RESEARCH ARTICLE

# Soil Organic Carbon Pool and Its Chemical Composition in *Phyllostachy pubescens* Forests at Two Altitudes in Jian-ou City, China

Haibao Ji[1,2], Shunyao Zhuang[1]*, Zhaoliang Zhu[1], Zheke Zhong[3]

1 State Key Lab of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province, China, 2 University of Chinese Academy of Sciences, Beijing, China, 3 China Bamboo Research Center, Chinese Academy of Forestry, Hangzhou, Zhejiang Province, China

* syzhuang@issas.ac.cn

## Abstract

*Phyllostachys pubescens* forests play an important role in soil organic carbon (SOC) sequestration in terrestrial ecosystems. However, the estimation and mechanism of SOC sequestration by *P. pubescens* forests remain unclear. In this study, the effect of *P. pubescens* forest distribution with elevation was investigated at two altitude sites in Jian-ou City, Southeast China. SOC storage was estimated and its chemical composition was obtained via ^{13}C-nuclear magnetic resonance (NMR), chemical classification, and spectral analysis. Results showed that the SOC contents and stocks were significantly higher at the high-altitude site than at the low-altitude site in the entire soil profile (0–60 cm). The C contents of the three combined humus forms exhibited similar responses to the elevation change, and all of these forms were higher at the high-altitude site than at the low-altitude site regardless of soil layer. However, the proportions of the three combined humus C showed no significant differences between the two altitudes. The results of ^{13}C-NMR showed that the SOC chemical composition did not significantly vary with elevation as well. This finding was consistent with the $E_{465}/E_{665}$ of the loosely combined humus. Overall, the results suggested that altitude should be considered during regional SOC estimation and that altitude affected the quantity rather than the quality of the SOC under the same *P. pubescens* vegetation.

## Introduction

The global soil organic carbon (SOC) storage is estimated at 1,550 Pg, which exceeds the cumulative pool of atmospheric C (760 Pg) and biotic C (560 Pg) [1]. Accordingly, any small change in the soil C pool size may considerably affect the atmospheric $CO_2$ content [2]. Forest ecosystems contain a globally significant amount of C, i.e., approximately half of the earth's terrestrial C (1,146 Pg), of which two-thirds (787 Pg) reside in forest soils [3, 4]. Mountain regions mostly containing forests represent heterogeneous environments that are vulnerable to climate change [5–7]. Understanding the relationship between mountain forest SOC and climate change is important. Changes in climatic variables, particularly precipitation and temperature along

altitudinal gradients in mountain forest ecosystems, influence the type of vegetation and, consequently, the amount, chemical composition, and turnover of SOC [8, 9]. Therefore, SOC stocks become altered with elevation [10] as precipitation increases and temperature decreases [9, 11, 12]. However, studies on soil C stocks along elevation gradients in alpine regions revealed different relationships with altitude, including a decrease, unimodal response, or no change [6]. Djukic et al. [13] stated that SOC stocks increase with elevation in low-elevation forest sites but decrease with elevation in high-elevation grassland sites in the Austrian Limestone Alps. In the Swiss Alps, Leifeld et al. [14] reported that soil C stocks do not correlate with change in elevation. The variability in relationships across studies revealed the likely importance of local variability in climate–altitude relationships [6]. Therefore, studying the characteristics of SOC in different climate zones is essential.

Bamboo comprises approximately 1,500 species and 87 genera in the Bambusoideae subfamily worldwide [15]. China, with 500 species in 48 genera, has a highly rich bamboo flora [16]. Bamboo plantations are an indispensable part of forest ecosystems, especially in Southern China. From 1949 to 2009, the total bamboo plantation area increased from 1.3 million hectares (M ha) to 6 M ha mainly because of reforestation and afforestation on wastelands; bamboo forests account for approximately 3% of the total forest area in China [17]. *Phyllostachys pubescens* comprises the most important portion of bamboo forests, populating approximately 70% of the total bamboo forest area in China. *P. pubescens* grows naturally in a subtropical monsoon climate zone [18]. It grows at elevations between 10 and 1700 m above sea level (asl), but most of the areas actually inhabited are less than 800 m asl in the hills and mountains [19, 20]. However, no sufficient evidence is available to reflect the relationship between the sequestration of SOC and the elevation of *P. pubescens* growth because of limited studies. Studying the parameters of SOC and elevation in *P. pubescens* forests may provide an accurate estimation of C on a regional scale.

Presently, SOC stability is emphasized in the understanding of the impact of soil C on global climate change [21, 22]. In the past, C stability in the soil was traditionally evaluated by determining the decomposition rate of SOC through incubation experiments conducted in the laboratory [23]. Recently, $^{13}$C nuclear magnetic resonance (NMR) technology has been increasingly used to assess C stability by analyzing the C chemical structure of the soil [22, 24, 25]. The NMR technique enables the acquisition of information on the C chemical structure of entire soil samples without any physical or chemical fractionation; the method is suitable for characterizing the chemical structure of natural SOC pools [25, 26]. As reported, *P. pubescens* forests possess a high amount and potential in SOC sequestration [17, 27]. However, the stability of the sequestered SOC in *P. pubescens* forests remains unknown, including the influence of the response of SOC to climate changes.

Soil humus forms are morphological patterns observed in the association between organic and mineral matter at the top of soil profiles; these forms serve as indicators of ecosystem carbon cycling [28]. Several scholars have analyzed the relationship between humus forms and SOC. For instance, Bonifacio et al. [29] suggested that humus forms reflect several mechanisms of organic matter stabilization and are clearly related to the capacity of the soil to store C in Northwestern Italy. Andreetta et al. [30] reported that humus forms hold a clear potential in evaluating the SOC status of Mediterranean forest soils. Soil humus comprises only a small portion existing in the free state; it is chiefly combined with minerals to form organic mineral complexes named combined humus [31]. Lu [32] showed that combined humus can be generally divided into three types on the basis of its variation in binding manner and tightness. These types include loosely, stably, and tightly combined humus, all of which differ in carbon-sequestration and soil fertility characteristics. Loosely, stably, and tightly combined humus are mainly composed of Al–Fe–humus complexes, Ca–humus complexes, and humin, respectively.

The humification index can reflect the chemical composition of soil organic matter (SOM) [33]. Zech et al. [34] demonstrated that increased humification results in increased alkyl C, aromatic C, and carboxyl C contents and decreased O-alkyl C content.

Considered as a "bamboo hometown" in China, Jian-ou possesses the largest area ($8.6 \times 10^4$ ha) inhabited by *P. pubescens*, which distributes in all towns of this city. Bamboo growth mostly stretches from 100 m to as high as 1,200 m asl in this area. According to the local forestry bureau[35], Jian-ou has $2.8 \times 10^8$ culms of bamboo forests. Bamboo plays an important role in the local socio-economy. In 2010, the economic income from the bamboo industry reached ¥5.3 billion, which accounted for 50% of the gross domestic product (GDP) of Jian-ou City [27].

Since the 1990s, numerous studies on SOC have been conducted in *P. pubescens* forests [17, 27, 36–38]. However, minimal information is available regarding the carbon-stock variation of *P. pubescens* with varying elevations. Moreover, soil C pools and their dynamics with *P. pubescens* on a regional scale have been studied less frequently. Therefore, the present study aimed to investigate the SOC pool, its chemical structure, and humification indices in *P. pubescens* forests at two altitudes in Jian-ou City, Southeast China for an improved understanding of SOC storage and turnover in *P. pubescens* forests.

## Materials and Methods

### Study site description

The study site was located in Jian-ou City (26°38′N–27°20′N, 117°58′E–118°57′E), northern part of Fujian Province, Southeast China. The area experiences a monsoonal subtropical climate with a mean annual temperature of 19.3°C and an average annual precipitation of 1,600–1,800 mm. The warmest and coldest months are July and January, respectively. The site has an average of 1,612 daylight hours and 286 frost-free days. Hilly landform dominates this region, which is near Wuyi Mountain, the likely center of origin of *P. pubescens* in China [19]. The soils in the experimental site were classified as "red soil" in the Chinese system of soil classification [39], equivalent to Ferralsols in the food and agriculture organization (FAO) soil classification system [40].

### Experimental design and soil sampling

Zhang et al. [41] divided the 0–1000 m elevation range into 10 elevation gradients of 100 m each in order to investigate the distribution area of *P. pubescens* forests in Jian-ou City. In their survey, they found the two elevation ranges (0–400 m and 400–1000 m) were occupied approximately 50% areas of *P. pubescens* forests among the 1000 m range, respectively. They also found that the bamboo mainly distributes in the range from 100–800 m. Hence, we selected the middle altitudes from the two elevation ranges as our sampling sites. Soil samples were collected from two bamboo forests at the 200 m (low-altitude site; LAS) and 761 m (high-altitude site; HAS) elevations asl. The sampling sites were homogeneously distributed in Jian-ou City, and permission to enter each site was given by Fujian Jian-ou Forestry Bureau, China. Three bamboo plots of 100 m$^2$ (10 m × 10 m) areas were chosen in each altitude site. In a 2012 bamboo survey by Ji et al. (unpublished data), the average culm densities of these selected *P. pubescens* forests were 3,600 and 3,900 culms ha$^{-1}$ at the two altitudes, respectively, and the average age of bamboo culms for these selected *P. pubescens* forests was between 1 and 2 years. Both culm density and culm age of these selected *P. pubescens* forests did not differ between elevations; however, the aboveground bamboo biomass increased with elevation, from 53 Mg ha$^{-1}$ (LAS) to 73 Mg ha$^{-1}$ (HAS).

Within each plot, soil samples were obtained from the 0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm layers from five randomly selected points. The soil samples from the five sampling

points within the same layer were mixed to form a composite sample. The samples were transported to the laboratory, air-dried, and then sieved (2 mm) to homogenize the sample and remove visible roots for further analysis. During the field soil sampling, soil bulk-density samples were collected using a bulk density corer with a 200 cm$^3$ volume.

There was no need of approval by Institutional Review Board (IRB) or Ethics Committee or by an Institutional Animal Care and Use Committee (IACUC) or equivalent animal ethics committee because our study was not human subject research and our object was *P. pubescens* forest which was a plantation but not an animal.

## Analysis of soil chemical and physical properties

Soil pH was analyzed using a pH meter in a 1:2.5 (w/v) soil/water extract. Total SOC was determined through the wet-combustion method with 133 mM $K_2Cr_2O_7$ and concentrated $H_2SO_4$ at 220–230°C; the total nitrogen (N) in the digest was measured using a semi-micro Kjeldahl method [32]. Available phosphorus (P) and potassium (K) were determined through the HCl–$NH_4F$ extraction–colorimetry method and the $NH_4OAC$ extraction–flame photometry method, respectively. Inorganic N ($NH_4^+$–N and $NO_3^-$–N) was analyzed through KCl extraction–colorimetry. The water content was measured by obtaining 10 g of fresh soil sample from the bulk density sample and drying this sample at 105°C to a constant weight. The water content of each sample was used to calculate the bulk density on the basis of the volume and total fresh weight of the soil within each soil corer. All methods described above followed those of Lu [32].

The SOC content ($C_c$) and SOC storage ($C_t$) in each soil layer were calculated using the following formula [42]:

$$C_c(g\,kg^{-1}) = 0.58 \times SOM$$

and

$$C_t(Mg\,ha^{-1}) = C_c \times BD \times D \times 0.1,$$

where SOM is the soil organic matter (g kg$^{-1}$), BD is the bulk density of the soil layer (g cm$^{-3}$), and D is the sampling depth of the soil layer (cm). The coefficient 0.58 transforms SOM into SOC.

## Analysis of combined humus forms

Combined humus forms are classified as described by Lu [32]. The extraction procedures for the different forms are listed as follows: (1) the loosely combined humus was extracted using 0.1 M NaOH; (2) the stably combined humus was extracted using 0.1 M $Na_4P_2O_7$ + 0.1 M NaOH mixed liquid (pH 13); and (3) the residue was considered as the tightly combined humus [32].

Both of the loosely and stably combined humus solution was measured at 465 and 665 nm using the ultraviolet spectrophotometer (Mapada UV-3100), respectively[43]. The $E_{465}/E_{665}$ ratio was calculated by dividing the absorbance of the sample at 465nm by that at 665nm. The $E_{465}/E_{665}$ ratio is related to the aromaticity and to the degree of condensation of the chain of aromatic carbons of the humic acids, and could be used as a humification index[44, 45]. Low $E_{465}/E_{665}$ ratio reflects a high degree of condensation of these structures while high ratio means presence of large quantities of aliphatic structures and low quantities of condensed aromatic structures [46]. This ratio also is inversely related to the degree of aromaticity, particle size, molecular weight, and acidity[47].

The loosely and stably combined humus C contents were measured by a liquid C/N analyzer, whereas the tightly combined humus C content was calculated by subtracting the sum of the loosely and stably combined humus C contents from the total humus C content [32].

## $^{13}$C-NMR analysis

Prior to $^{13}$C-NMR analysis, the soil samples were pretreated with HF to remove $Fe^{3+}$ and $Mn^{2+}$ from the soil and consequently increase the signal-to-noise ratio of the spectrum. The HF pre-treatment was conducted using the methods of Mathers et al. [48] and Zhang et al. [49] as follows. Air-dried soil (5 g) was placed in a 100 mL centrifuge tube, to which 50 mL of HF solution (10% v/v) was added. The tube was capped, shaken at 120 rpm for 1 h, and then centrifuged for 10 min at 3,000 rpm. Afterward, the clear solution was discarded, and the residue was again treated with HF. This procedure was repeated eight times, with the shaking time solely varying across the different cycles (1 h shaking time for the first four, 12 h for the next three, and 24 h for the last cycle). The soil sample was then washed with distilled water to remove the residual HF. For each wash, 50 mL of distilled water was added to the soil sample in the centrifuge tube, which was shaken at 120 rpm for 10 min, centrifuged at 3,000 rpm for 10 min, and discarded of clear solution. This process was repeated four times. The washed soil sample was dried at 40°C in an oven, ground to pass through a 0.15 mm sieve, and then stored for further analysis.

The HF-treated soil samples were analyzed with a cross polarization magic-angle-spinning (CPMAS) solid-state NMR spectroscopy. The CPMAS NMR spectra were acquired through a Bruker Avance 300 MHz NMR spectrometer (Spectrospin, Rheinstetten, Germany). The measurement employed a 7 mm CPMAS detector, a frequency of 75 MHz, a magic-angle-spinning frequency of 5,000 Hz, a contact time of 2 ms, and a recycle delay time of 2.5 s. The external standard used for chemical shift determination was glycine (carboxyl at 176.4 ppm). According to literature [50, 51], each NMR spectrum was divided into the following four regions representing the different chemical environments of a $^{13}$C nucleus: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–200 ppm). We obtained the relative content of the different C fractions by measuring the area under the curve for each region. Two indices of organic matter stability were calculated as follows: (1) A/O-A = alkyl C / O–alkyl C [50] and (2) aromaticity = aromatic C / (alkyl C + O-alkyl C + aromatic C)[51].

## Statistical analysis

Data were presented as the average of triplicates. Independent t test was used to assess the effects of altitude change on each soil layer. Each elevation gradient was treated as a block, and the statistical significance of the differences in effects of the elevation change on the nutrient contents, different C forms, humification indices, and the C chemical structure of the soil was determined. An alpha level of 0.05 for significance determination was used in all statistical analyses. Statistical analyses were performed using the SPSS software version 20 (IBM, Chicago, IL, USA).

## Results

### Soil chemical and physical properties

In the 0–10 cm and 10–20 cm soil layers, the pH was higher in the HAS than in the LAS ($P < 0.05$), but no significant difference in the other layers was found (Table 1). In the profile of the LAS, soil pH increased with increasing depth from 4.26 to 4.58. However, no significant difference in soil pH across different soil depths was observed in the profile of the HAS. The SOC contents decreased with increasing soil depths from 19.04 g kg$^{-1}$ to 9.72 g kg$^{-1}$ and from 34.37 g kg$^{-1}$ to 12.41 g kg$^{-1}$ in the LAS and HAS, respectively. In general, the SOC content was higher in the HAS than in the LAS. The soil total nitrogen exhibited a distribution pattern similar to that of the SOC. However, inorganic nitrogen, available phosphorus, available potassium, and bulk

**Table 1. Selected soil chemical and physical properties in *Phyllostachys pubescens* forests at two altitudes[a].**

| Soil profile | Elevation | pH | Organic C (g kg⁻¹) | Total N (g kg⁻¹) | Inorganic N (mg kg⁻¹) | Available P (mg kg⁻¹) | Available K (mg kg⁻¹) | Bulk density (g cm⁻³) |
|---|---|---|---|---|---|---|---|---|
| 0–10 cm | LAS | 4.26 b | 19.04 b | 1.16 b | 42.29 a | 15.91 a | 59.00 a | 0.90 a |
| | HAS | 4.66 a | 34.37 a | 1.94 a | 29.60 a | 10.29 a | 55.00 a | 0.75 a |
| 10–20 cm | LAS | 4.32 b | 16.84 b | 0.79 b | 35.24 a | 11.14 a | 49.33 a | 0.91 a |
| | HAS | 4.65 a | 27.19 a | 1.73 a | 25.01 a | 7.16 a | 39.67 a | 0.83 a |
| 20–40 cm | LAS | 4.46 a | 12.73 b | 0.91 b | 25.44 a | 8.43 a | 38.00 a | 1.11 a |
| | HAS | 4.62 a | 22.09 a | 1.65 a | 24.07 a | 5.45 a | 35.67 a | 0.98 a |
| 40–60 cm | LAS | 4.58 a | 9.72 a | 0.79 a | 18.36 a | 5.93 a | 26.33 a | 1.08 a |
| | HAS | 4.66 a | 12.41 a | 0.85 a | 15.58 a | 3.24 b | 20.67 a | 1.12 a |

LAS: low-altitude site; HAS: high-altitude site.

[a] Means with different letters indicate significant differences between the two altitudes for each parameter within each soil layer at $P = 0.05$ level according to the independent t test.

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density were higher at the LAS than at the HAS ($P > 0.05$). All of these indicators were closely related to the soil depth. The soil inorganic nitrogen as well as the available phosphorus and potassium decreased while the soil bulk density increased with increasing soil depth (Table 1).

## SOC storage

As shown in Fig 1, the SOC storage in the 0–60 cm layer amounted to 81.75 Mg hm⁻² in the LAS, which was lower than 118.6 Mg hm⁻² in the HAS ($P < 0.05$). In the 0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm layers, SOC storage was also lower in the LAS than in the HAS. However, the difference in SOC content between the two sites was significant only in the 0–10 cm and 20–40 cm layers and not in the 10–20 cm and 40–60 cm layers($P < 0.05$).

## Soil combined humus carbon forms

The three types of combined humus C (loosely, stably, and tightly combined humus C) are shown in Fig 2 and Table 2. The C contents of the loosely, stably, and tightly combined humus ranged from 4.5 g kg⁻¹ to 13.3 g kg⁻¹ (Fig 2A), 0.6 g kg⁻¹ to 1.2 g kg⁻¹ (Fig 2B), and 4.6 g kg⁻¹ to 19.8 g kg⁻¹ (Fig 2C), respectively. In the 0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm soil layers, the C content of the loosely combined humus was 80.3%, 64.2%, 74.9%, and 31.2% higher at the HAS than at the LAS, respectively (Fig 2A). Similarly, the C content of the stably combined humus was 57.7%, 66.3%, 41.5%, and 53.9% higher (Fig 2B), and the C content of the tightly combined humus was 82.3%, 59.0%, 69.9%, and 20.9% higher (Fig 2C) at the HAS than at the LAS in the respective soil layers. The combined humus forms were arranged on the basis of C content in the following order: tightly > loosely > stably combined humus C (Fig 2). All of the combined-humus C contents decreased with increasing soil depth at both sites (Fig 2). However, the proportions of the three combined humus C showed no significant differences between the two altitudes regardless of soil layer ($P > 0.05$) (Table 2).

The $E_{465}/E_{665}$ values of the loosely combined humus were higher at the LAS than at the HAS ($P > 0.05$) (Fig 3A). By contrast, those of the stably combined humus were higher at the HAS than at the LAS ($P > 0.05$) (Fig 3B).

## Chemical composition of SOC

The solid-state ¹³C CPMAS NMR spectra of soils in the 0–10 cm layer from the LAS and HAS are shown in Figs 4 and 5. The spectra shared similar patterns but differed in the relative
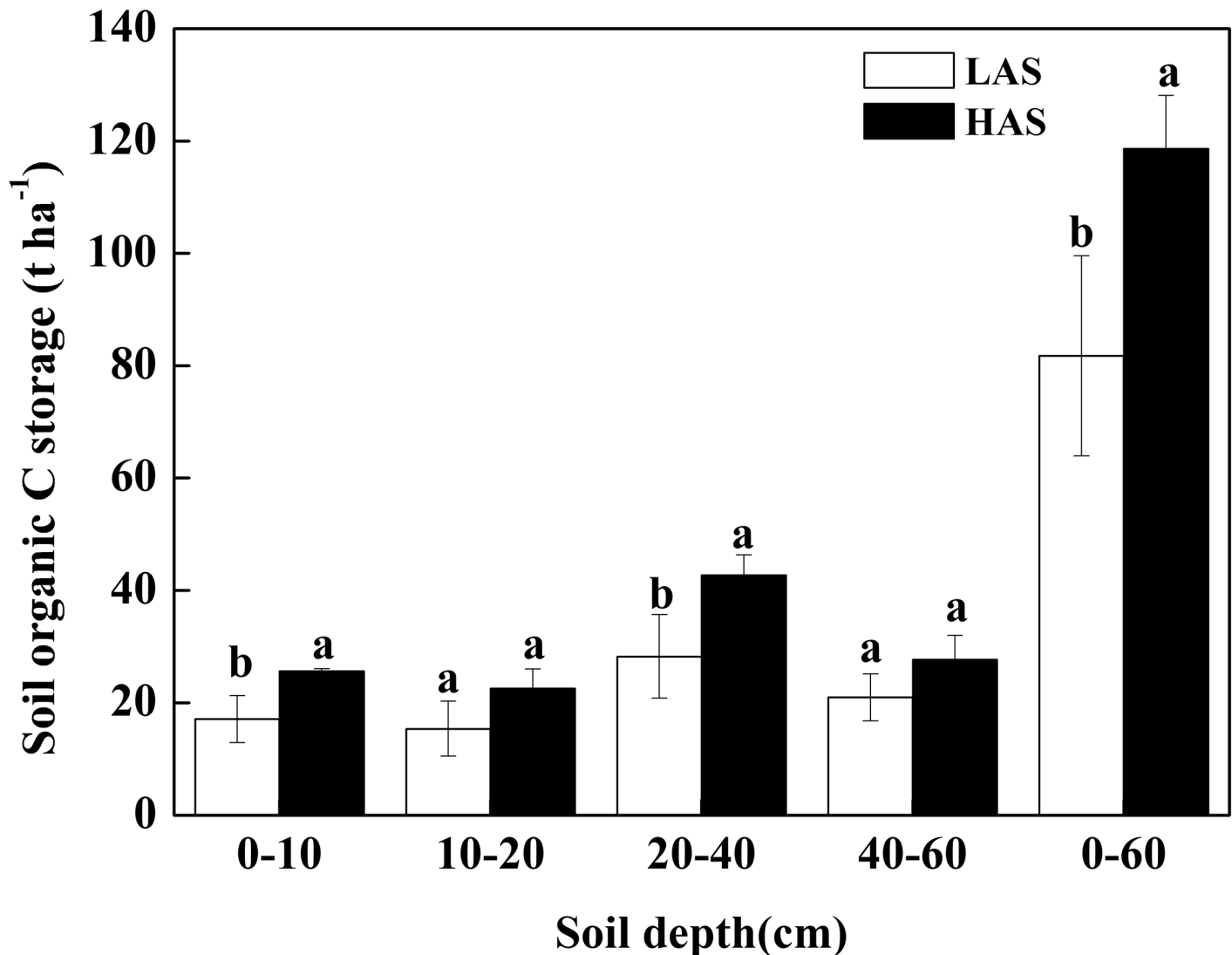
**Fig 1. Comparison of SOC storage in the different soil layers between two elevations.** LAS: low-altitude site; HAS: high-altitude site.

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intensity of the different chemical shift regions. The soil alkyl C, aromatic C content, alkyl to O-alkyl C ratio (A/O-A), and aromaticity were higher at the HAS than at the LAS, but no significant difference was found between the two sites ($P > 0.05$) (Table 3). However, the soil O-alkyl C content was significantly greater at the LAS than at the HAS ($P < 0.05$).

## Discussion

### Effect of altitude on soil properties

Soil pH is a function of parent material, weathering time, vegetation, and climate [52]. The weathering times and vegetation types of the two sites were similar; hence, the differences in soil pH could have resulted from the decreased nitrification in the higher elevation as shown by the low nitrate levels in the HAS (S1 Table). However, Dahlgren et al. [53] reported that soil pH decreases in an oak woodland-conifer forest elevational transect in the western US, and Smith et al. [52] showed that soil pH decreases over an elevation gradient in a shrub-steppe
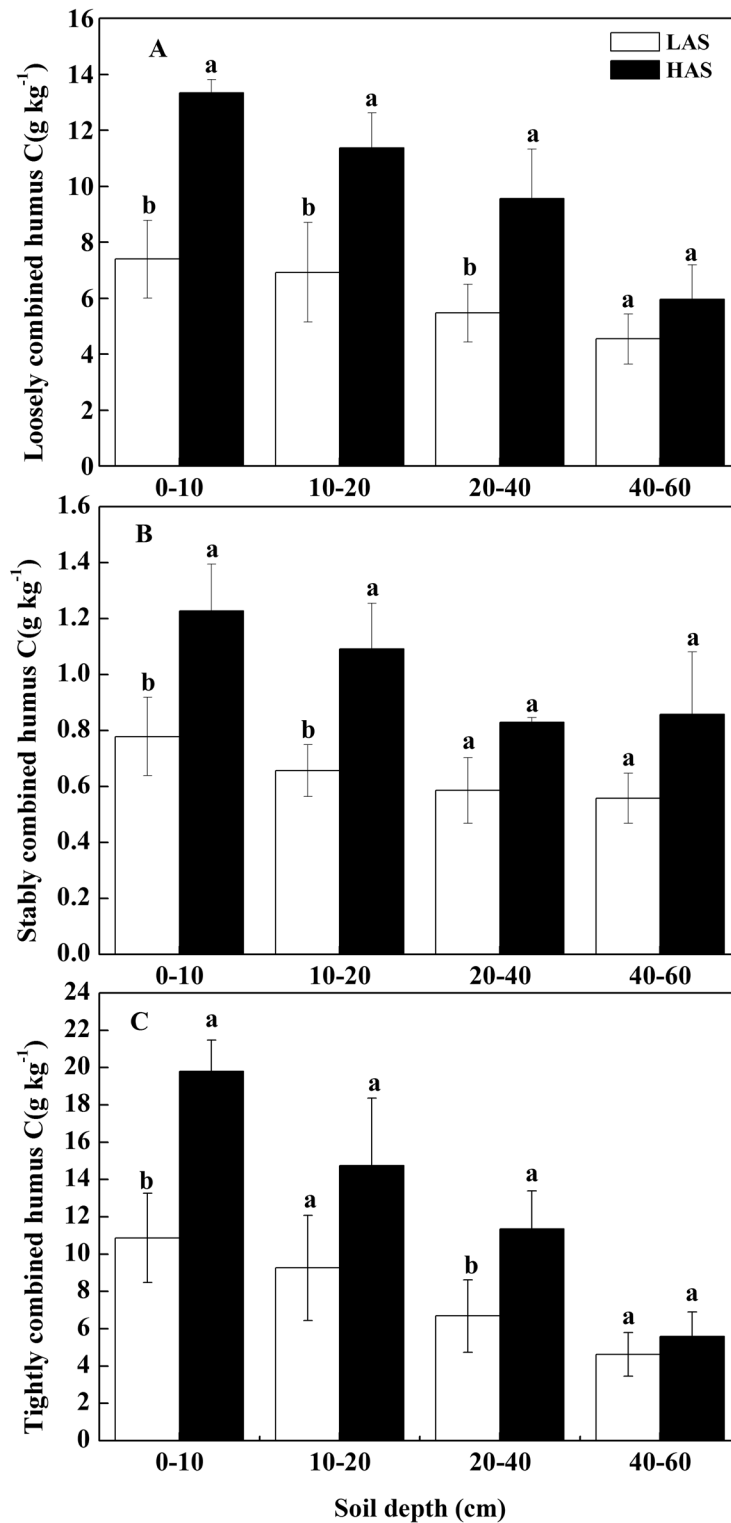
Fig 2. Comparison of the C contents of combined humus forms in the different soil layers between two elevations. C contents of (A) loosely combined humus, (B) stably combined humus, and (C) tightly combined humus. LAS: low-altitude site; HAS: high-altitude site.

**Table 2. Proportions of combined humus C in *Phyllostachys pubescens* forests at two altitudes[a].**

| Soil profile | Elevation | Soil Organic C (%) | | |
| --- | --- | --- | --- | --- |
| | | Loosely combined humus C | Stably combined humus C | Tightly combined humus C |
| 0–10 cm | LAS | 38.96 a | 4.12 a | 56.92 a |
| | HAS | 38.86 a | 3.56 a | 57.58 a |
| 10–20 cm | LAS | 41.36 a | 4.01 a | 54.64 a |
| | HAS | 42.25 a | 4.04 a | 53.71 a |
| 20–40 cm | LAS | 43.41 a | 4.63 a | 51.96 a |
| | HAS | 43.36 a | 5.12 a | 51.53 a |
| 40–60 cm | LAS | 46.86 a | 5.81 a | 47.33 a |
| | HAS | 47.96 a | 7.21 a | 44.84 a |

LAS: low-altitude site; HAS: high-altitude site.

[a] Means with different letters indicate significant differences between the two altitudes for each parameter within each soil layer at $P = 0.05$ level according to the independent t test.

ecosystem located in the eastern Washington State of the US. The discrepancies among different studies may be attributed to the variation in soil type, plant species, local climate, and other factors.

The obtained nitrogen contents showed a significant difference in the surface layers between the two altitude sites (Table 1). This result is consistent with the reports on the different types of mountain ecosystems [54, 55]. Low temperature and high moisture may have instigated such a result. However, the inorganic N, available P, and available K contents were higher at the LAS than at the HAS regardless of soil layer ($P > 0.05$), suggesting the decreased soil microbial activities in some extent.

## Effect of altitude on soil C storage and pools

The increase in SOC with increasing elevation had been previously reported in different types of mountain ecosystems [52, 56–58]. In the present study, the HAS contained higher SOC than the LAS in the 0–40 cm layers (Table 1). This SOC distribution pattern in the soil profile is expected because the underground portion of the *P. pubescens* plant is mainly distributed in the 0–30 cm layer, in which a greater amount of SOC secreted from this part is retained by the subsoil.

Incorporated with the bulk density, the SOC storage in the 0–60 cm layers was 45% higher in the HAS than in the LAS (Fig 1). Many researchers also reported an increase in SOC stocks with increasing altitude [59–64]. This increase likely originates from two sources as follows: (1) greater bamboo biomass along the elevation gradients and (2) decreasing litter decomposition with increasing altitude. The increase in tree biomass with increasing altitude indicates greater C input to the soil at high elevations. The result was comparable with a similar study performed in bamboo plantations, in which bamboo harvest reduced and litterfall input increased consequently with increasing altitude [65]. Smith et al. [52] found that the total soil C in a semi-arid shrub-steppe ecosystem increases with elevation probably because of the larger amount of plant biomass that results from greater precipitation at higher elevations. In general, temperature decreases with increasing elevation [10, 66, 67]. Low temperatures at high altitudes are useful in maintaining a low SOM decomposition rate [64]. Slowed litter decomposition and soil N mineralization at high elevations could presumably lead to the accumulation of total C content in the soils [54, 66], as we observed in the *P. pubescens* forests of the HAS. Therefore,
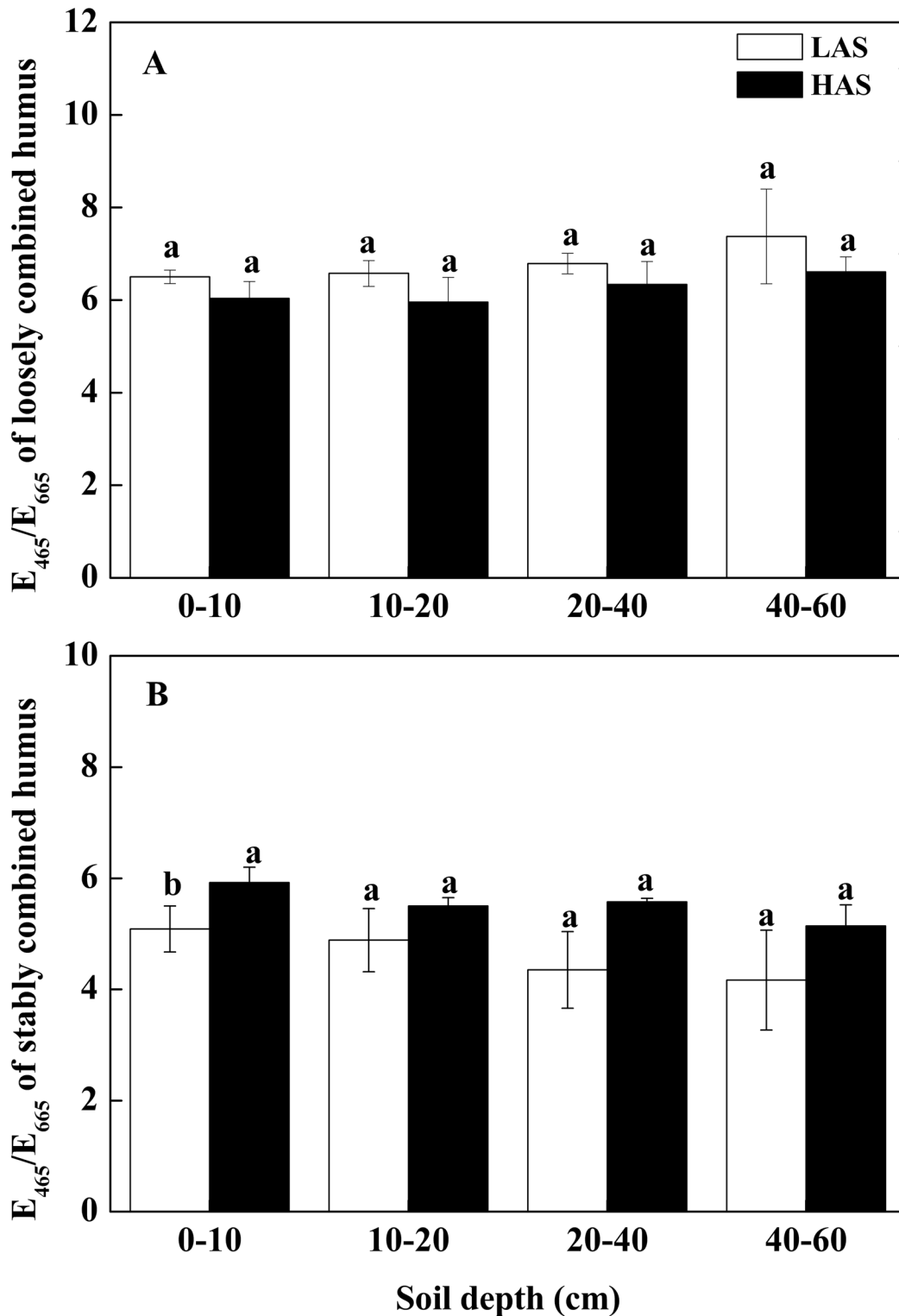
**Fig 3. $E_{465}/E_{665}$ ratios of combined humus. (A) Loosely combined humus. (B) Stably combined humus.** LAS: low-altitude site; HAS: high-altitude site.
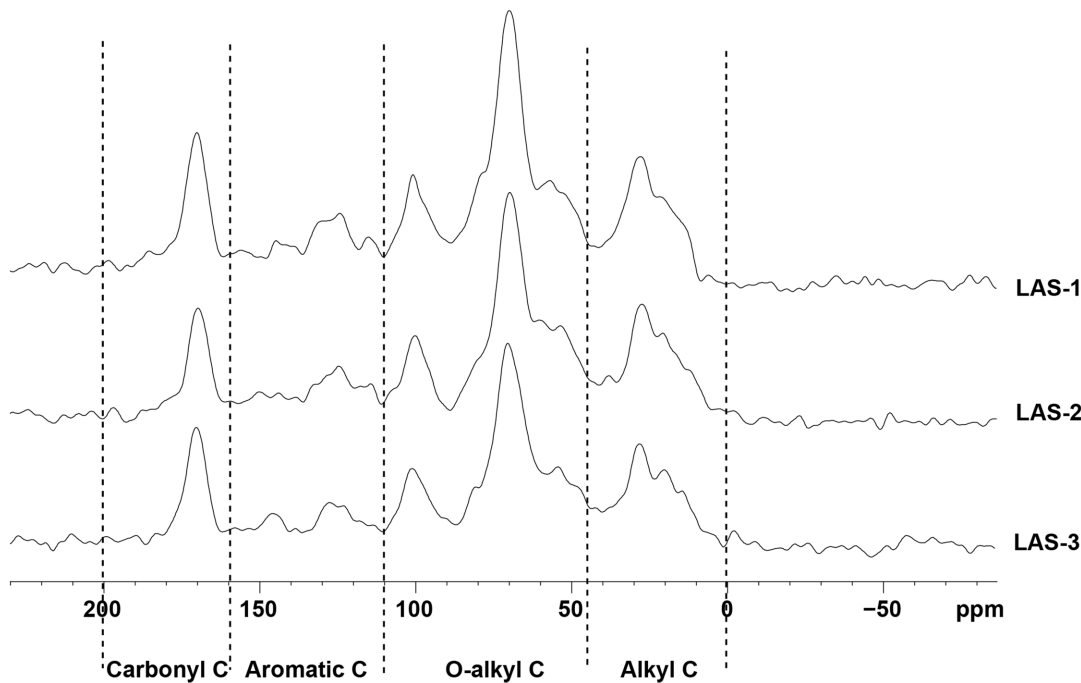
**Fig 4. Solid-state $^{13}$C-NMR spectra of soil samples from the LAS.** LAS: low-altitude site; HAS: high-altitude site.

elevation exerted a considerable influence on the SOC storage of *P. pubescens* forests. This altitude effect should be considered in future regional SOC storage estimation and management.

## Effect of altitude on SOC chemical composition

In this study, the different combined humus C contents showed similar responses to elevation change (Fig 2). The three combined humus C contents in all layers increased with elevation, similar to the SOC. Previous studies demonstrated that a relationship exists between SOC and organo-mineral complexes. In particular, Matus et al. [68] established a significant positive relationship between SOC and Al-complexed SOC in volcanic soils of Chile. Rasmussen et al. [69] found a highly significant positive correlation between Al–humus complexes and total C content in a California conifer forest, which could reflect the site's soil C dynamics and turnover. Our results showed that tightly combined humus C dominated the SOC in the soils of the three combined humus forms (Fig 2); this finding is consistent with the results of Ma et al. [70]. By contrast, Liu et al. [71] found that loosely combined humus C dominates the SOC in soils. The discrepancy among different studies could be caused by the differences in soil type, plant species, and other factors. However, the proportions of the three combined humus C showed no significant differences between the two altitudes (Table 2), suggesting that the three combined humus C structure was stable with the altitude in some extent.

The $^{13}$C-NMR technique has been used to assess the chemical characteristics of SOC [24]. Our results showed that O-alkyl C chiefly contributed to the SOC in the two altitude sites (Table 3); this finding is consistent with the results of Du et al. [58] and Zhang et al. [49]. By contrast, Jien et al. [72] found that alkyl C dominates the SOC in the soils of natural broad-leaved forests and adjacent coniferous plantations. The inconsistency among different studies could be caused by the differences in plant species, local climate, and management practices [73, 74].
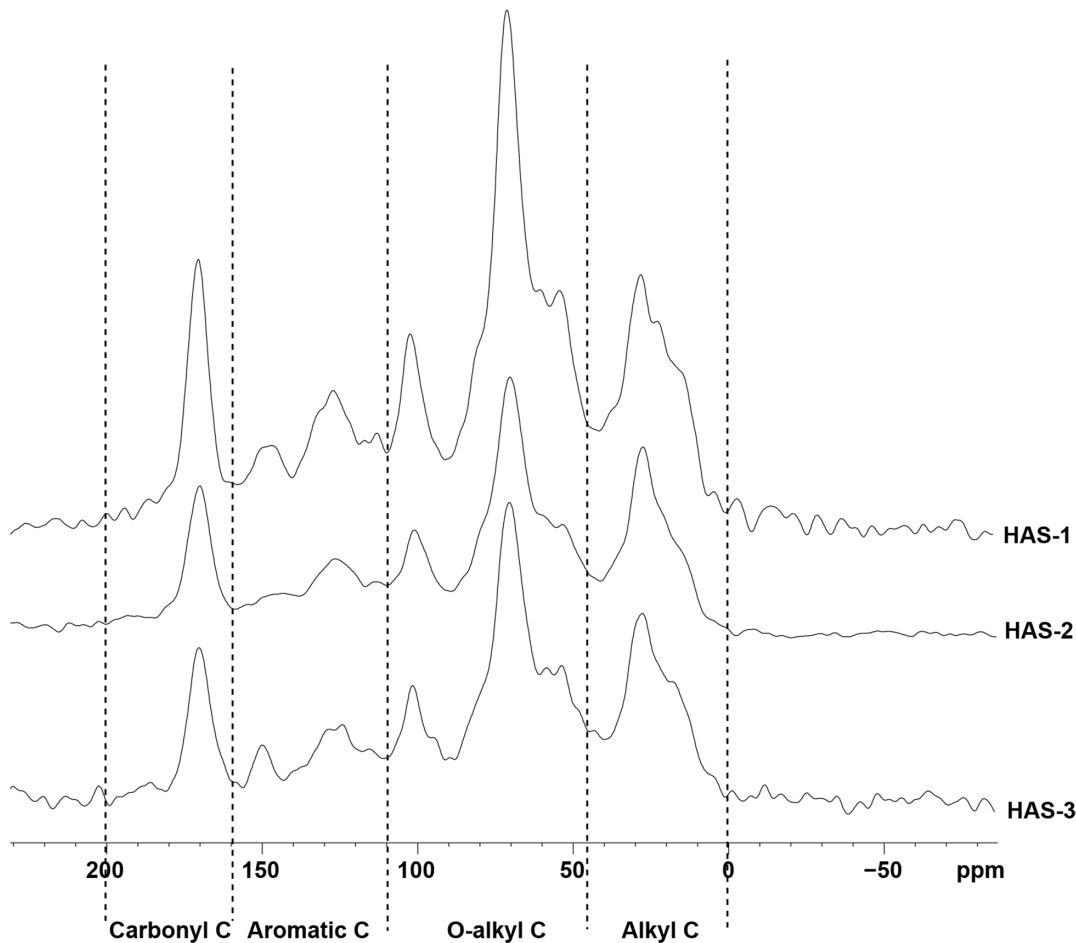
**Fig 5. Solid-state $^{13}$C-NMR spectra of soil samples from the HAS.** LAS: low-altitude site; HAS: high-altitude site.

In this study, we found that the alkyl C, aromatic C, and carbonyl C contents as well as the alkyl C to O-alkyl C ratio (A/O-A) and aromaticity of organic matter demonstrated no significant differences with elevation ($P > 0.05$) (Table 3). This finding is inconsistent with the results of Du et al. [58]. This discrepancy may be attributed to the differences in soil type because the soil type in our study did not differ in both altitudes evaluated. Some have argued that SOC stability is mainly dependent on soil type [75, 76] because of the minimal difference in soil chemical structure revealed by NMR spectroscopy, such as in different land-use types. In the present study, the O-alkyl C content was higher at the LAS than at the HAS (Table 3). This result

**Table 3. Distribution of soil organic carbon groups based on $^{13}$C-NMR spectra in *Phyllostachys pubescens* forests with elevation [a].**

| Elevation | Alkyl C (%) | O-alkyl C | Aromatic C | Carbonyl C | A/O-A | Aromaticity |
|-----------|-------------|-----------|------------|------------|-------|-------------|
| **LAS** | 21.80 a | 50.22 a | 14.44 a | 13.54 a | 0.43 a | 0.17 a |
| **HAS** | 24.58 a | 47.60 b | 15.79 a | 12.03 a | 0.52 a | 0.18 a |

LAS: low-altitude site; HAS: high-altitude site.

[a] Means with different letters indicate significant differences between the two altitudes for each parameter at the $P = 0.05$ level according to the independent t test.

implied that easily decomposed components, including polysaccharides and cellulose, enriched the LAS soil to a greater extent than the HAS soil [72].

The optical characteristics of humic substances were evaluated through the $E_{465}/E_{665}$ ratios, which provided rough estimations of the molecular dimensions, which decreased with increasing aggregated size of humic substances and degree of humification [72]. A low $E_{465}/E_{665}$ value indicates a high degree of humification [77, 78]. The $E_{465}/E_{665}$ of the loosely combined humus was higher at the LAS than at the HAS regardless of soil layer, but the difference was not significant ($P > 0.05$) (Fig 3A). Hence, the degree of condensation of the aromatic network and humification of loosely combined humus did not significantly differ. This result was consistent with that of the $^{13}C$ CPMAS NMR spectra showing no significant difference in aromatic C content and aromaticity with the different elevations (0–10 cm layer) (Table 3). However, although the $E_{465}/E_{665}$ of stably combined humus increased from the LAS to the HAS in all soil layers, the differences were not significant ($P > 0.05$) (Fig 3B). The sampling sites contain acidic soil; hence, the loosely combined humus mainly comprised the soil profiles. Accordingly, the $E_{465}/E_{665}$ of loosely combined humus is considered a more suitable index rather than that of the stably combined humus for identifying the aromaticity and humification degree of SOC.

## Effect of altitude on SOC dynamics and turnover in *P. pubescens* forests

Multiple environmental factors, namely, temperature, precipitation, N deposition, litter quality, and soil type, vary with altitude; each factor can potentially affect soil C sequestration and turnover [10]. Moisture plays an important role in the growth of *P. pubescens* and is the limiting factor for its productivity. Therefore, the greater precipitation at the higher elevation was associated with the larger amounts of bamboo biomass. Numerous studies indicated that the decomposition of labile SOM [79, 80] and soil respiration [81, 82] increase with temperature. The mean annual temperature shows a significant negative correlation with elevation [10, 65]. However, the SOC composition did not significantly differ between the two sites, as indicated by the proportions of the three combined humus C, the $^{13}C$ CPMAS NMR and $E_{465}/E_{665}$ results of the combined humus. Therefore, the high SOC sequestration in the *P. pubescens* forests of the HAS mainly resulted from the high precipitation, low temperature, and soil respiration associated with the high altitude.

## Conclusions

The SOC contents and stocks were significantly higher at the HAS than at the LAS in the entire soil profile (0–60 cm). The significant discrepancy implied that altitude should be considered in the regional C storage estimation of *P. pubescens* forests. The C contents of the three combined humus forms demonstrated similar responses to the elevation change in terms of C content; all of the forms showed higher C contents at the HAS than at the LAS regardless of soil layer. However, the proportions of the three combined humus C showed no significant differences between the two altitudes. The $^{13}C$ CPMAS NMR results also showed that the SOC chemical composition did not significantly vary with elevation. This finding is consistent with the $E_{465}/E_{665}$ values of the loosely combined humus. This result suggested that the SOC exhibited a similar pattern of chemical composition when covered by the same *P. pubescens* vegetation.

## Supporting Information

**S1 Table. The content of $NO_3^{-}–N$ in *Phyllostachys pubescens* forests at two altitudes.** LAS: low-altitude site; HAS: high-altitude site.
(DOC)

## Author Contributions

Conceived and designed the experiments: SYZ HBJ ZLZ. Performed the experiments: SYZ HBJ. Analyzed the data: SYZ HBJ. Contributed reagents/materials/analysis tools: SYZ HBJ ZLZ ZKZ. Wrote the paper: SYZ HBJ.

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