
37 climatic factors (i.e., MAT and MAP), and there is a linear relationship between soil aggregate  
38  $\Delta\delta^{13}\text{C}$  (calculated by subtracting PF from NF) and MAT and MAP. The soil aggregate C and N  
39 concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  show clear trends along the climatic transect. In addition, the  
40 impacts of LCC are more obvious in topsoil than in subsoil.

### 41 *Conclusion*

42 Our findings highlight that the impacts of LCC on soil C and N concentrations are related to  
43 climatic factors and that the turnover of soil C input and decomposition increase with increasing

44 MAT and MAP. Specifically, that the increased turnover of soil C input and decomposition in PF  
45 than NF was compensated by higher C inputs with increasing MAT and MAP.

46 Keywords: Land cover change; Soil aggregate; Soil C and N; Stable isotopes; Climatic factors.

## INTRODUCTION

Forest soils store a large portion of terrestrial carbon (C) (Dixon et al. 1994) but simultaneously may release large sums of C when they undergo land use or environmental changes (Guillaume et al. 2015; Houghton and Nassikas 2017). To fulfill societal needs for valuable timber and other economic forest products, natural forests (NFs) have been widely converted to intensively managed plantations (PFs) during the past several decades (Lin et al. 2018; Llorente et al. 2010; Straaten et al. 2015; Yang et al. 2019). There is substantial evidence showing significant differences in the quantity and quality of C input through litterfall or root exudation between NF and PF (Hertel et al. 2009; Lewis et al. 2016) and on management practices including tillage, fertilization, thinning, drainage, and harvesting (Jandl et al. 2007; Li et al. 2014). It is very important to know how land cover change (LCC) from NF to PF alters soil C and N cycles (Chen et al. 2016; Straaten et al. 2015). Thus, the influences of LCC on soil C and N have been widely investigated during the past two decades (Chen et al. 2004; Li et al. 2005; Lin et al. 2018; Straaten et al. 2015). Currently, however, there is still uncertainty regarding positive, negative or negligible effects on soil C and N at a large scale under various climatic conditions (Lewis et al. 2016).

Soil aggregates are the basic units of soil structure, and have an important role in controlling soil C and N stocks and stabilization (Tisdall and Oades 1982; Six and Paustian 2014; Zhu et al. 2017). Moreover, soil organic matter fractions can serve as early indicators for the change in soil C and N stocks induced by LCC (He et al. 2008; Leifeld and Kögel-Knabner 2005). A hierarchy model was proposed by Tisdall and Oades (1982), who stated that mineral particles are bound together to form microaggregates by various cements, including persistent organic materials, crystalline oxides and highly disordered aluminosilicates. These microaggregates, in turn, build

macroaggregates due to transient and temporal agents such as polysaccharides, roots, and fungal hyphae (Spohn and Giani 2011). Due to the various aggregate binding agents, the stability of soil aggregates and the allocated C and N in different-size aggregates have different sensitivities to land use and cover change (Tisdall and Oades 1982; Chen et al. 2017). Land cover change from natural forests to intensively managed plantations has been well known to strongly affect soil aggregation and C and N pools (Ashagrie et al. 2005; Chen et al. 2017; Goma-Tchimbakala 2009; He et al. 2008). However, fewer studies have focused on the efficiency and mechanism of aggregate C and N turnover associated with the input of plant residues and output of microbial mineralization.

Isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  in soils can change as a result of fractionation processes occurring during soil C and N cycles (Bol et al. 1999; Gerschlauer et al. 2019; Guillaume et al. 2015), thereby providing new information on soil C and N turnover controlled by biotic and abiotic factors (Garten et al. 2000; Ma et al. 2012; Ngaba et al. 2019; Wang et al. 2018). Generally, high litter inputs can lead to low values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in soils, whereas enhanced soil organic matter (SOM) mineralization can enrich soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Garten et al. 2000; Natelhoffer and Fry, 1998). Land use and cover change can alter C and N source inputs, SOM decomposition rates, soil erosion, and N cycle processes e.g., mineralization, nitrification and gas N loss (Chen et al. 2016; Guillaume et al. 2015; Hertel et al. 2009; Ross et al. 1999). Therefore, it has been suggested that the conversion of natural forests to plantations can lead to the changes in  $^{13}\text{C}$  and  $^{15}\text{N}$  in plant and soils at the stand scale (Deng et al. 2016; Llorente et al. 2010; Guillaume et al. 2015; Ngaba et al. 2019; Zeller et al. 2007). To our knowledge, however, no studies have systematically examined the isotopic signatures of  $^{13}\text{C}$  and  $^{15}\text{N}$  in soil aggregates

following the conversion of natural forests to managed plantations along a national-scale climate zone.

China is currently the country most affected by LCC from natural forests to intensively managed plantations in the world (Lin et al. 2018; Yang et al. 2009). The destruction of primary forests is regarded as a main factor causing significant loss of soil C and N pools (Lewis et al. 2016; Wang et al. 2016). Thus, this study focuses on soil aggregate C and N concentrations, and  $^{13}\text{C}$  and  $^{15}\text{N}$  abundances at the surface (0-10 cm) and subsurface (10-20 cm) layers in the NF and adjacent PF across five climate zones along an approximately 4200 km transect from northern to southern China. We aim to evaluate whether the impacts of LCC on soil aggregate C and N differ among climate zones and between topsoil and subsoil, and to determine the relationship between the changes in soil aggregate C and N concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with climatic factors, i.e., mean annual temperature (MAT) and mean annual precipitation (MAP). This study can provide new information on the effects of LCC under the background of global climate change. Based on the aforementioned studies, we hypothesized that (1) soil aggregate stability and C and N allocated in aggregates would decline in plantations because of intensive management disturbances, but (2) soil aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  would be enriched in plantations due to increasing SOM mineralization relative to adjacent natural forests. We also hypothesized that (3) the impacts of LCC on soil aggregate C and N would vary among climate zones, and the declines of soil aggregate C and N concentrations and the enrichments of aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  would increase with increasing MAT and MAP.

## MATERIALS AND METHODS

### *Study site and sampling*

The study sites are located in the eastern forest of China (18.44 - 52.92°N, 108.01-122.79°E) and have elevations of 275 to 800 m. In August 2017, soil samples were collected at five sites along a north-south transect in eastern China, including Mohe (MH), Qingyuan (QY), Huitong (HT), Dinghushan (DH), and Jianfengling (JF) (Fig. S1). At each site, natural forests (NFs) and paired adjacent plantations (PFs) converted from native vegetation were selected to ensure comparable stand conditions. The characteristics of climatic conditions, soil types and vegetation compositions in each stand are shown in Table 1. Three plots of 20 m × 20 m were set up in each stand and soils were taken at surface (0-10 cm) and subsurface (10-20 cm) layers, packed in zip locked polythene bags and taken back to the laboratory immediately for analysis of soil aggregate distribution, soil C and N concentrations, and isotopic abundances of <sup>13</sup>C and <sup>15</sup>N.

### *Soil aggregate fractionation*

The aggregate size distribution and stability are determined using a wet sieving protocol (Kemper and Rosenau 1986). In summary, soils are air-dried for 2 weeks, and a sieve of 8 mm is used to remove roots, coarse plant residues and any stones >8 mm. Then, 40 g air-dried soil is used for aggregate size fractionation by wet sieving and is placed on a set of six nested sieves of 5, 2, 1, 0.5, 0.25 and 0.053 mm. The sieves are shaken vertically for 30 min at a rate of 35 strokes min<sup>-1</sup> in a water bucket. The soil aggregate fractions retained in each sieve were carefully washed off the sieve into a preweighed aluminous container, oven-dried at 105°C and weighed. A portion of soil aggregate <0.053 mm retained in water was also collected and oven dried at 105°C for determination of soil C, N and isotopic abundances of <sup>13</sup>C and <sup>15</sup>N, but the mass was calculated from the total soil mass minus the dry mass of the other six aggregates. Each fraction

was agitated for 18 h with 5% sodium hexametaphosphate (1:3 soil: liquid ratio) to correct the presence of coarse fragments and sands.

#### *Soil C and N concentrations and natural abundance of $^{13}\text{C}$ and $^{15}\text{N}$ analysis*

Soil C and N concentrations and stable isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  were measured using an isotope ratio mass spectrometer (IRMS) (IsoPrime 100, Isoprime Ltd., UK) connected to a CN elemental analyzer (Vario MICRO cube, Elementar, Germany). Carbon and nitrogen stable isotope abundances are calculated as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) using the following formula:

where  $R_{\text{sample}}$  is the  $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$  ratio in the samples and  $R_{\text{standard}}$  is the  $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$  ratio in the standard. The Pee Dee Belemnite (PDB) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N} = 0.0\text{‰}$ ) are used as the standards for carbon and nitrogen, respectively. Internal standards (L-histidine, glycine, D-glutamic and acetanilide) are used to check the precision of isotopic compositions. In general, the analytical precisions for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are better than 0.2‰.

#### *Statistical analysis*

All statistical analyses were conducted using R software (version 3.5.1). Analysis of variance (ANOVA) was performed to test the effects of climate zone, land use, soil depth and soil aggregate size on the mass proportion of soil aggregate size fractions, C and N concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Comparisons among different climate zones were tested using Tukey's honest significant difference at  $p < 0.05$ . All data were subjected to the assumptions of normality and homogeneity of variance before conducting the ANOVA procedure. Regression analysis is performed to fit the relationships between climatic factors (i.e., MAT and MAP) and the changes



in soil aggregate C and N and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  calculated from NF minus PF, and among soil aggregate C and N and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in natural forests and plantations.

## RESULTS

### *Soil aggregate mass proportion and mean weight diameter*

Climate zones have significant influences on the mass proportion of each aggregate fraction (Table 2; Fig. 1). However, forest type and soil depth do not differ in the mass proportion of each fraction (Table 2), except that the averaged mass proportion of 0.053–0.25 mm microaggregate across five sampling sites and two depths is significantly higher in PF (21.4%) than in NF (17.4%). There is a forest type  $\times$  climate zone interaction on the mass proportion of 0.5–1 mm aggregate size (Table 2,  $p=0.03$ ). Mean weight diameters (MWD) are not different between NF and PF but vary among climate zones with a significantly lower MWD in the south subtropical (SST) zone (Table 2, Fig. S2).

### *Soil aggregate C and N concentration*

In general, soil C and N concentrations ( $\text{g kg}^{-1}$  aggregate) are almost uniformly distributed across all 7 aggregate fractions (Fig. 2a, b), except for a lower C concentration in the  $<0.053$  mm fraction than in the 1–2 mm fraction ( $p=0.03$ ). The impacts of LCC on soil aggregate C and N concentrations vary among climate zones (Table 3). The averaged C concentration over aggregate sizes and depth is higher in PF than NF in the cold temperate (CT) zone ( $p<0.001$ ), but there are lower aggregate C concentrations in PF in both mid-temperate (MT) ( $p=0.02$ ) and tropical (TRO) zones ( $p=0.03$ ) compared to NF (Fig. 3a). The soil aggregate N concentration decreases from NF to PF in the MT ( $p=0.02$ ), SST ( $p<0.001$ ) and TRO ( $p=0.03$ ) zones, but PF has a higher aggregate N concentration than NF in the CT zone (Fig. 3b).

Climate has a strong influence on soil aggregate C and N concentrations (Table 3) but differs between NF and PF. In plantations, soil aggregate C decreases from the CT zone to the mid-subtropical (MST) zone but then slightly increases in the SST and TRO zones. However, NF has the highest aggregate C in the MT zone (Fig. 3a). The soil aggregate N concentrations in both NF and PF all increase from the CT to MT zones but then decline in the MST, SST and TRO zones (Fig. 3b). Furthermore, the changes in soil aggregate C concentration ( $\Delta C$ ) and N concentration ( $\Delta N$ ) calculated by subtracting PF from NF show nonlinear relationships with mean annual temperature (MAT) and precipitation (MAP) (Fig. 4a-d). In addition, although soil aggregate C and N all decrease with increasing soil depth in both NF and PF (Table 3; Fig. 2a, b), the declines of aggregate C and N concentrations are larger in NF (14.38 C g kg<sup>-1</sup> and 0.85 N g kg<sup>-1</sup>) than PF (9.67 C g kg<sup>-1</sup> and 0.48 N g kg<sup>-1</sup>).

#### *Soil aggregate $\delta^{13}C$ and $\delta^{15}N$ content*

Soil  $\delta^{13}C$  does not differ across all aggregate fractions (Table 3; Fig. 2c), but soil  $\delta^{15}N$  is significantly higher in the <0.053 mm aggregate size than in the other fractions (Fig. 2d). Soil aggregate  $\delta^{13}C$  and  $\delta^{15}N$  are significantly affected by forest type, soil depth and climate zone, and there are significant interactions between forest type and site location, and between forest type and soil depth (Table 3). The mean values of  $\delta^{13}C$  over aggregate sizes and soil depth are enriched after natural forests are converted to plantations, except in the CT zone (Fig. 3c). Similarly, PF has higher values of soil aggregate  $\delta^{15}N$  than NF in the CT, MT and TRO zones, but there are no differences in soil aggregate  $\delta^{15}N$  between NF and PF in either the MST or SST zones (Fig. 3d).

Mean values of soil  $\delta^{13}C$  over seven aggregate fractions in NF and PF show similar trends along climate zones and increase from the CT zone to MST zones but then decline to the TRO

zone (Fig. 3c). Furthermore, soil aggregate  $\Delta\delta^{13}\text{C}$  has negative relationships with increasing MAT and MAP (Fig. 4 e, f). However, the trend of soil aggregate  $\delta^{15}\text{N}$  along climate zones differs between NF and PF. The soil aggregate  $\delta^{15}\text{N}$  in the NF increases from the CT to MST zones and then declines to the TRO zone (Fig. 3d). For PF, however, the soil aggregate  $\delta^{15}\text{N}$  values in the CT, MT, MST and TRO zones are not different but are significantly higher than those in the SST zone. The soil aggregate  $\Delta\delta^{15}\text{N}$  at both soil depths shows nonlinear relationships with MAP (Fig. 4h), but there is no clear relationship between MAT and the different soil aggregate  $\delta^{15}\text{N}$  at 10-20 cm (Fig. 4g). Moreover, there are forest type  $\times$  soil depth interactions on both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in soil aggregates (Table 3). Soil  $\delta^{13}\text{C}$  are significantly enriched along the soil depth in NF ( $p<0.001$ ), but there is no difference in soil  $\delta^{13}\text{C}$  between the two soil layers in PF ( $p=0.08$ , Fig. 2c). Compared to the surface layer (0-10 cm), soil aggregate  $\delta^{15}\text{N}$  is enriched by 28% and 14% in NF and PF, respectively, at the 10-20 cm depths (Fig. 2d).

#### *Relationships among soil C and N concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$*

The statistical analyses show a positive correlation between soil C and N concentration in both NF ( $r=0.91$ ,  $p<0.001$ ) and PF ( $r=0.78$ ,  $p<0.001$ ) (Fig. 5a). Moreover, there is a significantly positive correlation between soil aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in NF ( $r=0.71$ ,  $p<0.001$ ) but not in PF ( $r=0.19$ ,  $p=0.12$ ) (Fig. 5b). In NF, soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are negatively correlated with aggregate C and N concentrations, respectively (Fig. 5c, d). However, soil  $\delta^{15}\text{N}$  is not correlated with soil N concentration (Fig. 4d), and there is a weak negative correlation between soil aggregate  $\delta^{13}\text{C}$  and C concentration in PF (Fig. 5c).

## DISCUSSION

### *Soil aggregate size distribution, and aggregate C and N concentrations as affected by LCC*

In this study, we found an increase in microaggregates (0.053-0.25 mm) and silt + clay (<0.053 mm) but a decreasing mass proportion of macroaggregates (> 0.25 mm) in the intensively managed plantations relative to adjacent natural forests. These findings supported our first hypothesis that LCC from natural forests to plantations could decrease the stability of soil aggregates, and agreed with a previous study by Yang et al. (2009) who also found that converting old-growth native forests to *Castanopsis Kawakamii* and *Cunninghamia lanceolata* Lamb. plantations decreased the mass proportion of macroaggregates in southern China. Compared to natural forests, intensively managed plantations and croplands are usually exposed to various anthropogenic disturbances, e.g., site preparation, tree planting and furrow fertilization, which can disintegrate large aggregates into smaller aggregates and resulting in an increase in the proportion of small aggregates in soils (Abrishamkesh et al. 2011; Richards et al. 2009). Consequently, the excluded disturbances related to land use and cover change have been widely suggested to be prone to physical restoration of aggregate stability (Blanco-Canqui and Lal 2004; Pohl et al. 2012; Six et al. 1999). In addition, soil aggregation is mediated by complex factors, such as abiotic (clay minerals, carbonates, exchangeable cations), biotic (plant roots, soil fauna and microorganisms), and environmental (soil temperature and moisture) factors (Bronick and Lal 2005; Pohl et al. 2012; Tisdall and Oades 1982). In the present study, the changes in soil aggregate size distribution might be related to the reduction in SOC in plantations (Chen et al. 2004; Lin et al. 2018), and furthermore, the higher plant diversity, vegetation cover and root density in natural forests were likely beneficial for forming and stabilizing macroaggregates in soils (Le Bissonnais et al. 2018; Pohl et al. 2012).

The changes in C and N in soil aggregates can usually be used as early indicators of soil C and N changes (Leifeld and Kögel-Knabner 2005) and provide us with the mechanisms of soil C and N pool stabilization induced by LCC (Gelaw et al. 2015; Six et al., 2000; Zhu et al. 2017). We observed that the averaged soil C concentration across aggregate fractions declined significantly in the plantations in the MT and TRO zones compared with the natural forests. Similarly, several previous studies also observed a net loss of soil C and N after land conversion from natural forest to plantations (He et al. 2008; Lyu et al. 2017; Richards et al. 2009). This result is suggested to be related to the reduction of C inputs into soils and the increasing organic matter decomposition caused by the disturbances of site preparation and burning before plantation establishment (Chen et al. 2016; Deng et al. 2016; Goma-Tchimbakala 2009). Moreover, soil aggregation is an important mechanism for the stabilization of soil organic matter, and in less disturbed systems, the formation of macroaggregates can stabilize and protect soil organic C (Gelaw et al. 2015; Li et al. 2005; Six et al., 2000; Zhu et al. 2017). However, the soil aggregate C concentration was higher in PF than in NF in the cold temperate zone, and no differences in the soil aggregate C concentration were observed in the MST and SST zones after LCC. Our results indicated that land conversion from NF to PF had a strong influence on soil C and N dynamics, but the impacts varied among climate zones with positive, negative, or even no influence. Such large spatial-scale site-to-site variations in SOC were also observed by Lewis et al. (2016), who found that SOC was significantly higher in the pine plantation at two of the eight sites, was higher in the native vegetation at two sites and did not differ at four sites. They explained that the variance among sites was due to the different plantation species, the different environmental drivers (e.g., rainfall) and the higher degree of variability between sites (e.g.,

different plantation age, site preparation and management methods, soil types, and native vegetation types).

It is not surprising to us that soil aggregate C and N concentrations decreased with soil depth in both NF and PF. However, there were forest type  $\times$  soil depth interactions on soil aggregate C and N concentrations, and the vertical differences in soil aggregate C and N between the two layers decreased in plantations compared with natural forests. This result indicated that land conversion altered the vertical distribution of soil aggregate C and N. Compared to subsoil, topsoil C and N are usually more sensitive to land cover and land use change (Gaudinski et al. 2000; Ngaba et al. 2019) because of enhanced topsoil erosion after natural forest conversion to plantations (Guillaume et al. 2015). In addition, the smaller differences in aggregate C and N concentrations along soil depth in plantations could be related to intensive management practices, such as digging, scarification, or stump lifting, resulting in mixing topsoil into the subsurface layer (Mobley et al. 2015).

#### *Soil aggregate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as affected by LCC*

Stable isotopes of  $^{13}\text{C}$  and  $^{15}\text{N}$  in soil aggregate fractions are an excellent integrative and potentially powerful tool for understanding the mechanisms of soil C and N changes after land cover change (John et al. 2005; Llorente et al. 2010). According to the concept of aggregate hierarchy, microaggregates are bound together into macroaggregates by transient binding agents and temporary binding agents (Tisdall and Oades 1982; Six et al. 2000). The consequences of this aggregate hierarchy are an increase in C concentration but a depletion of  $\delta^{13}\text{C}$  with increasing aggregate-size fraction (John et al. 2005). However, our data did not support the aggregate hierarchy model. Although a lower C in the  $<0.053$  mm fraction was observed in the present study, we did not observe significant differences in soil  $\delta^{13}\text{C}$  with increasing aggregate-

size fraction. Similar to the results in our study, John et al. (2005) also found that soil  $\delta^{13}\text{C}$  was uniformly distributed across soil aggregate fractions in forest soil. Some recent studies suggested that the formation of fine mineral-stabilized organic matter derived recently from litter plays an important role in SOM formation and stability (Cotrufo et al. 2015), which might explain the lower values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  because of the new plant C inputs in microaggregate fractions.

The mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  across soil aggregates were generally higher in the plantations than in natural forests. This result is consistent with our second hypothesis and with another previous study by Zeller et al. (2007) that found that soil under Norway spruce displayed a lower  $\delta^{13}\text{C}$  value than soil under a natural stand. In our study, there were significantly negative relationships of soil  $\delta^{13}\text{C}$  and C concentrations, and  $\delta^{15}\text{N}$  and N concentrations in NF, which was supported by other previous studies (Evans and Belnap 1999; Garten et al. 2000). This suggests that the enriched soil  $\delta^{13}\text{C}$  values in the plantations might be related to increased SOM decomposition due to site disturbances (Chen et al. 2016; Deng et al. 2016; Natelhoffer and Fry 1998). Moreover, the rate of litter input and the litter's isotope abundance can also influence the isotopic abundances of soil C and N (Natehoffer and Fry 1988). Usually, the litter decomposition rate is slower in plantations due to the lower litter quality compared with natural forests (Li et al. 2005), which results in fewer inputs of depleted litter C in plantations. One of our previous studies observed that the isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  in forest floor litter in NF were generally lower than those in PF (Ngaba et al. 2019) and which furthermore led to the higher values of soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in plantations.

In addition, our results showed that there are increasing trends of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with soil depth in natural forests. The explanations of soil  $\delta^{13}\text{C}$  along soil depth are generally grouped into three categories: mixing, selective decomposition and preservation of compounds enriched in

$\delta^{13}\text{C}$ , and kinetic fractionation during humification (Bird et al. 2002a; Diochon and Kellman 2008; Garten et al. 2000). However, these depth trends of soil  $\delta^{13}\text{C}$  were not observed in plantations, and the magnitude of the  $^{15}\text{N}$  shift from the surface to depth was less in PF than in NF. The declined gradients of soil aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in plantations might be related to the mechanisms of topsoil erosion, mechanical mixing between soil layers, and increasing C mineralization in topsoil with disturbances compared to intact natural forests (Diochon and Kellman 2008; Eshetu et al. 2000; Guillaume et al. 2015).

*Changes in soil aggregate distribution, C and N concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among climate zones*

For our third and last hypothesis, we found that climate zone had significant impacts on the mass proportion of each soil aggregate fraction, the averaged C and N concentrations, and isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  across aggregates. Though the trends of soil aggregate C and N concentrations, and aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  along the climate zones were slightly different between NF and PF, we found that the soil C and N concentrations generally increased from the cold temperate zone to the mid-temperate zone, declined to the subtropical zone, and then again exhibited a slight increase in the tropical zone. In the present study, the lower C and N concentrations in NF in the cold temperate zone compared to the mid-temperate zone might be related to the short-term restoration of the natural forests at the site where one serious fire disaster occurred in 1987. Usually, climate factors can influence soil C and N dynamics through biotic processes of forest productivity and decomposition of SOM (Post et al. 1985). In our study, however, the pattern of forest biomass productivity cannot explain the trends of soil C and N along the north-south transect in eastern China, considering that net ecosystem productivity decreased with increasing latitude (Yu et al. 2013; Wen and He 2016). Many previous studies



have reported that the decomposition of SOC increases with increasing MAT, thereby causing soil C and N loss and enrichment of soil isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  (Bird et al. 2002b; Garten et al. 2000; Homann et al. 2007; Högberg 1997). However, the relationships of soil C and N turnover with MAP are more complex and usually show an inflection point (Jobbágy and Jackson 2000; Wang et al. 2018). The decomposition rate of soil organic matter can decline when MAP is very high and anaerobic conditions dominate (Wang et al. 2018). In addition, a decline in water use efficiency at wetter sites can promote the depletion of heavy C and N isotopes in soils (Austin and Vitousek 1998; Peri et al. 2012).

It is interesting that our data show the changes in soil C and N concentrations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  induced by LCC are related to MAT and MAP. In the plantations soil C becomes more enriched in  $^{13}\text{C}$  with increased MAT and MAP, suggesting greater decomposition in the plantations compared to the natural forests with increased MAT and MAP (Fig. 4e, f). And yet, soil C pools converged to the same value between the natural forests and plantations at the highest MAT and MAP (Fig. 4a, b), suggesting that C inputs in plantations kept up with higher decomposition rates at these sites. Although there are many previous studies reporting positive, negative or no impacts of land cover change from natural forests to plantations on soil C and N pools in various climate zones (Chen et al. 2004; Guillaume et al. 2015; Li et al. 2014; Lin et al. 2018; Lewis et al. 2016; Yang et al. 2019), there is still little research comparing the different effects of LCC along climatic factors. Liao et al. (2012) reported larger declines in soil C in the temperate zone than in the tropical zone after land cover change from natural forests to plantations. In their review on the changes in SOC induced by LCC worldwide, Guo et al. (2002) found that the release of soil C was different among areas with precipitation <1000 mm, 1000-1500 mm and >1500 mm. Several mechanisms might explain the varied impacts of LCC on soil

C and N. First, the soil temperature sensitivity ( $Q_{10}$ ) of soil C and N was different between natural forests and plantations (Yang et al. 2017), thereby resulting in the varied response of soil C and N between natural forests and plantations with increasing MAT. Second, soil C accumulation and  $^{13}\text{C}$  abundance are affected by the quantity and quality of plant C sources (Nadelhoffer and Fry 1988; Hatton et al. 2015). The differential rates of productivity and vegetation composition between natural forests and plantations varied among climate zones (Brown et al. 2020; Cai et al. 2019), which might also contribute to the differences in soil C and N changes induced by LCC among climate zones with increasing MAT and MAP.

## CONCLUSION

Our results indicated that the impacts of LCC on soil aggregate C and N, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  varied among climate zones with increasing, decreasing or no influence on soil aggregate C and N because of the site-to-site variances of the linked differences in climatic conditions, soil texture and dominant vegetation. Moreover, our results implied that the changes in soil C and N concentrations, and isotopic abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  are related to climatic factors and that at large spatial scales, natural environmental factors (e.g., MAT and MAP) had a greater influence on soil C and N dynamics compared to the anthropogenic disturbance of natural forests converting to managed plantations. Considering the limits of our study, in the future, more studies are needed to rule out the relative contribution of climatic factors on soil C and N dynamics compared to other factors, such as vegetation production and composition, soil texture, and plantation age.

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#### AUTHOR CONTRIBUTIONS

M.N. elaborated in sampling, and obtained and analyzed data; M.N. and R.B. wrote the first draft of the paper. Y.H. designed study concept. R.B. and Y.H. critically revised the final paper.

#### ADDITIONAL INFORMATION

Competing Interests: The authors declare no competing interests.

Table 1 Characteristics of the sampled natural forests and paired plantations at five sites along a north-south transect in eastern China

	Mohe (MH)	Qingyuan (QY)	Huitong (HT)	Dinghushan (DH)	Jianfengling (JF)
Location	N 52.92, E 122.79	N 41.85, E 124.93	N 26.85, E 109.60	N 23.17, E 112.52	N 18.44, E 108.01
Elevation (m)	448	597	427	275	800
Climate zone	Cold temperate (CT)	Mid-temperate (MT)	Mid-subtropical (MST)	South subtropical (SST)	Marginal tropical (TRO)
MAT (°C)	-4.14	5.91	17.17	21.08	19.80
MAP (mm)	436	794	1256	1955	2499
Soil classification	Gelic Histosol	Haplic Alisol	Haplic Acrisol	Humic Acrisol	Humic Acrisol
Soil texture	Loam	Loam	Silt clay	Clay loam	Sandy clay
Natural forest type	CBM	DBL	EBL	MEB	TMF
Natural forest dominant species	<i>Larix gmelinii</i> , <i>Betula platyphylla</i>	<i>Quercus mongolica</i> , <i>Acer mono</i> , <i>Carpinus cordata</i>	<i>Castanopsis hystrix</i> , <i>Cyclobalanopsis glauca</i> , <i>Machilus pauhoi</i>	<i>Castanopsis chinensis</i> , <i>Cryptocarya chinensis</i> , <i>Schima superba</i>	<i>Livistona saribus</i> , <i>Gironniera subaequalis</i> , <i>Cryptocarya chinensis</i>
Plantation forest type	<i>Larix gmelinii</i>	<i>Pinus koraiensis</i> .	<i>Cunninghamia lanceolata</i>	<i>Pinus massoniana</i>	<i>Pinus massoniana</i>
Plantation age	29	38	33	30	30

CBM, coniferous broad-leaf mixed forest; DBL, deciduous broad-leaved forest; EBL, evergreen broad-leaved forest; MEB, monsoon evergreen broad-leaved forest; TMF, tropical montane rain forest; MAT, mean annual temperature; MAP, Mean annual precipitation.

Table 2 The ANOVA results of forest type, climate zone and soil depth on the mass proportion of each soil aggregate fraction and the mean weight diameter.

	>5 mm	2-5 mm	1-2 mm	0.5-1 mm	0.25- 0.5 mm	0.053-0.25 mm	<0.053 mm	MWD
Forest type	0.89	0.73	0.15	0.07	0.83	*	0.08	0.57
Climate zone	***	***	***	***	***	***	*	***
Soil depth	0.64	0.64	0.55	0.05	0.79	0.08	0.10	0.35
Type × Climate	0.55	0.17	0.33	*	0.59	0.55	0.09	0.58
Type × Depth	0.11	0.86	0.48	0.95	0.33	0.80	0.63	0.23
Climate × Depth	0.37	0.42	0.17	0.98	0.09	0.06	0.10	0.92
Type × Climate × Depth	0.18	0.72	0.44	0.30	0.23	0.52	0.04	0.33

\*\*\* indicates a significant difference at the  $p<0.001$  level (2-tailed); \* indicates a significant difference at the  $p<0.05$  level (2-tailed).

MWD, mean weight diameter

Table 3 The ANOVA results of forest type, climate zone, soil depth and aggregate size on soil aggregate C and N concentration (g kg<sup>-1</sup> aggregate), and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$

Variance	Df	C concentration		N concentration		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		F value	Pr (>F)	F value	Pr (>F)	F value	Pr (>F)	F value	Pr (>F)
Forest type	1	0.27	0.60	2.40	0.12	27.16	***	43.55	***
Climate zone	4	42.94	***	48.99	***	63.52	***	14.15	***
Soil depth	1	112.64	***	98.94	***	26.17	***	88.26	***
Aggregate size	6	3.26	**	1.22	0.30	1.63	0.14	10.99	***
Type $\times$ Climate	4	11.35	***	12.28	***	15.67	***	10.29	***
Type $\times$ Depth	1	5.76	*	5.16	*	4.33	*	4.65	*
Climate $\times$ Depth	4	1.69	0.15	2.59	*	0.99	0.41	3.67	**
Type $\times$ Size	6	0.52	0.80	0.87	0.52	0.20	0.98	0.27	0.95
Climate $\times$ Size	24	1.29	0.17	1.75	*	0.40	0.99	0.93	0.56
Depth $\times$ Size	6	1.09	0.37	1.04	0.40	0.58	0.74	1.03	0.41

\*\*\* indicates a significant difference at the  $p<0.001$  level (2-tailed); \*\* indicates a significant difference at the  $p<0.01$  level (2-tailed);

\*indicates a significant difference at the  $p<0.05$  level (2-tailed).

## Figure Captions

Figure 1 Mass proportion of each soil aggregate fraction in natural forests and plantations among five climate zones along a north-south transect in eastern China. NF, natural forest; PF, plantation forest. CT, cold temperate zone; MT, mid-temperate zone; MST, mid-subtropical zone; SST, south subtropical zone; TRO, marginal tropical zone.

Figure 2 Distribution of soil (a) C and (b) N concentrations ( $\text{g kg}^{-1}$  aggregate) and (c)  $\delta^{13}\text{C}$  and (d)  $\delta^{15}\text{N}$  among aggregate size fractions under natural forests and plantations. The different lowercase letters indicate significant differences among aggregate size fractions. NF, natural forest; PF, plantation forest.

Figure 3 Impacts of land cover change from natural forests to plantations on soil aggregate (a) C and (b) N concentrations and (c)  $\delta^{13}\text{C}$  and (d)  $\delta^{15}\text{N}$  among the five climate zones. The different lowercase letters on bars indicate significant differences among climate zones under natural forests and the different capital letters on bars indicate significant differences among climate zones under plantations. \*\*\*, \*\* and \* indicate significant differences between natural forest and plantation in each climate zone at the level of  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$ , and ns indicates a non-significant difference, respectively. NF, natural forest; PF, plantation forest. CT, cold temperate zone; MT, mid-temperate zone; MST, mid-subtropical zone; SST, south subtropical zone; TRO, marginal tropical zone.

Figure 4 The regression of the changes in soil aggregate C and N concentrations and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  calculated from natural forest subtracting plantation with mean annual temperature (MAT) and mean annual precipitation (MAP).



Figure 5 Relationships of (a) soil aggregate C and N concentrations, (b) soil aggregate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , (c) soil aggregate C concentration and  $\delta^{13}\text{C}$ , and (d) soil aggregate N concentration and  $\delta^{15}\text{N}$ . Each point presents the mean value of three replication plots (n=3). NF, natural forests; PF, plantations. The solid line indicates the regression of natural forests, and the dotted line indicates the regression of plantations.

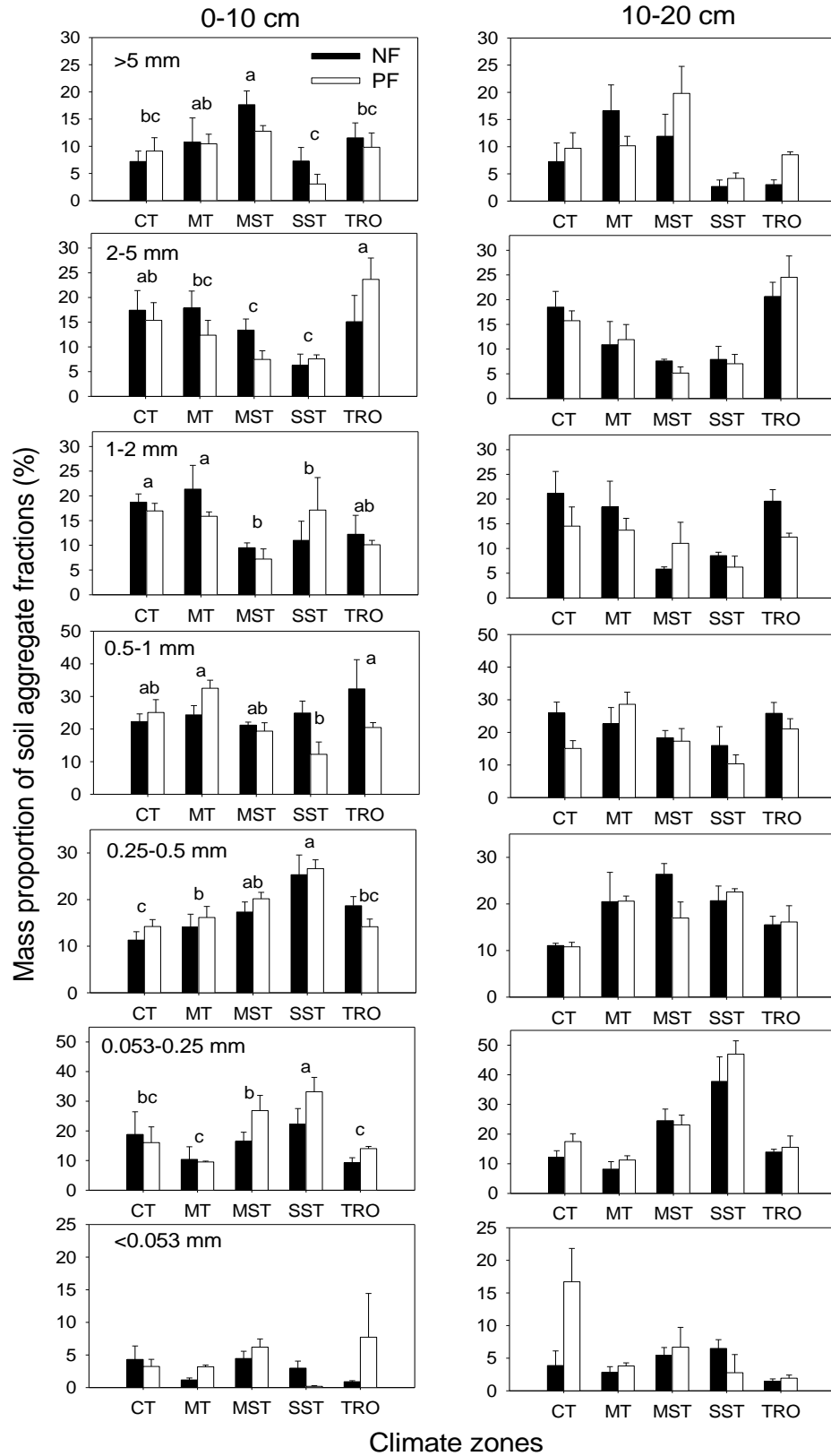


Figure 1

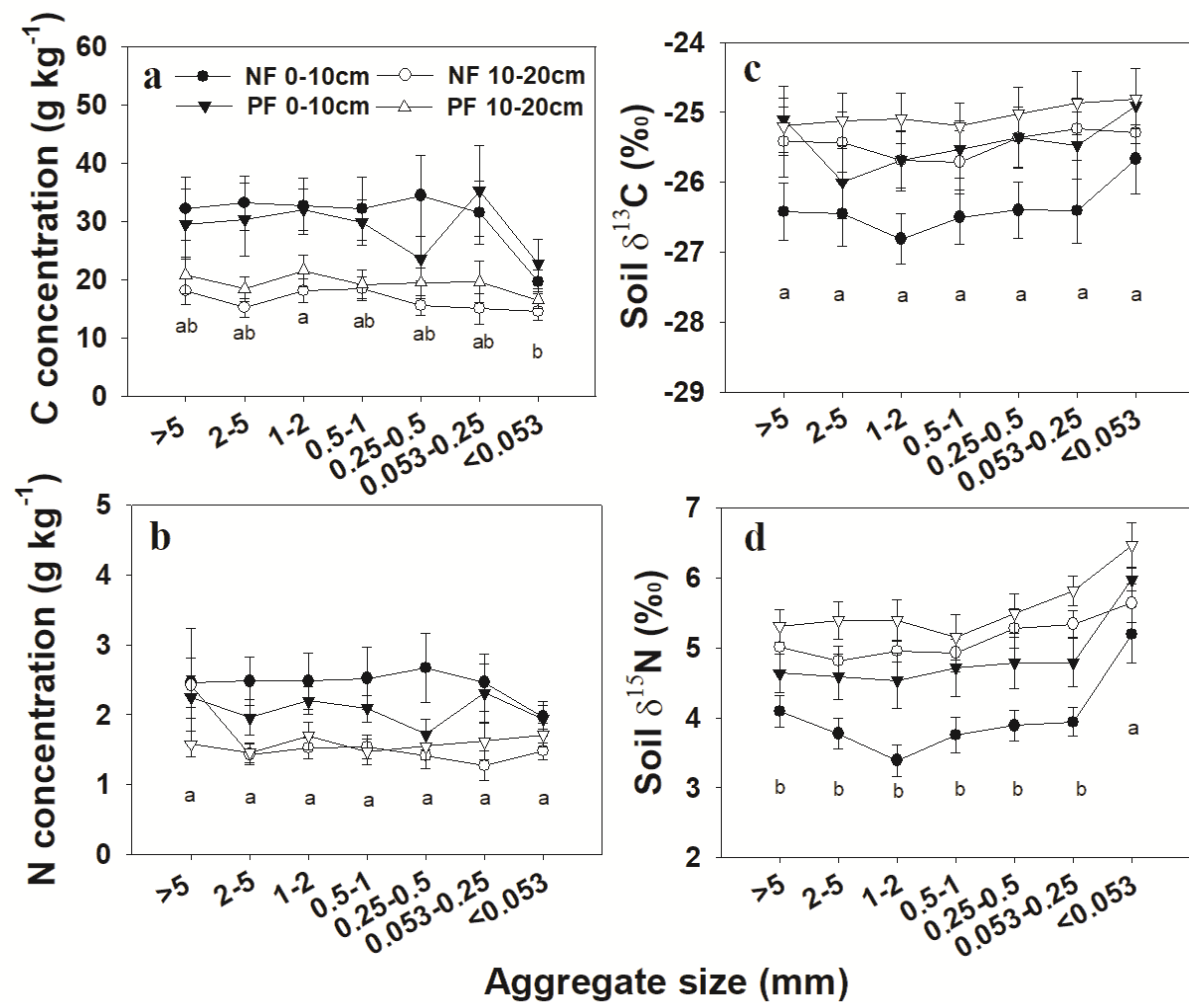


Figure 2

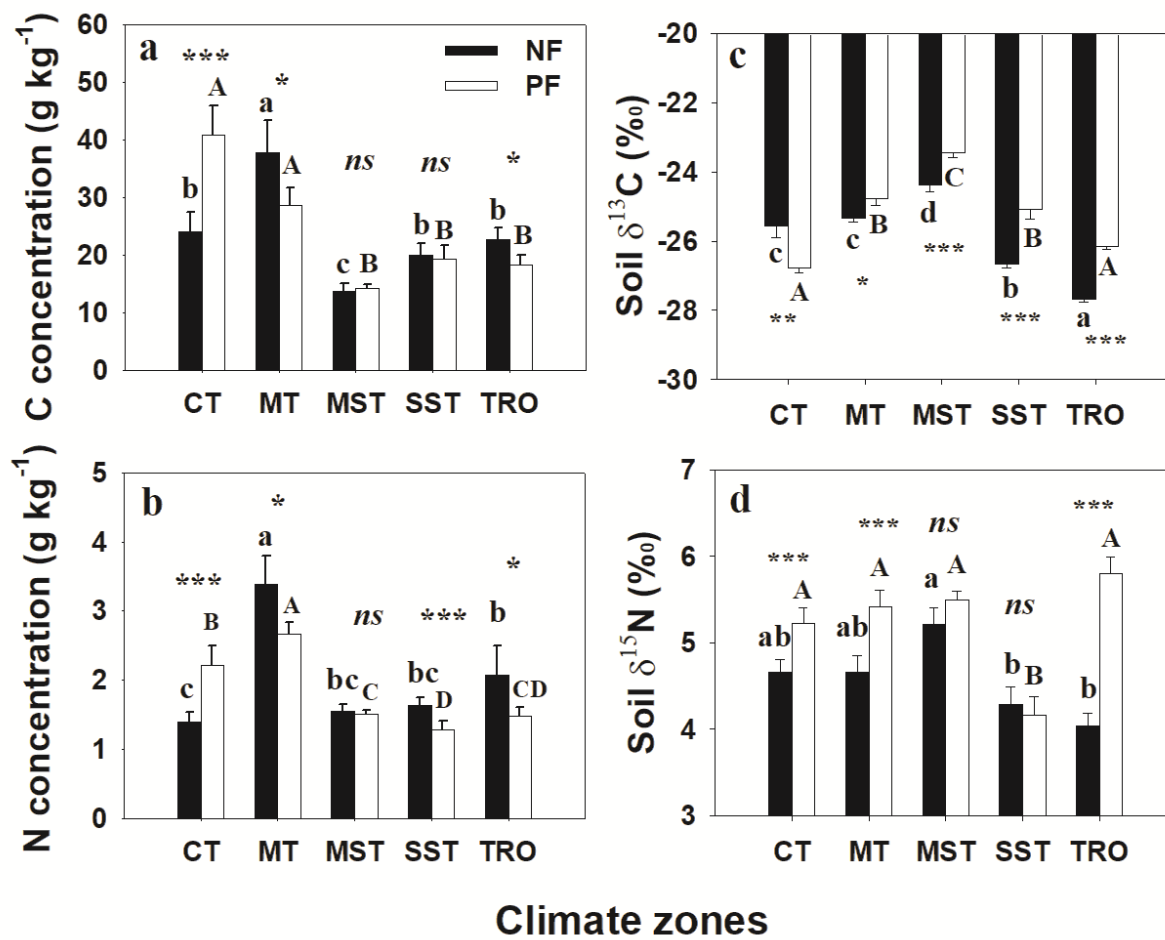


Fig. 3

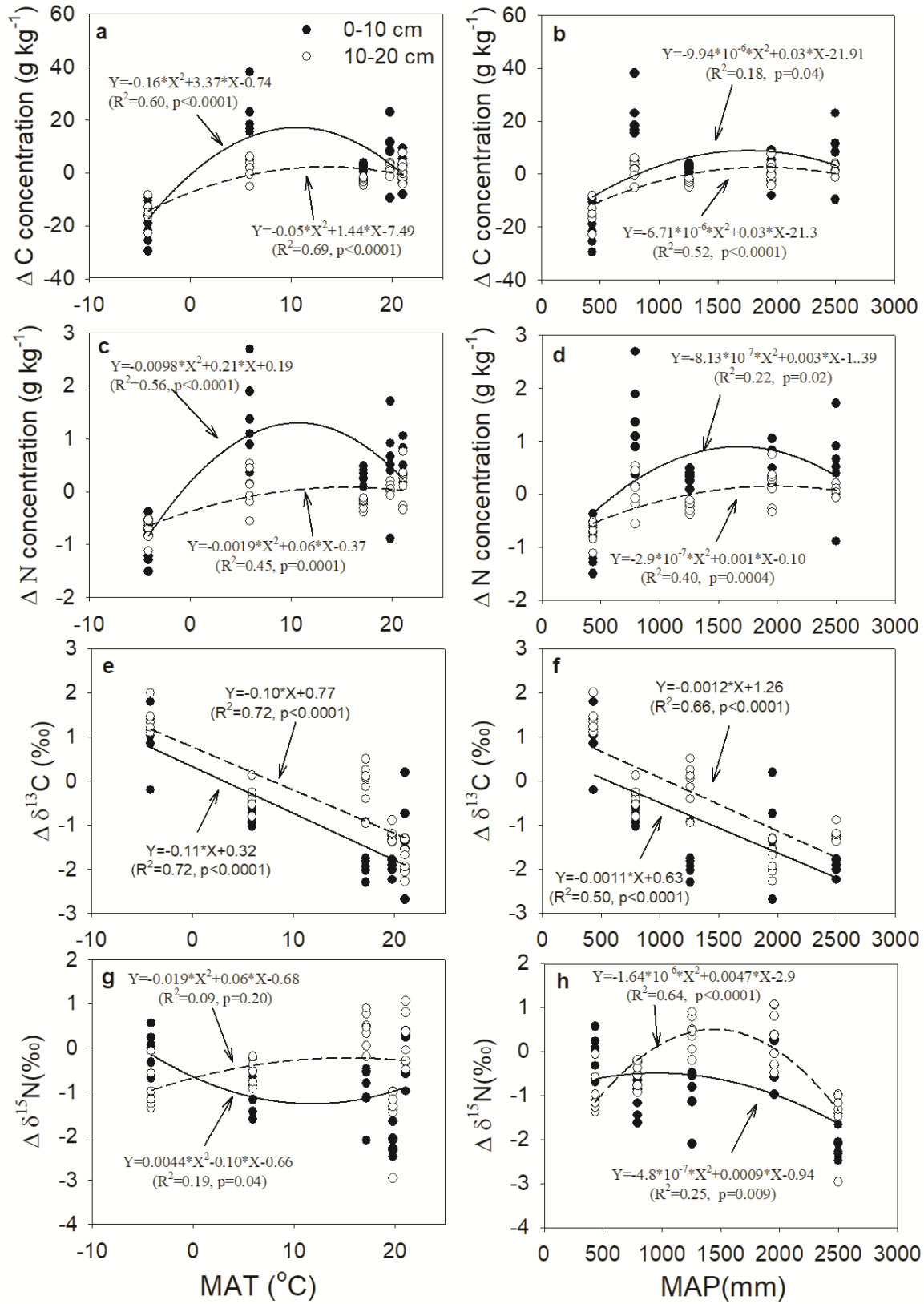


Fig. 4

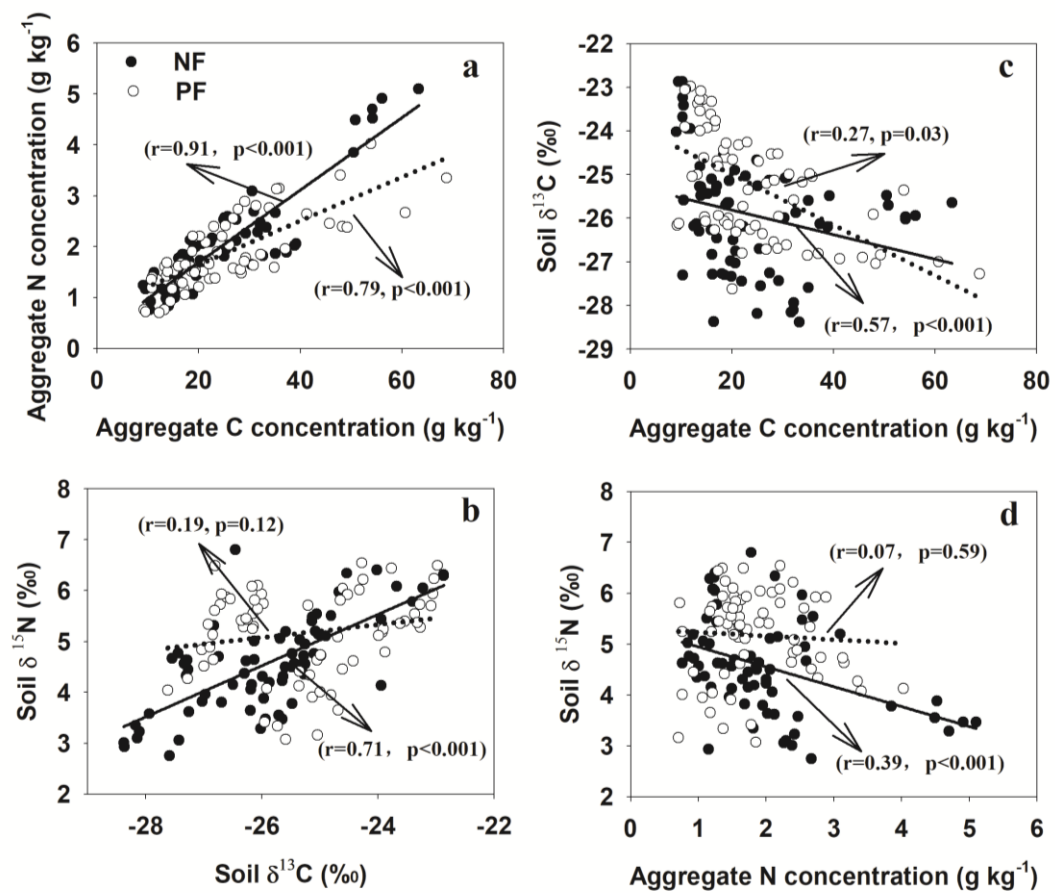


Fig. 5

## **Supplementary Figure Captions**

Figure S1 The locations of the studied sites in five climate zones along a north-south transect in eastern China. Mohe (MH), Qingyuan (QY), Huitong (HT), Dinghushan (DH), Jianfengling (JF).

Figure S2 Mean weight diameter of soil aggregate at (a) 0–10 cm and (b) 10–20 cm layers in natural forests and plantations in five climate zones along a north-south transect of eastern China. The different lowercase letters on bars indicate significant differences in the climate zones. NF, natural forest; PF, plantation forest. CT, cold temperate zone; MT, mid-temperate zone; MST, mid-subtropical zone; SST, south subtropical zone; TRO, marginal tropical zone.

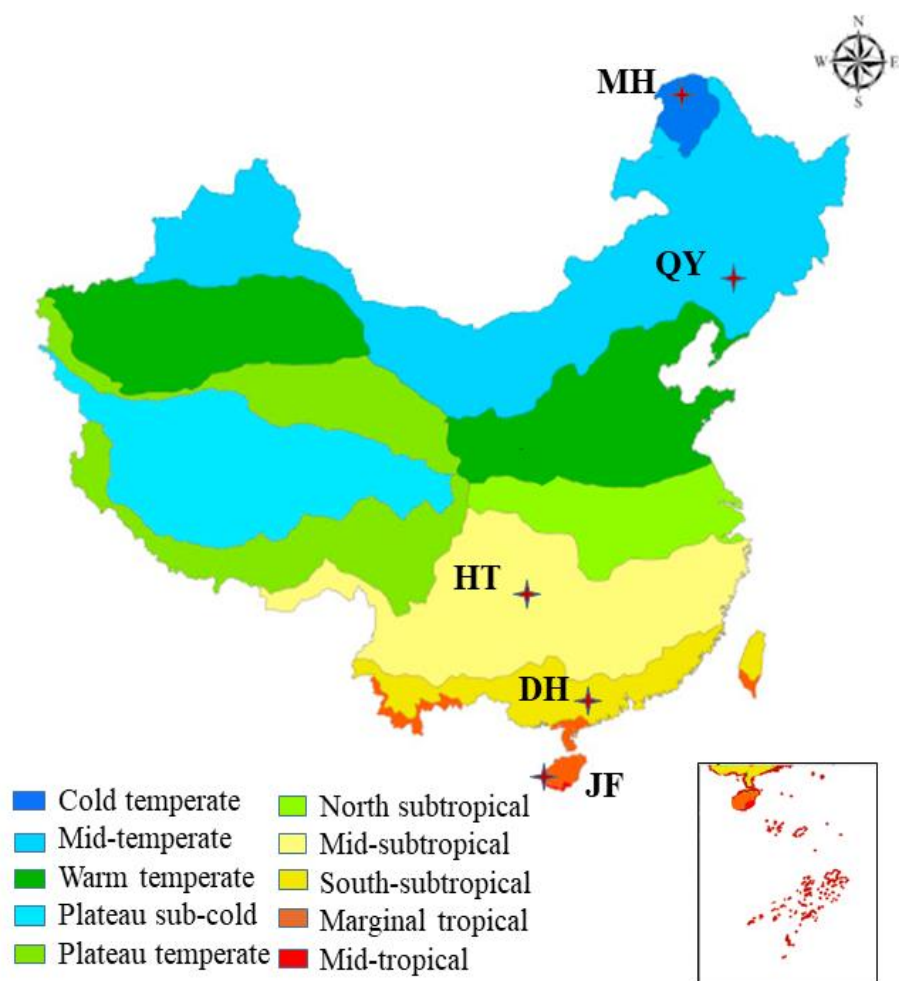


Figure S1



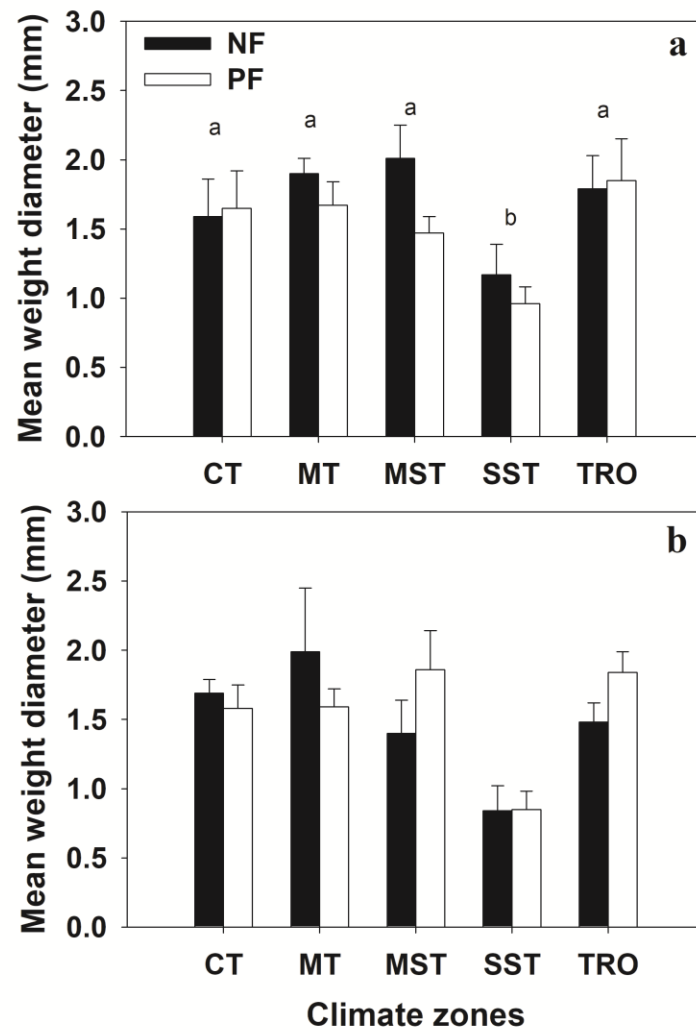


Figure S2