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Surface soil organic carbon in temperate and sub-tropical oriental oak stands of East China

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Abstract

Forest ecosystems contain large amounts of soil organic carbon (SOC) which is a major component of biogeochemical cycles that may be sensitive to environmental change. We used a combination of nuclear magnetic resonance (NMR) spectroscopy and elemental and isotopic composition to examine the influence of soil properties and climatic factors on the quantity and degree of decomposition of SOC for organic and surface mineral horizons in seven oriental oak (*Quercus variabilis*) forest sites arranged across a 11° latitudinal gradient in East China. Lacking Oa horizons, the two southernmost sites contained lower amounts of SOC in the forest floor horizon but otherwise latitudinal trends were not consistent. The SOC stock in the 0-10 cm mineral horizon exhibited no clear trend along the gradient and had a negative association with clay + silt content. Based on higher alkyl/O-alkyl (A/O) ratio and alkyl/methoxyl (A/M) ratio, the SOC at the 0-10 cm depth appeared to be relatively more decomposed in three of the four southern sub-tropical sites. However, the degree of SOC degradation also decreased strongly with increasing soil pH ($R^2 = 0.90$, $P = 0.001$). Soil OC exhibited increases in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and decreases in C/N with depth for all the seven sites, indicating an increase in its extent of decomposition. Our analysis indicated that the A/M ratio from NMR provided the best indication of the extent of SOC degradation along the latitudinal transect whereas the elemental and isotopic composition better reflected patterns with soil depth.

Keywords

Soil organic matter, soil properties, climate, CP-MAS ^{13}C NMR, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

1. Introduction

35 Forest ecosystems in the Northern Hemisphere cover 2×10^9 ha and contain
36 large amounts of carbon (C) (390 Pg), representing a huge reservoir of C on a global
37 basis, mainly in the form of soil organic carbon (SOC, 260 Pg) (Goodale et al. 2002).
38 Eswaran et al. (1993) estimated that 1576 Pg of OC is stored in soils globally, with ~
39 506 Pg (32%) of this in soils of the tropics, and ~40% of the C in soils of the tropics is
40 in forest soils. Any change in the size and the turnover rate of soil C pools may
41 potentially alter the atmospheric CO₂ concentration and the global climate (von
42 Lützow et al. 2006).

43 Forest soil C storage is broadly associated with climate, management and
44 disturbance history, tree species composition, microbial community composition and
45 activity and soil physical properties (Johnsen et al. 2013). For example, climate
46 influences the accumulation and stabilization of soil C pools as the biological activity
47 that affects C turnover is temperature dependent (Pendall et al. 2004; Whitby and
48 Madritch 2013). Tree species composition also has an important effect on the
49 quantity and quality of SOC pools (Laganiere et al. 2013; Wang et al. 2013), e.g., by
50 influencing soil microclimatic conditions (Hobbie et al. 2006), and by producing leaf
51 and root litter in variable amounts and quality (Berg 2000). Xu et al. (2016)
52 highlighted that clay content was the most important variable in regulating
53 decomposition of SOC at a large scale (mean annual temperature ranging from -15 to
54 26°C). Despite its pivotal role, our understanding of forest SOC and its controlling
55 factors remains incomplete.

56 A variety of methods have been used to characterize the complex chemistry of
57 SOC. Solid-state ¹³C Nuclear Magnetic Resonance with cross-polarization and magic

angle spinning (CP-MAS ^{13}C NMR) provides a first approximation of the relative abundance of organic C functional groups (Baldock et al. 1997; Clemente et al. 2012). The relative distributions of these broad C categories in SOC vary with decay (Yuste et al. 2012); for example, as the extent of degradation increases, the alkyl C spectral region increases concomitantly with the decrease in the O-alkyl C spectral region, and thus the alkyl/O-alkyl (A/O) ratio has been widely used to indicate the degree of organic matter decomposition (Baldock et al. 1997). Another indicator of the extent of SOC decomposition is the stable isotopic composition ($\delta^{13}\text{C}$) because reaction rates are slower for heavy than for lighter isotopes, enriching the remaining SOC with heavy isotopes (Guillaume et al. 2015). Indicating increases in stabilized SOC at greater soil depths, notably, both the A/O ratio and the $\delta^{13}\text{C}$ of SOC increase with depth in many soil profiles (Quideau et al. 2000; Garten Jr 2006; Schneckenberger and Kuzyakov 2007). Using these methods researchers have also observed that SOC may be less decomposed at higher latitudes at least in sub-arctic zones (Norris et al. 2011; Sjogersten et al. 2003). To our knowledge no such studies have been conducted comparing temperate and sub-tropical climatic zones.

In the present study, samples of the forest floor layers (Oe and Oa) as well as surface mineral soil horizons (0-2, 2-5, and 5-10 cm) were taken from seven oriental oak forest sites in East China, three in the warm temperate climatic zone and four in the sub-tropical zone, and assessed by CP-MAS ^{13}C NMR, elemental and isotopic analyses. Oriental oak (*Quercus variabilis*) is a common deciduous broadleaf tree species in East Asia (24° to 42° N and 96° to 140° E). Its distribution encompasses a wide range of annual temperature and precipitation (Kang et al. 2011), providing a

81 natural climatic gradient for studying SOC dynamics across two zones with control
 82 for tree species composition. We focused on the organic and surface mineral soil
 83 horizons which contain large amounts of carbon that may be sensitive to
 84 environmental change (Du et al. 2014; Liski et al. 1999). The aims of this study were
 85 (1) to investigate soil OC stock and concentration along the climatic gradient; (2) to
 86 investigate the degree of SOC decomposition, as indicated by CP-MAS ^{13}C NMR and
 87 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, along both the latitudinal and depth gradients; and (3) to examine the
 88 role of soil properties and climatic factors determining SOC quantity and degree of
 89 decomposition along the gradient.

90 **2. Materials and methods**

91 *2.1. Site descriptions*

92 In July 2014, seven oriental oak stands were sampled across a large latitudinal
 93 range spanning 1500 km in East China, extending from Beijing (40.25°N, 117.12°E) to
 94 Jiangxi (29.09°N, 115.62°E) (Fig. 1). Three sites (PG, HYS and BA) are located in the
 95 warm temperate zone based on the conventional boundary at 35° N latitude, while
 96 four sites (HZY, XY, HS, YS) are in the subtropical zone. All the sites are natural,
 97 second-growth, monospecific oriental oak stands which have not been disturbed by
 98 direct human activities and wildfires for at least the last five decades. Among the
 99 seven sites, only Site HZY had an important herbaceous layer. Soils in the sites
 100 encompass three soil orders (USDA Soil Taxonomy); entisols at three sites (PG, HYS,
 101 BA), inceptisols at two sites (HZY, XY) and ultisols at the most southerly sites (HS, YS).
 102 Climatic conditions of these sites change from cooler and drier in the north (mean
 103 annual temperature (MAT) from 11.1°C, and mean annual precipitation (MAP) from

492mm) to warm and wet in the south (MAT 16.9°C and MAP 1759 mm) (Table 1).
Combining MAP and MAT, annual actual evapotranspiration (AET) was estimated
using formula (1) (Ding 2013):

$$(1) AET = MAP \div \sqrt{[0.9 + (MAP \div E_0)^2]}; E_0 = 300 + 25 \times MAT + 0.5 \times MAT^3$$

AET: annual actual evapotranspiration, mm; *MAT*: mean annual temperature, °C;
MAP: mean annual precipitation, mm; *E₀*: maximum annual potential of
evapotranspiration with sufficient soil water, mm.

2.2. Field Sampling

At each site, forest structure was characterized in a 20 m x 20 m plot positioned
at mid-slope in the study area and canopy height and stem diameter at breast height
were measured for each tree (Table 1). Five parallel transects were established into
each site with 10 m spacing between them and with 10 sampling points on each
transect at 5 m spacing (i.e. 50 x 50 m grid). The forest floor horizons (Oe and Oa)
were collected with hand spades in a circle 13 cm in diameter to the top of the
mineral soil; the boundary between forest floor and mineral soil was usually distinct,
marked by high stoniness and by color and texture. Mineral soil samples were
collected with a 2.5-cm corer at 0-2, 2-5, and 5-10 cm depths. For each of the five
transects, the samples from the 10 points were composited by horizons. Oa horizons
were not observed at the two southernmost sites, HS and YS.

Ten additional 2.5-cm diameter soil cores of the whole 0-10 cm mineral horizon
were collected on each transect, and composited by transect for measuring soil
pH, %C, %N and texture. Fifteen soil cores (three per transect) were excavated to 10
cm depth using a bulk density corer. Soil samples were placed in polyethylene bags,

127 stored on ice and transferred to the laboratory within 24 h. Mineral soils were sieved
128 (2 mm), and mass of the fine and coarse fractions was measured. The Oe and Oa
129 horizons were chopped into small pieces (< 5 mm).

130 2.3. *Soil physico-chemistry*

131 Using the 0-10 cm bulk mineral soil, soil pH was measured at a soil-to-water
132 ratio of 1:2.5 (m/v); soil particles were classified into silt, clay and sand using the
133 Bouyoucos hydrometer method (Elliott et al. 1999). Bulk density of the 0-10 cm
134 mineral soil was calculated by dividing the dry weight (dried at 105°C until constant
135 weight) by the soil core volume (corrected with the volume of stones (> 2 mm)).
136 Forest floor C stock was calculated as the product of dry mass and C concentration,
137 while 0-10 cm mineral soil C stock was estimated using the concentration data and
138 bulk density measures, and both values were scaled to kg m⁻². Both carbon stock and
139 carbon concentration were used to indicate SOC quantity.

140 2.4. *NMR analysis*

141 Two of the five replicates for each depth and site were used for NMR analysis.
142 Prior to NMR analysis, each of the 42 mineral soil samples (3 depths × 2 replicates ×
143 7 sites) were treated with hydrofluoric acid (HF) to concentrate the organic matter
144 and remove paramagnetic minerals (Simpson and Simpson 2012). About 5 g of the
145 sample was shaken with 30 mL 10% HF for 2 h. After centrifugation for 10 min, the
146 supernatant was removed. The procedure was repeated five times. The remaining
147 sediment was washed five times with 30 mL deionized water to remove residual HF
148 before freeze drying. Using two parallel samples, the CP-MAS ¹³C NMR spectra for
149 each mineral horizon at each site were acquired twice on a Bruker Avance 400 MHz

150 NMR spectrometer, equipped with a 4 mm broadband CP-MAS probe.
151 Approximately 100 mg of sample was packed into a 4 mm zirconium rotor with a
152 Kel-F cap. The acquisition parameters were as follows: spectral frequency of 100.613
153 MHz for ^{13}C and 400.13 MHz for ^1H , spinning rate of 5 kHz, ramp-CP contact time of
154 1.5 millisecond, 1 s recycle delay, 17128-100967 scans per sample and exponential
155 line broadening of 50 Hz. Glycine was used as an external standard to calibrate the
156 chemical shifts.

157 After correcting the spinning sidebands of carboxyl carbon, the spectra were
158 integrated using Topspin V. 2.1 and assigned based on Mathers et al. (2007). Eight
159 structural regions were integrated: alkyl carbon (0-45 ppm), N-alkyl/methoxyl carbon
160 (45-60 ppm), carbohydrate carbon (60-90 ppm), Di-O-alkyl carbon (90-110 ppm), aryl
161 carbon (110-145 ppm), O-aryl carbon (145-165 ppm), carboxyl carbon (165-190 ppm),
162 and ketone carbon (190-210 ppm). The alkyl region (0-45 ppm) contains carbon in
163 compounds such as lipids, cutin and suberin. The O-alkyl C region (45-110 ppm)
164 typically corresponds to more easily degraded OM constituents, such as those found
165 in carbohydrates (Supplementary Fig. S1)(Simpson and Simpson 2012). The
166 Alkyl/O-alkyl (A/O) ratio, which usually increases with progressive degradation, was
167 calculated by dividing the area of the alkyl region (0-45 ppm) by the area of the
168 O-alkyl (45-110 ppm) region (Simpson et al. 2008). The alkyl/methoxyl (A/M) ratio
169 also was calculated based on the area of the alkyl region (0-45 ppm) and the
170 N-alkyl/methoxyl region (45-60 ppm). Both the A/O and A/M ratios were used to
171 indicate the degree of SOC decomposition.

172 2.5. Elemental and isotopic analyses

The sieved mineral soil horizons were air-dried, and the Oa and Oe horizons were dried at 65°C until constant weight, before isotopic analysis. The isotopic composition, total carbon and nitrogen of these samples were measured on a Vario EL III Elemental Analyzer coupled to an Isotopic Ratio Mass Spectrometer (Elementar Analysensysteme GmbH, Germany). Elemental results were converted to an oven-dry weight basis. Isotopic results were expressed in the δ -notation, as the ‰ variation from the standard reference material, Pee Dee Belemnite (PDB) for C and atmospheric N₂ for N.

2.6. Statistics

Statistical tests were performed using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). While the other data were calculated as arithmetic means of five replicates with standard errors, NMR data were calculated as arithmetic means of two replicates. To perform the following statistical methods, NMR compositions at the 0-10 cm depth were calculated by summing the weighted values of 0-2, 2-5, and 5-10 cm depths (i.e. multiplied by 0.2, 0.3, and 0.5, respectively).

A general linear model was applied to evaluate differences between climatic zones of carbon stock and the various indexes of degree of SOC decomposition. For 0-10 cm mineral horizon, Pearson correlations were used to evaluate the inter-relationships among the soil and climate variables. Stepwise multiple regression analyses were conducted to evaluate the effects of soil and climate factors on SOC concentration and degree of degradation, where MAP, MAT, and AET were included as climatic variables, while clay + silt content and pH were included as soil properties.

196 3. Results

197 3.1. Soil OC concentration and stock

198 Forest floor organic C stock differed significantly between zones ($P < 0.001$,
199 Supplementary Table S1). At the most southerly sites (HS and YS), where Oa horizons
200 were lacking, forest floor OC stock was particularly low (0.17 and 0.28 kg m⁻²,
201 respectively) and low forest floor OC stock was also observed at a third sub-tropical
202 site (site HZY) that had a thin Oa horizon (Fig. 2). However, one of the sub-tropical
203 sites (site XY) had comparatively well-developed forest floor horizons, similar to the
204 temperate zone sites. The 0-10 cm mineral SOC stock exhibited no significant
205 difference between climatic zones ($P = 0.359$), and it did not show any clear trend
206 with latitude (Fig. 2). Based on stepwise regression analysis the only soil or climatic
207 factor that had a significant effect on mineral soil OC concentration was silt + clay
208 content: % C decreased with increasing silt + clay across the seven sites ($R^2 = 0.66$, P
209 = 0.026, Table 2).

210 3.2. Soil organic C: NMR analysis

211 For the 0-10 cm mineral soil, the percentage of signal intensity attributable to
212 alkyl C was significantly lower ($P = 0.021$) in the northern sites than in the southern
213 sites (Table 3, Supplementary Table S1). The percentage of alkyl C was significantly
214 correlated with both pH and AET (Table 4). The relative contribution of methoxyl C to
215 total signal intensity also was significantly different between zones ($P = 0.038$,
216 Supplementary Table S1) and generally higher in the northern sites than in the
217 southern sites (Table 3); it was strongly correlated with pH, and significantly
218 correlated with MAP and AET (Table 4). The contributions of carbohydrate C,

219 di-O-alkyl C and the whole O-alkyl regions were neither significantly different
220 between zones nor correlated with the environmental variables (Table 4,
221 Supplementary Table S1).

222 Neither index of degree of decomposition, the A/O and A/M ratios, showed any
223 consistent trend with increasing soil depth (Table 3). However, averaged across the
224 0-10 cm mineral layer, both the A/O ratio and the A/M ratio decreased significantly
225 with increasing soil pH ($P < 0.05$, Table 4), whereas no effect of soil texture (silt + clay)
226 was observed. The A/M ratio also was correlated with climatic variables, as
227 significant positive relationships with MAP and AET were observed ($P < 0.05$, Table 4).
228 Stepwise regression analysis also showed that pH was the most important variable in
229 controlling the A/M ratio across these sites ($R^2 = 0.90$, $P = 0.001$, Table 2).

230 *3.3. Soil organic C: Elemental and isotopic composition*

231 The C/N ratios ranged from 10.16 in the 5-10 cm horizon of site PG to 30.16 for
232 the Oe horizon at site HYS (Fig. 3a). The C/N ratios decreased with depth in the soil
233 profiles, and declined more dramatically in the forest floor than in the mineral soil.
234 The $\delta^{13}\text{C}$ ranged from -28.38‰ in the Oe horizon at site YS to -17.63‰ for the 5-10
235 cm horizon at site HZY (Fig. 3b). For a given horizon, the values of $\delta^{13}\text{C}$ were higher in
236 the northern sites than in the southern sites with the striking exception of site HZY.
237 The $\delta^{15}\text{N}$ ranged from -4.86‰ in the Oe horizon at site XY to 5.97‰ for the 2-5 cm
238 horizon at site PG (Fig. 3c). Similar to $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$ values also showed a
239 generally increasing trend with soil depth. However, no consistent trend was found
240 for $\delta^{15}\text{N}$ values among sites within a given horizon.

241 **4. Discussion**

242 4.1. Soil organic C quantity

243 We observed especially low forest floor carbon stocks in three of the
244 sub-tropical oak forest stands (Fig. 2). Accumulation of SOC in surface organic layers
245 is mainly driven by rates of C input (litterfall and fine root turnover), by the
246 biochemical quality of litter and by decomposition and mixing rates (Jandl et al.
247 2007). Focusing on one single tree species, the influence of litter quality was
248 minimized in the present study. Although we did not measure litterfall, all the stands
249 had closed canopies and in fact the largest trees and highest forest basal area were
250 found in the three sub-tropical sites with low forest floor carbon stock (Table 1);
251 hence, it seems unlikely that low litterfall inputs explain this pattern. More likely,
252 higher temperature and moisture accelerate soil organic matter decay and reduce
253 the accumulation of SOC in the organic layer; for example, Meentemeyer (1978)
254 demonstrated the dependence of litter decay on site evapotranspiration, and
255 distinctly higher annual actual evapotranspiration (AET) was estimated for the
256 sub-tropical sites (Table 1). Another possible contributor might be soil invertebrates,
257 e.g., earthworms and millipedes, which mix the forest floor into mineral soil (Nielsen
258 and Hole 1964). Lin et al. (2009) observed that both the amount and diversity of
259 forest floor macrofauna showed increasing trends from north to south in eight
260 typical forest ecosystems across China. These increases might contribute to a faster
261 litter decomposition and a reduced stock of forest floor C in the south.

262 Carbon stock in surface mineral soil showed no clear trend along the latitudinal
263 gradient (Fig. 2). The factors controlling the accumulation of SOC in mineral layers
264 are more complex than in the organic layer. In addition to the degree of microbial

processing, it also depends on both the aboveground and belowground C sources (Laganiere et al. 2013) as well as mechanisms of stabilization associated with clay minerals and aggregation (Schmidt et al. 2011). We found a significantly negative effect of clay + silt content (i.e., a positive effect of sand content) on SOC concentration. Carter et al. (1998) proposed that the capacity of a soil to protect and accumulate SOM in organomineral particles is not always positively related to the clay and silt content per se but rather to the degree to which this capacity level is filled. Once the clay plus silt is saturated with organic matter, additional SOM would be found in macroaggregates, possibly as sand-sized macroorganic matter (Carter 2002). Also, although we did not measure it, certain clay minerals, especially short-range ordered minerals, are particularly effective in SOC sequestration (Kögel-Knabner et al. 2008) and perhaps the proportion of these minerals differs among the sites. We also found a weak negative relationship between pH and mineral soil C concentration ($R^2 = 0.48$, $P = 0.085$). One possible explanation might be that SOC had a higher solubility at high pH (Yin et al. 1996; Tavakkoli et al. 2015), and more dissolved OC could lead to the reduced accumulation of SOC. Our findings suggested that soil properties such as texture and pH were closely associated with SOC accumulation.

4.2. Soil organic C composition based on NMR

The A/O ratio is widely used as an indicator of the degree of SOC degradation; however, the A/O ratio did not show consistent patterns in some studies (Blumfield et al. 2004; Lorenz et al. 2004). In the present study, among the three parts in the O-alkyl region (methoxyl, carbohydrate, Di-O-alkyl), we only found a significant

288 correlation between methoxyl C and the soil and climate variables (Table 4). Mathers
289 et al. (2007) suggested that the presence of a large amount of lignin will protect
290 some of the polysaccharide C from microbial attack and thus the O-alkyl C region
291 may overestimate the quantity of readily decomposable C when substantial amounts
292 of lignin are present; this might explain why the relative content of carbohydrate C in
293 our study was not sensitive to climate and soil variables. To better investigate the
294 links between the degree of SOC degradation and environmental variables, we
295 evaluated the alkyl-to-methoxyl (A/M) ratio. Showing similar patterns across sites,
296 the A/M ratio had closer associations with soil and climatic factors than the A/O ratio
297 (Table 4).

298 The alkyl/O-alkyl (A/O) ratio typically increases with progressive biodegradation
299 of labile OM components (Clemente et al. 2012). Contrary to previous reports of an
300 accumulation of alkyl C and hence an increased A/O ratio with increasing soil depth
301 (Christensen 2001; Nierop et al. 2001), in our study neither the A/O ratio, the A/M
302 ratio nor the alkyl C abundance showed any consistent variation with depth. We
303 speculate that a low A/O ratio of root detritus which is most abundant at these
304 shallow depths might contribute to this lack of depth pattern as suggested by Norris
305 et al. (2011). The limited depth sampling in the present study might also preclude
306 the detection of NMR patterns that occur at greater soil depths.

307 Instead, we found that both the A/M ratios and the A/O ratios were apparently
308 lower in the four northern sites for the 0-10 cm mineral soil compared to the three
309 southern sites, suggesting that the SOC in the northern sites is less decomposed
310 compared to the southern sites. In a comparable study along a boreal forest (*Pinus*

311 *banksiana*) transect, Norris et al. (2011) observed a higher A/O ratio in the south,
312 and Kane et al. (2005) noted that the proportional amount of labile SOM in the
313 mineral soil decreased with increasing temperature in boreal *Picea mariana* forest.

314 The 0-10 cm mineral soil pH had a strong negative effect on both the A/M ratio
315 and the A/O ratio across sites. Sinsabaugh (2010) stated that the laccases of white
316 rot fungi primarily involved in lignin breakdown generally have lower pH optima
317 (4.0-5.0); while the laccases of brown rot and coprophilic fungi, mainly active in
318 polymerizing soluble phenols and thereby contributing to humification, generally
319 have higher pH optima (6.0-7.5). With pH values ranging from 4.0 to 5.0, lignin in the
320 0-10 cm mineral soil at the three southern sites may be more susceptible to
321 microbial attack than in the northern sites. We thus speculate that a faster
322 breakdown of lignin might contribute to the higher A/M ratio and A/O ratio in the
323 southern sites, but further investigation is needed. Besides the strong negative effect
324 of pH, we also observed positive relationships between MAT, MAP and especially
325 AET on the A/M ratio. Our finding that pH was negatively affected by MAP and AET (r
326 = -0.78 and $r = -0.76$, $P < 0.05$; Table 4) suggests that differences in SOC composition
327 involve direct impacts of soil pH plus more complex interactions between climate
328 and soil properties.

329 4.3. Soil organic C: Stable isotopes and C/N ratio

330 Decreases in C/N ratio and increases in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with depth are
331 suggestive of increasing SOC stabilization with depth in our sites. One commonly
332 held theory is that the $\delta^{13}\text{C}$ increases with depth are reflective of isotopic
333 discrimination that occurs during microbial decomposition (Garten Jr 2006;

334 Nadelhoffer and Fry 1988), whereby microbes consume SOC, respire the light
335 isotope (^{12}C), and incorporate the heavier isotope (^{13}C) into biomass that is
336 subsequently deposited in the soil OM complex. As this process proceeds SOC
337 particles decompose into smaller sizes, which then physically migrate downward
338 (Acton et al. 2013; Kayler et al. 2011). The C/N ratio of soil OM also decreases with
339 progressive biodegradation because plant-derived OM contains less nitrogen than
340 microbial-derived OM (Baumann et al. 2009). Thus, as plant material is degraded, the
341 proportion of carbon declines and nitrogen increases (Simpson and Simpson 2012).

342 With one exception (site HZY) soil $\delta^{13}\text{C}$ values were higher in the temperate
343 zone than in the sub-tropical sites for a given horizon (Fig. 3b). Grass understory
344 vegetation beneath *Quercus variabilis* was only observed in sub-tropical site HZY;
345 thus, the exceptionally high $\delta^{13}\text{C}$ there might be explained by a historical influence of
346 C4 grasses contributing to SOC, as $\delta^{13}\text{C}$ is much higher for C4 than C3 plant tissue
347 (Vitarello et al. 1989). A simple interpretation of the slightly lower $\delta^{13}\text{C}$ in the
348 other sub-tropical than the temperate sites is not possible as Balesdent et al. (1993)
349 indicated wide variation in tree tissue $\delta^{13}\text{C}$ within a single climatic region. However,
350 in the present study, leaf $\delta^{13}\text{C}$ value had a strong negative relationship with mean
351 annual precipitation ($R^2 = 0.91$, $P = 0.001$; Supplementary Fig. S2) and decreased
352 from north to south. Therefore, it seems likely that differences in soil $\delta^{13}\text{C}$ between
353 the temperate and sub-tropical zones reflected differences in detrital sources rather
354 than degree of decomposition.

355 In summary, to our knowledge, this is the first regional investigation on SOC
356 quantity and degree of decomposition encompassing warm-temperate to

sub-tropical forest zones with control for tree species composition. We have explored two methods to characterize the degree of SOC degradation. The NMR analysis provided the best index of degree of SOC degradation across the climatic gradient whereas isotopic composition ($\delta^{13}\text{C}$) better reflected the degree of SOC decomposition along the soil depth gradient. Site differences in litter isotopic composition might have masked the latitudinal variation in SOC degradation. Thus, our results highlight the importance of using different techniques for characterizing SOC. No fully consistent latitudinal patterns were observed for SOC quantity. The alkyl/methoxyl ratio, based on NMR spectra, appeared to provide the most informative index of degree of SOC decomposition. This index was strongly related to soil pH, indicating a greater degree of SOC degradation at lower soil pH in the wetter sub-tropical sites, which may reflect the effect of climate on pH together with the effect of pH on laccase enzymes. Further study of the interactions among macroclimate, soil development and SOC under controlled conditions of vegetation composition could contribute to improved understanding of controls on SOC accumulation in forest soils.

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Table 1 Selected climate, stand and mineral soil characteristics at seven forest study sites composed of *Quercus variabilis* in China.

Selected properties	Site						
	PG	HYS	BA	HZY	XY	HS	YS
Latitude (°N)	40.25	39.48	37.09	34.02	32.12	30.12	29.09
Longitude (°E)	117.12	115.48	113.83	117.06	114.01	118.29	115.62
Altitude (m)	260	516	801	117	131	450	360
MAP (mm)	591	530	492	688	1045	1701	1759
MAT (°C)	11.5	11.1	12.7	14.9	15.2	16.1	16.9
AET(mm)	454	423	421	548	677	810	853
Stand density (stem hm ⁻²)	2267	3200	800	1422	1066	978	933
DBH (cm)	12.43	12.83	14.22	22.85	16.84	28.17	28.07
Basal area (m ² hm ⁻²)	29.15	43.69	17.20	60.01	24.84	70.52	61.93
Canopy height (m)	11.2	9.4	12.6	16	13.6	13.0	18
Clay (%)	23.8±1.6	20.4±0.8	13±0.3	34.3±0.9	16.7±0.9	26.8±1.4	29.2±0.6
Silt (%)	53.9±1.3	47.0±2.2	35.6±1.3	53.2±1.4	43.7±1.0	49.3±1.1	24.8±1.1
Sand (%)	22.3±0.3	32.7±1.8	51.4±1.5	12.5±0.7	39.6±1.5	23.9±1.3	46±0.5
pH (in water)	6.24±0.10	6.69±0.20	5.95±0.11	7.58±0.13	4.50±0.04	4.74±0.03	4.35±0.05
Soil density (g cm ⁻³) *	0.93±0.04	0.99±0.03	1.01±0.04	1.26±0.02	1.02±0.05	0.93±0.02	0.98±0.03
C (%)	2.37±0.12	3.45±0.28	3.15±0.12	2.04±0.04	3.82±0.45	2.73±0.13	3.99±0.17
N (%)	0.22±0.01	0.28±0.02	0.27±0.01	0.18±0.00	0.28±0.03	0.22±0.01	0.30±0.01
Total OC stock (kg m ⁻²)	3.41±0.20	4.32±0.29	4.07±0.12	2.95±0.09	4.72±0.48	2.71±0.13	4.18±0.17

Note: MAT, mean annual temperature; MAP, mean annual precipitation; AET, annual actual evapotranspiration. The Climatic data was an average of the last five years recorded by the local ecological stations. DBH, stem diameter at breast height. Values were mean ± standard error, n=5, *n=15. Total OC stock, including both organic horizons and 0-10 cm mineral soil.

Table 2 Stepwise multi-regression analysis of SOC quantity and composition with soil and climatic variables

SOC quantity and composition	Variables entered	R ²	n	P	Regression
C concentration (%)	Clay + silt	0.66	7	0.026	$y = -0.04x + 5.94$
A/O ratio	pH	0.73	7	0.014	$y = -0.04x + 0.84$
A/M ratio	pH	0.90	7	0.001	$y = -0.37x + 5.25$

Note: Variables used in the regression analysis: clay + silt content, pH value, mean annual temperature, mean annual precipitation, annual actual evapotranspiration. For C stock, none of the five variables entered into the model.

Table 3 SOC composition (%) in different soil depths at seven study sites usingCP-MAS ¹³C NMR. Sites are arranged in order of decreasing latitude.

Site	Depth (cm)	Alkyl	N-alkyl/Methoxyl	Carbohydrate	Di-O-alkyl	Aryl	O-aryl	Carboxyl	Ketone	A/O ratio	A/M ratio
PG	0-2	26.41	9.96	29.28	8.31	9.44	4.38	10.17	2.05	0.56	2.65
	2-5	24.41	9.89	30.28	8.46	9.36	4.19	11.16	2.25	0.50	2.48
	5-10	27.28	9.48	28.65	7.69	10.11	4.12	10.78	1.89	0.60	2.88
HYS	0-2	26.08	10.83	29.68	7.85	9.01	3.93	10.78	1.84	0.54	2.42
	2-5	28.20	11.03	30.00	7.47	8.83	3.13	10.22	1.13	0.58	2.57
	5-10	29.15	9.50	28.88	7.42	9.31	3.37	10.82	1.55	0.64	3.07
BA	0-2	27.76	10.28	29.84	7.77	8.54	3.70	10.26	1.85	0.58	2.70
	2-5	27.33	10.00	29.96	7.57	8.51	3.80	10.81	2.03	0.58	2.74
	5-10	27.25	9.03	30.54	7.98	9.43	3.42	10.49	1.85	0.57	3.02
HZY	0-2	24.85	10.31	30.95	8.65	8.33	4.08	10.69	2.15	0.50	2.41
	2-5	26.88	10.33	30.57	8.14	7.57	3.50	10.98	2.03	0.55	2.60
	5-10	27.63	10.11	31.07	8.08	7.56	3.02	10.88	1.65	0.56	2.73
XY	0-2	28.94	9.02	30.09	7.87	8.27	4.21	9.40	2.20	0.62	3.21
	2-5	30.62	8.19	29.40	7.74	8.33	3.63	9.94	2.15	0.68	3.75
	5-10	33.20	8.43	28.61	7.36	7.79	3.42	9.32	1.87	0.75	3.94
HS	0-2	27.55	9.49	34.65	8.46	6.87	2.52	9.12	1.34	0.53	3.00
	2-5	31.18	8.51	32.38	7.75	7.56	2.29	9.10	1.25	0.64	3.67
	5-10	30.21	8.54	32.99	7.59	7.76	1.92	9.71	1.28	0.62	3.54
YS	0-2	30.35	8.48	30.12	7.54	8.31	3.14	10.29	1.77	0.66	3.58
	2-5	28.94	8.85	29.84	7.61	8.79	3.00	11.05	1.93	0.63	3.29
	5-10	31.11	7.88	29.52	6.87	9.17	2.25	11.54	1.66	0.70	3.95
PG		26.25	9.70	29.27	8.04	9.75	4.19	10.77	2.03	0.56	2.71
HYS		28.25	10.22	29.38	7.52	9.10	3.41	10.63	1.48	0.59	2.79
BA		27.38	9.57	30.22	7.82	8.98	3.59	10.54	1.90	0.58	2.87
HZY	0-10	26.85	10.22	30.90	8.21	7.72	3.37	10.87	1.86	0.54	2.63
XY		31.58	8.48	29.15	7.57	8.05	3.64	9.52	2.02	0.70	3.74
HS		29.97	8.72	33.14	7.81	7.52	2.15	9.40	1.29	0.61	3.47
YS		30.31	8.29	29.73	7.22	8.89	2.66	11.14	1.76	0.67	3.68

Note: Values were mean of two replicates.

Table 4 Pearson correlations (*r*) among SOC and soil and climatic variables across seven oak forest study sites in China (N=7).

	Clay + silt (%)	pH	MAT (°C)	MAP (mm)	AET (mm)
C stock (kg m ⁻²)	-0.75	-0.32	-0.17	-0.16	-0.12
C concentration (%)	-0.81*	-0.69	0.25	0.37	0.36
Alkyl C (%)	-0.41	-0.85*	0.67	0.72	0.76*
N-alkyl/Methoxyl C (%)	0.47	0.97**	-0.75	-0.83*	-0.85*
Carbohydrate C (%)	0.37	-0.09	0.46	0.47	0.43
Di-O-alkyl C (%)	0.71	0.68	-0.30	-0.51	-0.47
O-alkyl C (%)	0.64	0.51	-0.03	-0.10	-0.14
A/O ratio (%)	-0.55	-0.86*	0.57	0.61	0.66
A/M ratio (%)	-0.45	-0.95**	0.75	0.82*	0.85*
Clay + silt (%)		0.55	-0.06	-0.12	-0.08
pH			-0.61	-0.78*	-0.76*
MAT (°C)				0.86*	0.93**
MAP (mm)					0.98**

Note: **P < 0.01; *P < 0.05.

Figure captions

Fig. 1. Distribution of the seven oriental oak forest sites along a 1500-km climatic gradient in East China. Three sites (PG, HYS, BA) are in the warm temperate zone, four sites (HZY, XY, HS, YS) are in the sub-tropical zone.

Fig.2. Soil organic carbon stock (kg m^{-2}) in organic and mineral layers in seven oriental oak stands. Values were mean $\pm$ standard error, $n=5$.

Fig. 3. Elemental and isotopic composition of organic matter in forest floor and mineral soils in oriental oak stands along the latitudinal gradient. (a), C/N ratios; (b), $\delta^{13}\text{C}$; (c), $\delta^{15}\text{N}$. Values were mean $\pm$ standard error, $n=5$.

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Fig. 1

Fig. 2

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Fig. 3

Supplementary Table S1 Results of analysis of variance for the effects: (A) on carbon stock of zone; (B) on isotopic composition of zone for each depth layer; and (C) SOC composition (0-10 cm) of zone

Subject	Effect	DF	F value	$P_r > F$
A. Carbon stock (kg m^{-2})				
Forest floor horizon	Zone	1	34.88	< 0.001
0-10 cm mineral horizon	Zone	1	0.86	0.359
B. Isotopic composition				
Oe				
C/N ratio	Zone	1	13.86	0.001
$\delta^{13}\text{C}$	Zone	1	69.30	< 0.001
$\delta^{15}\text{N}$	Zone	1	1.66	0.206
Oa*				
C/N ratio	Zone	1	7.78	0.010
$\delta^{13}\text{C}$	Zone	1	42.54	< 0.001
$\delta^{15}\text{N}$	Zone	1	18.36	< 0.001
0-2 cm				
C/N ratio	Zone	1	7.52	0.010
$\delta^{13}\text{C}$	Zone	1	7.70	0.009
$\delta^{15}\text{N}$	Zone	1	0.09	0.767
2-5 cm				
C/N ratio	Zone	1	13.23	0.001
$\delta^{13}\text{C}$	Zone	1	0.58	0.454
$\delta^{15}\text{N}$	Zone	1	2.30	0.139
5-10 cm				
C/N ratio	Zone	1	15.98	< 0.001
$\delta^{13}\text{C}$	Zone	1	0.25	0.620
$\delta^{15}\text{N}$	Zone	1	1.99	0.168
C. SOC composition (0-10 cm)				
Alkyl C (%)	Zone	1	7.11	0.021
N-alkyl/Methoxyl C (%)	Zone	1	5.42	0.038
Carbohydrate C (%)	Zone	1	2.08	0.175
Di-O-alkyl C (%)	Zone	1	0.17	0.689
O-alkyl C (%)	Zone	1	0.01	0.916
A/O ratio	Zone	1	3.66	0.080
A/M ratio	Zone	1	8.05	0.015

Note: * three sites in the warm temperate zone, and two sites in the sub-tropical zone.

Supplementary Fig. S1 Representative CP-MAS ¹³C NMR spectra of different mineral soil horizon samples (0-2, 2-5, and 5-10 cm) at seven oriental oak sites. *, spinning sideband of carboxyl C (165-190 ppm).

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Supplementary Fig. S2 Linear relationship between mean annual precipitation and oriental oak leaf delta ¹³C along the gradient

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