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Effects of precipitation on soil organic carbon fractions in three subtropical forests in southern China

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Abstract

Aims
The aim of this study was to investigate the effects of precipitation changes on soil organic carbon (SOC) fractions in subtropical forests where the precipitation pattern has been altered for decades.

Methods
We conducted field manipulations of precipitation, including ambient precipitation as a control (CK), double precipitation (DP) and no precipitation (NP), for 3 years in three forests with different stand ages (broadleaf forest [BF], mixed forest [MF] and pine forest [PF]) in subtropical China. At the end of the experiment, soil samples were collected to assay SOC content, readily oxidizable organic carbon (ROC) and non-readily oxidizable organic carbon (NROC), as well as soil microbial biomass carbon (MBC), pH and total nitrogen content. Samples from the forest floors were also collected to analyze carbon (C) functional groups (i.e. alkyl C, aromatic C, O-alkyl C and carbonyl C). Furthermore, fine root biomass was measured periodically throughout the experiment.

Important Findings
Among the forests, ROC content did not exhibit any notable differences, while NROC content increased significantly with the stand age. This finding implied that the SOC accumulation observed in these forests resulted from the accumulation of NROC in the soil, a mechanism for SOC accumulation in the mature forests of southern China. Moreover, NP treatment led to significant reductions in both ROC and NROC content and therefore reduced the total SOC content in all of the studied forests. Such decreases may be due to the lower plant-derived C inputs (C quantity) and to the changes in SOC components (C quality) indicated by C functional groups analyses under NP treatment. DP treatment in all the forests also tended to decrease the SOC content, although the decreases were not statistically significant with the exception of SOC and ROC content in PF. This finding indicated that soils in MF and in BF may be more resistant to precipitation increases, possibly due to less water limitations under natural conditions in the two forests. Our results therefore highlight the different responses of SOC and its fractions to precipitation changes among the forests and suggest that further studies are needed to improve our understanding of SOC dynamics in such an important C sink region.

Keywords: rainfall pattern, labile organic carbon, soil organic carbon, stable organic carbon, forest floor

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INTRODUCTION

Changes in the intensity and patterns of precipitation are occurring around the world (IPCC 2013; Knapp *et al.* 2008). In China, floods and droughts have been observed to be more frequent during the past 40 years (Piao *et al.* 2010), including in southern China (Zhou *et al.* 2011), where the forest soil has been identified as a significant C sink (Piao *et al.* 2009;

Zhou *et al.* 2006). Changes in precipitation patterns could consequently modify the magnitude of the C sink in southern China, or even worse, transform the C sink into a C source, as precipitation has been commonly considered as a modifier of soil C cycling (Aanderud *et al.* 2010; Goebel *et al.* 2011; Young and Ritz 2000) by changing soil moisture and underground hydrological processes (Heisler and Weltzin 2006). However, the potential effects of changes in precipitation patterns on soil organic carbon (SOC), especially on its different fractions, have not yet been well assessed in forest ecosystems.

Because precipitation influences soil moisture and hydrological processes such as surface runoff and ground water infiltration (Heisler and Weltzin 2006), which are important controlling factors in SOC cycling, changes in precipitation patterns have great potential to influence SOC content and its dynamics (Aanderud *et al.* 2010). For example, Meier and Leuschner (2010) observed that SOC decreased by ~25% more in beech forests with annual precipitation >900 mm year^{-1} than in those with precipitation <600 mm year^{-1}. It has also been reported that on the one hand, soil moisture could affect SOC accumulation by influencing the quantity of plants' C input to soils (Zhou *et al.* 2008), as well as the decomposition rate of those C inputs (O'Brien *et al.* 2010). On the other hand, water availability and its spatial distribution in soil matrix can affect the spatial accessibility and degradability of SOC for decomposers, and then change the process of SOC decomposition (Goebel *et al.* 2011; Young and Ritz 2000). Moreover, water infiltration may transport substrates from the litter layer to mineral soil, consequently changing the fate of organic C (Lee *et al.* 2004). A study of Mediterranean woodland demonstrated that enhanced soil moisture during the summer accelerates C cycling through stimulated annual stem primary production, litter fall, soil respiration and net annual plant-derived C input to the soil (Cotrufo *et al.* 2011), of which all are related to SOC cycling. Little, however, is known about how SOC in subtropical forests responds to precipitation changes compared to the well-studied temperate forests and grasslands (Brando *et al.* 2008).

Subtropical forest ecosystems store large proportions of living terrestrial C and soil C across the world (Brown and Lugo 1982). Most of these forests are and will continue to experience changes in precipitation patterns due to global warming and land use shifts (Knapp *et al.* 2008). In the Dinghushan Natural Reserve in southern China, for example, annual no-rain days have significantly increased while annual light-rain days have decreased significantly since 1980 (Zhou *et al.* 2011). The changed precipitation patterns have led to a decrease in soil moisture of ~10%, but an increase of 143% in surface runoff in the wet season from April to September (Zhou *et al.* 2011). Thus, there is an urgent need to understand how precipitation changes influence soil C dynamics in these subtropical forests.

SOC is a continuum of C-containing fractions with different decomposition rates (Bradford *et al.* 2008; Kögel-Knabner 2002). Different SOC fractions may therefore respond differently to environmental changes (von Lützow *et al.* 2007). For example, soil readily-oxidized carbon (ROC) has relatively higher turnover rate and is more responsive to management practices, whereas soil non-readily-oxidized carbon (NROC) is relatively less responsive (Blair *et al.* 1995). The potentially different responses of SOC fractions make it important to how these fractions respond to environmental changes. Meanwhile, litter is an important source of SOC, and the analysis of litter C functional groups allows us to understand the changes in the organic C composition of forest litter and can therefore provide explanations for the different responses of SOC fractions (ROC and NROC) to manipulated precipitation treatments. The C fractions of litter input could influence the SOC composition significantly (Quideau *et al.* 2000). Therefore, the analysis of C functional groups can shed light on why SOC fractions respond differently to precipitation changes (Kögel-Knabner 2002). Using this assay, Ono *et al.* (2011) showed that different SOC compositions across forests could be attributed to different supply rates of aliphatic and aromatic carbons from the litter to the topsoil in the forests they studied.

For all of the above reasons, we conducted a precipitation manipulation experiment in three subtropical forests in southern China, testing SOC content and its fractions under three precipitation manipulations in the forests. Moreover, fine root biomass and C functional groups on the forest floor were measured to explore the potential reasons for observed patterns of SOC and its fractions under different precipitation treatments. In doing so, we aimed to test the following two assumptions: (i) SOC content and its fractions (ROC and NROC) would vary across the studied forests; and (ii) the SOC fractions would exhibit different responses to precipitation treatments depending on the forest type, e.g. an ersatz drought resulting from the no precipitation (NP) treatment may decrease ROC and NROC, while high soil moisture resulting from double precipitation (DP) treatment may stimulate the accumulation of SOC.

MATERIALS AND METHODS

Site descriptions

The study was carried out in the Dinghushan Biosphere Reserve (112°10′E and 23°10′N), Guangdong Province, South China. The reserve occupies an area of 1200 ha and experiences a typical subtropical humid monsoon climate, with a mean annual temperature and relative air humidity of 21.5°C and 80%, respectively. The mean annual rainfall is 1956 mm with a distinct seasonality, i.e. >80% of precipitation falls in the wet season from April to September and the remaining 20% in the dry season from October to March. The reserve comprises three types of typical forests: an old-growth monsoon evergreen broadleaf forest (BF), a mixed pine and broadleaf forest (MF) and a pine forest (PF) (Deng *et al.* 2012). The BF, at ~200–300 m above sea level, is distributed

in the core area of the reserve and has been undisturbed for >400 years. Major species in the BF include *Cryptocarya chinensis, Machilus chinensis* (Champ. ex Benth.) Hemsl., *Syzygium superba, C. chinensis* (Hance) Hemsl., and *Syzygium rehderianum* Merr. et Perry in the canopy and sub-canopy layers. The MF is distributed between the core area and the periphery of the reserve at an elevation of 200–300 m. It originated from clear-cuts and subsequent pine plantation in the 1930s, and then the gradual invasion of some pioneer broadleaf species. The dominant species in the canopy layer of the MF are *Pinus massoniana, Schima superba* Gardn. et Champ, *Castanopsis chinensis* Hance, and *Craibiodendorn scleranthum* var. *kwangtunense* (S. Y. Hu) Judd. The PF is distributed in the periphery of the reserve at an elevation of ~200 m. It was planted in the 1950s. The dominant species in the PF is *P. massoniana* Lamb. The amount of litter was 981.8, 702.5 and 587.5 g m^{-2} $year^{-1}$ for the BF, MF and PF, respectively (Chen *et al.* 2012b). Soils at all the three forest sites are shallow ultisol overlying sandstone and shale bedrocks. The soil bulk density was 0.901, 1.031 and 1.323 g cm^{-3} in the BF, MF and PF, respectively. Other soil characteristics of the three forests are summarized in Table 1.

Experimental design

Experimental treatments started in December 2006 at the three aforementioned subtropical forests, with three quadrants replicated in each of the studied forests. In each forest, we selected quadrants with a similar slope aspect, slope degree, slope position and plant community to conduct precipitation manipulations to minimize the heterogeneity derived from different environmental factors. In each quadrant, three plots were established to receive ambient precipitation (CK), DP and NP, respectively. Each plot had a dimension of 3 × 3 m^2, and the buffering distance between plots was >1 m. Precipitation was intercepted in the NP plots using clear polyvinyl chloride (PVC) plates as a roof at a height of 1.0–1.5 m above the floor; precipitation was redistributed to the corresponding DP plots homogenously with PVC tubes. Around each NP plot, thick PVC plates were inserted underground to a depth of 15 cm to prevent outside surface runoff from flowing into the plot. Litter falling on the PVC roofs of the NP plots was collected and returned to the plots three times per month. Soil moisture at a depth of 5 cm was randomly measured five times within each plot on a half-month basis throughout the experiment using an MPKit (ICT, Australia).

Field sampling

Soil samples from 0 to 10 cm were collected in August 2010, using a circle soil auger with an inner diameter of 2.5 cm. In each plot, five random soil cores were mixed into one composite sample, resulting in a total of three samples for each treatment area in each forest. Finally, 27 samples were obtained for soil chemical analysis. Fresh soil samples were passed through a 2-mm sieve to remove rocks and plant roots. Sub-samples were air-dried and ground to perform the analyses of SOC, C fractions and general properties measurements.

Forest floor samples were collected from litter layers, fermentation layers and humus layers in the established plots in August 2010. In each plot, three subplots of 20 × 20 cm were selected randomly for sampling. The litter layer was sampled first, followed by fermentation layer and then the humus layer, following their accessibility (Kanerva and Smolander 2007). The fresh litter layer consisted of fresh or slightly decomposed litter from living plants; the fermentation layer consisted of partly decomposed litter, the origins of which were mostly identifiable; and the humus layer consisted of decomposed organic matter whose origins could not be identified (Kanerva and Smolander 2007). Nine samples from the same layer with the same treatment in each forest were homogenously mixed into one composite sample. A total of 27 samples were collected from the forest floor. The samples were dried at 60°C to a constant weight, ground and sieved through a 149-μm mesh for the C functional groups analysis.

Root samples were collected from 0 to 20 cm depth using a circle stainless–steel auger with an inner diameter of 10 cm in February and August of 2007 and February, April, August, and October of 2008 (Deng *et al.* 2012). One sample was collected in each plot; thus, a total of 27 soil cores were collected for fine root biomass measurements at each sampling

Table 1: effects of precipitation on general soil properties in the BF, MF and PF

Forest type	Treatment	Soil moisture (%)	pH	Total *N* (g kg^{-1})	MBC (mg kg^{-1})
BF	NP	15.77 ± 2.94^{a}	3.77 ± 0.17^{a}	1.81 ± 0.19^{a}	483 ± 82^{a}
	DP	30.36 ± 11.08^{b}	3.98 ± 0.03^{b}	1.80 ± 0.35^{a}	705 ± 93^{b}
	CK	27.40 ± 10.97^{c}	3.97 ± 0.03^{b}	1.80 ± 0.35^{a}	711 ± 89^{b}
MF	NP	14.30 ± 2.51^{a}	3.99 ± 0.07^{a}	1.45 ± 0.15^{a}	347 ± 74^{a}
	DP	30.98 ± 10.12^{b}	3.99 ± 0.07^{a}	1.67 ± 0.11^{ab}	697 ± 149^{b}
	CK	28.46 ± 10.44^{c}	3.97 ± 0.08^{a}	2.05 ± 0.30^{b}	524 ± 20^{c}
PF	NP	7.77 ± 3.17^{a}	3.92 ± 0.14^{a}	1.25 ± 0.32^{a}	287 ± 56^{a}
	DP	21.11 ± 8.69^{b}	4.1 ± 0.06^{a}	1.35 ± 0.24^{a}	542 ± 100^{b}
	CK	18.13 ± 8.50^{c}	4.06 ± 0.10^{a}	1.26 ± 0.25^{a}	442 ± 87^{c}

Soil moisture (mean ± standard deviation in the table) was measured from Jan 2007 to Jan 2009. Soil MBC was assayed in the 2008 wet season (Huang *et al.* 2011a). Within each forest, values with different letters are significantly different at the $P < 0.05$ level by LSD test.

time. After sending the samples to the laboratory, these soil cores were washed carefully over a 2-mm sieve to remove soil. Roots (≤3 mm) were hand-picked and dried at 60°C until they reached a constant weight.

Soil analyses

Soil pH was measured in a 1:2.5 soil/water suspension. SOC was determined by dichromate oxidation and titration with ferrous ammonium sulfate. Total N was measured using the Kjeldahl method (Liu 1996).

Soil ROC was determined using the $KMnO_4$ oxidation method, as described by Blair *et al.* (1995). In detail, the air-dried soil was ground and further passed through a 149-µm sieve. Sub-samples of soil containing 15–30 mg C were weighed into 100 ml plastic centrifuge tubes, and then 25 ml of 333 mmol L^{-1} $KMnO_4$ solution were added to each tube. The tubes were shaken for 1 hour at 250 r min^{-1} and centrifuged for 5 min at 2000 r min^{-1}. The supernatants were then diluted 250 times with deionized water. Finally, the absorbance of the samples and standards was read on a UV spectrophotometer at a wavelength of 565 nm. Changes in the concentration of $KMnO_4$ were used to calculate the soil ROC concentration, assuming that 1 mmol MnO_4 was consumed in the oxidation of 9 mg C. NROC was measured as the amount of C amount that could not be oxidized by $KMnO_4$, as indicated by the differences between the SOC and ROC concentrations of each sample.

Forest floor samples were analyzed using cross-polarization magic-angle-spinning (CPMAS) solid state ^{13}C NMR spectroscopy (Avance 300 MH) at a frequency of 75.5 MHz. The important condition parameters used in the NMR included a spinning rate of 12 kHz, contact time of 35 ms, recycle time of 5 s and zero-filled of 2000 data points. Chemical shift values were determined using glycocoll at 176.03 ppm as an external reference. To quantify the different types of C, the CPMAS ^{13}C NMR spectra were divided into four chemical shift regions, i.e. alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm) and carboxyl C (160–210 ppm). The integration of the peaks within each of the chemical shift regions allowed us to estimate the relative C contents, expressed as the percent ratio of the peak area of these groups to the total peak area in each composite sample. The recalcitrance index was determined as the ratio of (alkyl C + aromatic C)/(O-alkyl C + carbonyl C) (Ostertag *et al.* 2008).

Statistical analyses

Data analyses were carried out using SPSS 11.5 for Windows. A two-way analysis of variance (ANOVA) was used to study the effects of forest type, precipitation treatment and their interaction on soil pH, SOC, ROC and NORC separately. Once significant effects were detected, multiple comparisons with the LSD method were performed to assess significances among different groups. A repeated measures ANOVA was used to examine the effects of the different experimental treatments on fine root biomass over time. Pearson correlation coefficients were calculated to show the relationships between soil parameters. Differences were considered to be significant when $P < 0.05$.

RESULTS

SOC and its fractions

Soil contained significantly higher SOC content in the CK plots in BF and MF than in PF, showing averages of 29.16 ± 5.34, 27.84 ± 2.95 and 19.07 ± 0.43 g kg^{-1} in BF, MF, and PF, respectively (Fig. 1a and Table 2). The NP treatment significantly decreased SOC content in all the studied forests compared to the CK plots ($P < 0.01$, Fig. 1a). The DP treatment tended to

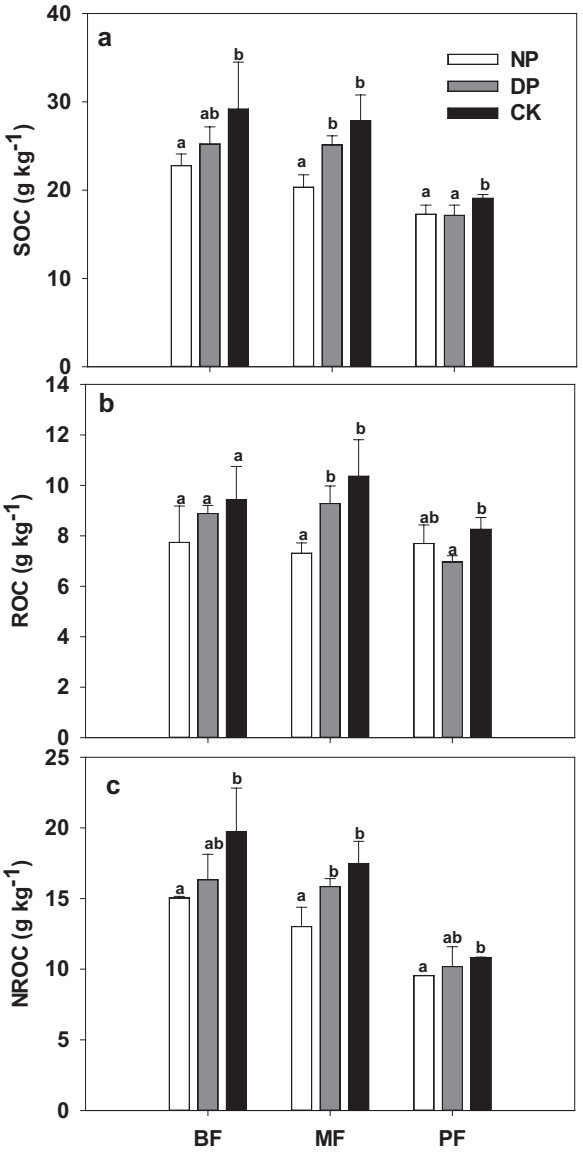

Figure 1: effects of precipitation on SOC, ROC and NROC in the BF, MF and PF. Error bars are standard errors ($n = 3$). Within each forest, values with different letters are significantly different at the $P < 0.05$ level by LSD test.

Table 2: analyses of variance on the effects of forest type, precipitation treatment and their interactions on soil pH, SOC, ROC and NROC

	pH	SOC	ROC	NROC
Forest	3.560*	45.388**	6.346**	56.801**
Treatment	4.562*	17.405**	9.986**	13.375**
Forest × treatment	1.412	2.284	2.566	1.694

The numbers in the cells are *F*-values and asterisks indicate the level of significance (*$P < 0.05$; **$P < 0.01$).

decrease SOC content, but the change was significant only in PF ($P = 0.10$ in BF, $P = 0.14$ in MF and $P = 0.01$ in PF, Fig. 1a).

Consistent with the clear trend of SOC content, soil ROC in the CK plots was also significantly higher in both BF and MF than in PF (Fig. 1b and Table 2) and was on average 9.43 ± 1.32, 10.36 ± 1.64 and 8.26 ± 0.47 g kg^{-1} in BF, MF and PF, respectively. Similarly, the NP and DP treatments appeared to decrease ROC content, yet the differences were only significant between the NP and CK plots in MF ($P = 0.01$) and between the DP and CK plots in PF ($P = 0.04$, Fig. 1b).

Soil NROC content was significantly different among the three forests (19.74 ± 3.08, 17.48 ± 1.56 and 10.81 ± 0.04 g kg^{-1} in the CK plots in BF, MF and PF, respectively, Fig. 1c and Table 2). The NP treatment decreased NROC significantly in all three forests ($P < 0.05$ in BF, $P < 0.05$ in MF and $P < 0.05$ in PF, Fig. 1c); however, the difference was not significant between the DP and CK treatments in the forests studied ($P = 0.9$ in BF, $P = 0.16$ in MF and $P = 0.23$ in PF, Fig. 1c).

Moreover, forest types and precipitation manipulations significantly affected the content and fractions of SOC ($P < 0.05$, Fig. 1); however, the interactions between the two were not significant for SOC content or for the content of its fractions ($P > 0.05$, Fig. 1).

Other soil physiochemical properties

In the CK plots, both BF and MF had significantly higher soil moisture than PF ($P < 0.05$, Table 1). In all the three forests, the NP treatment significantly decreased soil moisture ($P < 0.05$), while the DP treatment significantly increased soil moisture (Table 1). The NP treatment also decreased soil pH significantly in BF ($P < 0.05$), but did not affect soil pH in either MF or PF ($P > 0.05$, Table 1).

The total N content in BF and in MF was significantly higher than the total N content in PF under the CK treatment ($P < 0.05$), but it was not significantly different between BF and MF (Table 1). None of our experimental treatments changed the soil total N concentration significantly in either BF or PF, while the NP treatment significantly decreased the total N in MF ($P < 0.05$, Table 1).

Soil MBC in BF was significantly higher compared to PF or in MF ($P < 0.05$, Table 1); it was slightly higher in MF than in PF but the difference was not significant ($P > 0.05$, Table 1). Moreover, under the NP treatment, MBC was lower than that in the CK plots in all three forest types. The DP treatment significantly increased MBC in both PF and MF.

Root biomass

In CK plots, root biomass was significantly higher in BF and MF than in PF ($P < 0.05$), but there was no significant difference between BF and MF ($P > 0.05$, Fig. 2). In all three forests studied, the NP treatment significantly decreased root biomass throughout the entire investigation period ($P < 0.05$), while the DP treatment did not change root biomass significantly ($P > 0.05$), with the exception of PF in February 2008 ($P < 0.05$, Fig. 2).

Forest floor layer

The relative intensities of C functional groups of the forest floor samples are shown in Fig. 3 and Table 3. Under the CK treatment, the samples' total organic C was dominated by O-alkyl C in the three studied forests (39.98–54.50%, Fig. 3). In each of

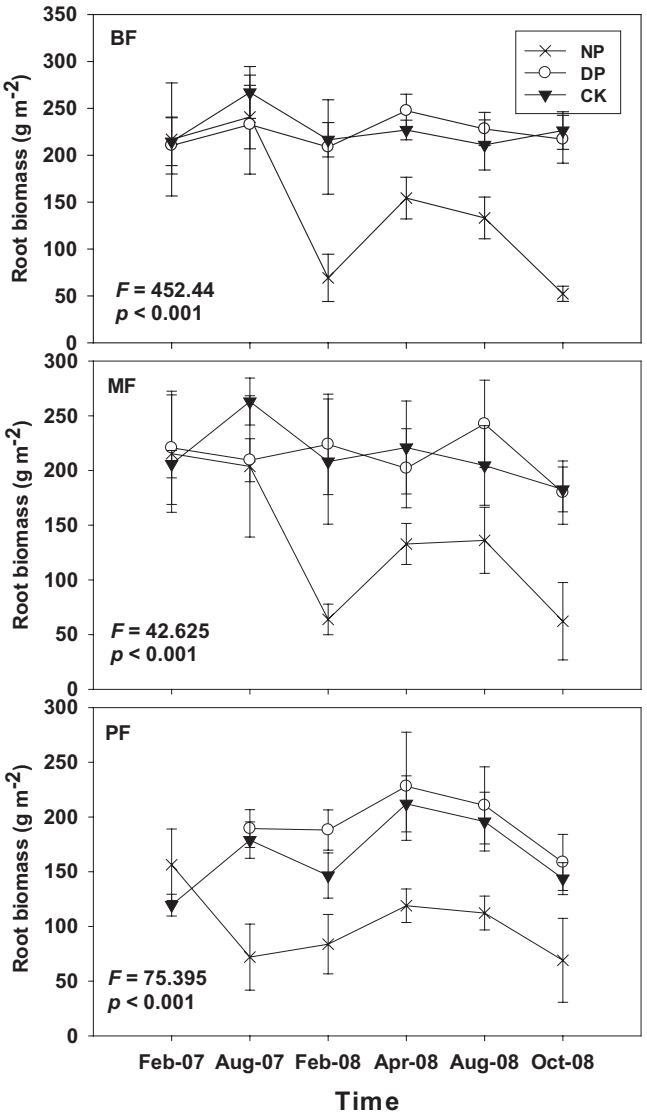

Figure 2: root biomass (≤3 mm) under the precipitation treatments at the three forest sites. The *F* and *P* values indicate the significance of the treatment effect on fine root biomass over time by repeated measures ANOVA.

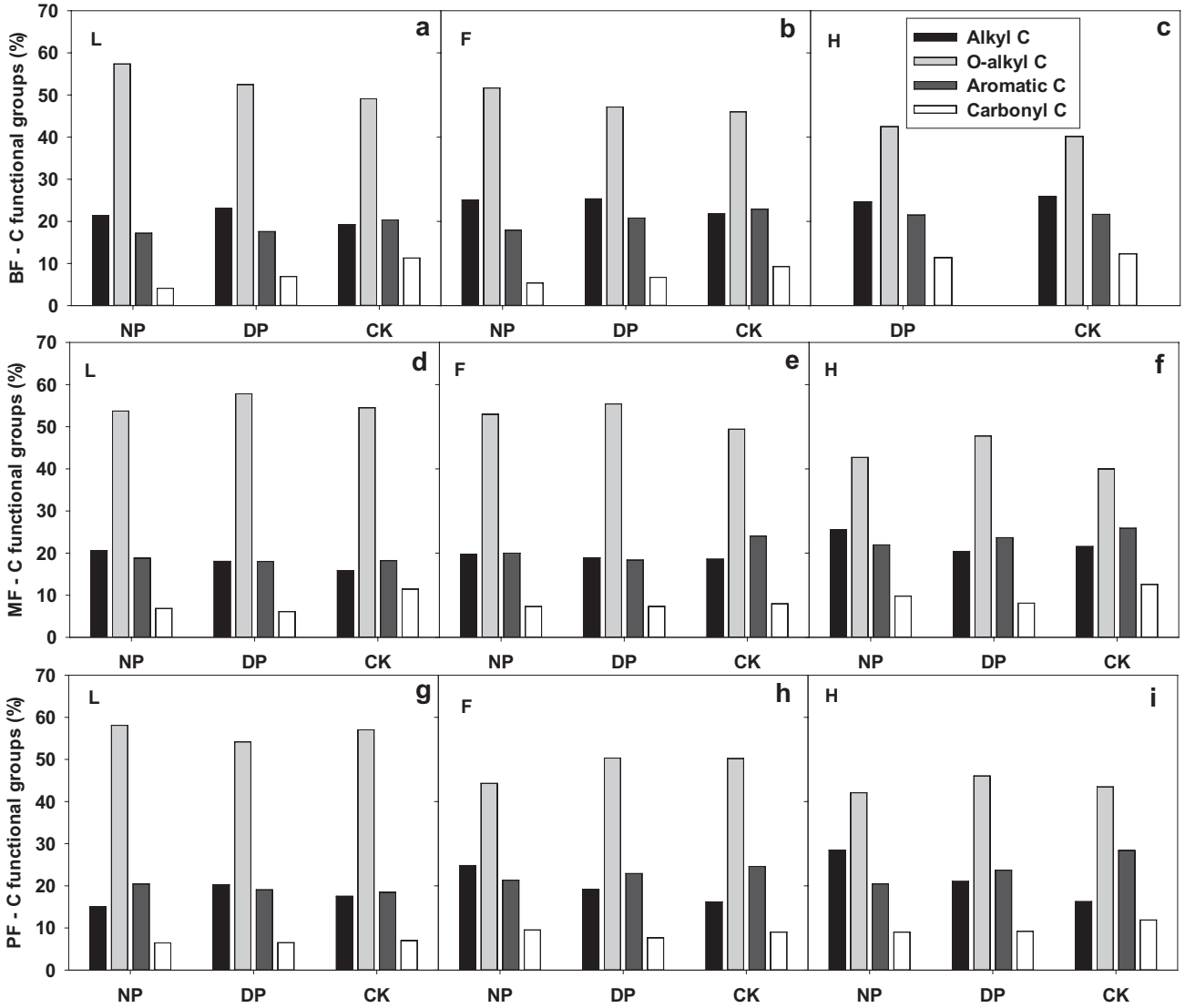

Figure 3: distribution of C functional groups from CPMSA ^{13}C-NMR spectra of forest floor layers under different treatments among the three forests. L = fresh litter layer; F = fermentation layer; H = humus layer.

Table 3: effects of precipitation on the recalcitrance index in the forest floor in the BF, MF and PF

Litter layers	Treatment	BF	MF	PF
L	NP	62.64	65.09	54.95
	DP	68.42	56.43	64.82
	CK	65.56	51.64	56.18
F	NP	75.32	65.90	85.72
	DP	85.68	59.38	72.50
	CK	80.85	74.23	68.88
H	NP	—	90.44	95.73
	DP	85.58	78.87	80.91
	CK	90.68	90.29	80.60

The recalcitrance index (%) was determined as (alkyl C + aromatic C)/(O-alkyl C + carbonyl C). Abbreviations: L = fresh litter layer; F = fermentation layer; H = humus layer.

the forests, O-alkyl C intensity varied with the layer, with the fresh litter layer having the highest content, followed by the fermentation layer and then the humus layer (Fig. 3). However, Alkyl C in BF and in MF exhibited different trends from that of O-alkyl C, i.e. the intensity in the fermentation and humus layers was higher compared to that of the fresh litter layer (Fig. 3a–f). The intensity of aromatic C was not obviously different among the layers in BF, but it was higher in the fermentation and humus layers than in the fresh litter layer in the other two forests (Fig. 3). The Carbonyl C intensity was the highest in the humus layers (Fig. 3). The recalcitrance index varied by layer but was the same for all three forests: humus layers > fermentation layers > fresh litter layers (Table 3). The recalcitrance index in the humus layers was higher in BF and MF than PF (Table 3).

For BF, the intensities of alkyl C and O-alkyl C in the fresh litter layer and in the fermentation layer under the NP and

DP treatments were higher compared to the CK treatment. Aromatic C and carbonyl C under the NP and DP treatments had relatively lower intensities than that under the CK treatment (Fig. 3a–c). In fresh litter and fermentation layers, the recalcitrance indices increased with the increased precipitation, with the highest values in the DP plots and the lowest in the NP plots, while DP treatment decreased the recalcitrance index in the humus layer (Table 3). The humus layers disappeared under the NP treatment in this forest.

For MF, the NP treatment increased the intensity of alkyl C and the recalcitrance index, but decreased the intensity of carbonyl C in the fresh litter layer (Fig. 3d–f and Table 3). Aromatic C and the recalcitrance index in the fermentation layer were lower in the NP plots than in the CK plots. In the humus layer, the NP treatment increased the intensities of alkyl C and O-alkyl C, but decreased the intensity of aromatic C. Alkyl C and O-alkyl C in the fresh litter layer showed a relative increase under the DP treatment, while carbonyl C was lower here than in the CK plots. The DP treatment increased the O-alkyl C, while aromatic C showed a relative decrease in the fermentation layer and humus layer. In both the fermentation and humus layers, the DP treatment decreased the recalcitrance index.

For PF, the NP treatment decreased the intensity of alkyl C and increased the intensity of aromatic C in the fresh litter layer (Fig. 3g). Compared to the CK control plots, alkyl C and the recalcitrance index in the fermentation layer were relatively higher under the NP treatment, and aromatic C was relatively lower (Fig. 3h and Table 3). In humus layer, the NP treatment increased alkyl C and the recalcitrance index but decreased aromatic C and carbonyl C (Fig. 3i and Table 3). In both the fermentation and humus layers, O-alkyl C intensity increased under the DP treatment. The DP treatment decreased aromatic C and carbonyl C. In both the fresh litter and fermentation layers, the DP treatment increased the recalcitrance index; however, there was no obvious difference in the humus layer between the DP and CK treatments.

DISCUSSION

Effect of forest types on SOC and its fractions

Among our study sites, PF contained significantly lower SOC content than the other two forests. On the one hand, this may be attributed to the relatively lower litterfall and root C inputs to the soil in PF compared to MF and BF. On the other hand, more forest gaps in younger forests with a developing canopy limit the shaded area and therefore increase soil temperature while decreasing soil moisture. The relatively lower soil moisture can consequently contribute to the slower decomposition rates of litterfall and roots (Martin *et al.* 2004; Ostertag *et al.* 2008), possibly due to limited microbial activity and lower substrate transformation capacity when water supply is limited. Likewise, a lower forest canopy, as well as lower soil moisture, was also detected in PF rather than in MF and in BF (Zhou *et al.* 2006). The lower soil moisture is likely to restrict the decomposition of litter and roots (Zhang *et al.* 2008), and then reduce the transfer of plant-C into mineral soil in PF. The two possible decreases in C inputs in PF may result in the observed pattern of SOC among forests, compared with what was observed in MF and BF. Likewise, previous studies have reported that SOC content increased with forest age in different forest ecosystems (Huang *et al.* 2011b; Li *et al.* 2013; Pregitzer and Euskirchen 2004), an observation that is consistent with the present study.

Underlying the clear increase in SOC with the forest development, we observed that different SOC fractions showed different dynamics within these forests. This is an important finding that could improve our understanding of the mechanisms of SOC accumulation in forests. For ROC, the proxy for labile SOC in our study (Blair *et al.* 1995), there was no significant difference among the different forest types. The content of soil ROC is generally determined by the balance of plant-derived C inputs and outputs (Tirol-Padre and Ladha 2004; Yang *et al.* 2009). In our study sites, the amounts of litterfall and root biomass (≤3mm) in BF and MF were higher than in PF (Chen *et al.* 2012b), indicating the higher plant-derived C inputs in the former two forests. This seemed to be beneficial for ROC accumulation in those areas. Compared with PF, however, BF and MF had significantly higher soil moisture and MBC, which could have facilitated the decomposition of ROC in consideration of labile SOC as preferable-utilized substrates by soil microorganisms (Blair *et al.* 1995; Chen *et al.* 2012a). As a result, the synchronism in the enhancements of ROC inputs and outputs could be the explanation for why a significantly higher ROC accumulation was not observed in BF and in MF versus in PF.

Additionally, in spite of the lower litterfall production in PF, the relative intensity of O-alkyl C in fresh litter in PF was higher than that in both MF and BF. The O-alkyl C group, mainly comprising polysaccharides, was commonly regarded as a proxy for easily oxidizable carbohydrates (Alarcón-Gutiérrez *et al.* 2008; Tirol-Padre and Ladha 2004). The higher relative intensity of O-alkyl C in PF implied a higher percentage of ROC inputs to total C inputs, and could also contribute to the similar ROC content in PF compared to the other two forests.

The stable organic C fraction, indicated by the NROC content in this study, was significantly higher in BF and MF than in PF, a pattern in line with the results from the C functional group analysis. The higher recalcitrance index signified greater contributions to SOC content from alkyl C and aromatic C groups, which are usually regarded as slower-decomposed organic C groups (Ostertag *et al.* 2008). In our study, we observed a much higher recalcitrance index in humus layers in BF and MF than in PF, implying relatively higher percentages of these two relatively recalcitrant organic C groups in both BF and MF. Combined with the observed higher inputs of litterfall and roots in these two forests, these factors may contribute to the faster accumulation of stable organic C in the mineral soil in MF and BF.

Effects of decreased precipitation on SOC content and its fractions

Decreased soil moisture under the NP treatment had a negative effect on soil ROC content in these forests, although the decreases in PF and in BF were not significant. At first this pattern surprised us because, as discussed previously, lower soil moisture may be beneficial for ROC accumulation due to the lower decomposition activity, which was indicated by the decreased MBC content under the NP treatment in this study (Chen *et al.* 2012a; Deng *et al.* 2010). However, a reduction in ROC under the NP treatment was anticipated when we counted the roots biomass. As the main input of soil ROC, the decreases in the production of this plant-derived C offset the possibility of ROC accumulation resulting from the lower decomposition activity (lower C output) under the NP treatment. This is in line with the observations of O'Brien *et al.* (2010). Moreover, the decreased root biomass may reduce the labile C input to mineral soil in the forms of root excretions, and consequently decrease ROC content. In addition, a reduction in soil moisture could limit the downward movement of C from the litter layer to the soil (Lee *et al.* 2004), consequently decreasing soil ROC in all the three forest types.

As a proxy of stable organic C fractions, NROC is relatively less responsive to environmental changes, owing to the decomposition recalcitrance of the C group to soil microorganisms (Blair *et al.* 1995). Our results, however, showed that NROC content decreased under the NP treatment in all three forests, a finding that appears to contradict the aforementioned traditional interpretation. The reductions in NROC content likely resulted from the reduction in the production of litterfall and roots, i.e. reductions in the NROC inputs led to the decrease in NROC content. Alternatively, changes in the decomposability of litterfall under the NP treatment could also contribute to such a pattern, as residual OM was more recalcitrant in the wet and warm climate than in the cold and dry climate according to Hilli *et al.* (2008), in spite of the supply of the same standard litter. This is also supported by our results from the CPMAS ^{13}C-NMR analyses: from the fermentation to humus layers, aromatic C under the NP treatment was lower than that at the CK plots, but the NP treatment was not observed to have the same influence in the fresh litter layer. Aromatic C compounds are commonly considered to be less decomposable by microorganisms (Ostertag *et al.* 2008). A decrease in the relative intensity of aromatic C under the NP treatment could thus imply the decreased accumulation of non-labile C in mineral soil.

The SOC pool comprises the two fractions, i.e. ROC and NROC in the present study. Compared with CK plots, the NP treatment decreased the contents of both ROC and NROC, subsequently resulting in a long-term decrease in SOC in all three forests.

Effects of a precipitation increase on SOC content and its fractions

The DP treatment tended to decrease the contents of SOC, ROC and NROC, but it was not significant except for the decrease in the SOC and ROC content in PF. Water content is a key factor controlling SOC turnover in PF because soil moisture is quite low in this forest (Zhou *et al.* 2005). This is also indicated by the increased soil MBC content under DP treatment in PF, which likely implies that soil microbial growth was impressed by the water limit in this forest under natural precipitation (comparing the MBC content in the CK treatment with that in the DP treatment). The increased soil MBC under the DP treatment could be responsible for the decreases in the contents of ROC and SOC because of the higher microbial decomposition capacity under this condition (Li *et al.* 2013). This is consistent with previous studies in the same region (Chen *et al.* 2012a; Deng *et al.* 2010, 2012). Moreover, PF has higher soil bulk density in 0–10 cm layers. The higher bulk density may be another reason for the decreased ROC and SOC in this forest, as lower soil porosity would result in a slow water infiltration rate and consequently enhance surface runoff (Levy *et al.* 1997), thus leading to more ROC and SOC losses from soil erosion.

Contrary to the SOC and ROC contents in PF, the DP treatment did not exhibit a significant influence on the SOC or ROC content in BF and MF. This was likely due to the relatively higher soil moisture in these two forests even under CK treatment; therefore, water limitation did not occur in BF and MF. As a result, increased precipitation cannot increase soil moisture adequately to change the ROC and SOC content significantly. Alternatively, the higher soil water content under natural conditions (CK treatment in this study) in both BF and MF itself may imply that soils in these two forests are more resilient or persistent to precipitation increases (Deng *et al.* 2011; Huang *et al.* 2011b), when the increased soil water content does not exceed the water thresholds, over which oxygen limitation will occur in the soil system (Davidson *et al.* 1998). This stronger resilience or persistence is also indicated by our observations of the fine root biomass and other soil properties including pH, and total N, as they were not significantly different between the DP and CK treatments in both BF and MF.

Moreover, the DP treatment did not affect the NROC content significantly in any of the studied forests. This is also consistent with the results of the C functional group analysis showing that no significant difference were observed in alkyl C, aromatic C and the recalcitrance index between the DP and CK treatments. The DP treatment in our study sites did not lead to any notable changes in the fine roots biomass or in the percentage of recalcitrant C functional groups in the litter, resulting in the NROC patterns observed above (Kögel-Knabner 2002; Ono *et al.* 2011).

In conclusion, NROC but not ROC content increased significantly from the young PF to the mature BF, implying that an increase in total SOC observed among these forests was mainly due to the increase of NROC. In all three forests, the NP treatment exhibited negative effects on total SOC due to decreases in C inputs (including litterfall and root productions) and changes in C quality (SOC fractions and C functional groups). However, the DP treatment did not significantly change the

contents of total SOC and its fractions in both BF and MF, indicating that the BF and MF had a higher capacity to resist the effects of a precipitation increase on soil carbon storage. Our results suggest that the SOC fractions would respond differently to precipitation changes in subtropical forests in southern China depending on the forest types and that there may be a mechanism underlying the observed SOC accumulation in mature forests in this region. Further studies are of high importance to better understand the underlying mechanisms of the various responses of SOC fractions to changes in precipitation. Such studies could improve our predictions of ecosystem C cycling under future precipitation changes in southern China.

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