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## Lithological control on phytolith carbon sequestration in moso bamboo forests

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Phytolith-occluded carbon (PhytOC) is a stable carbon (C) fraction that has effects on long-term global C balance. Here, we report the phytolith and PhytOC accumulation in moso bamboo leaves developed on four types of parent materials. The results show that PhytOC content of moso bamboo varies with parent material in the order of granodiorite (2.0 g kg<sup>-1</sup>) > granite (1.6 g kg<sup>-1</sup>) > basalt (1.3 g kg<sup>-1</sup>) > shale (0.7 g kg<sup>-1</sup>). PhytOC production flux of moso bamboo on four types of parent materials varies significantly from 1.0 to 64.8 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>, thus a net 4.7 × 10<sup>6</sup>–310.8 × 10<sup>6</sup> kg CO<sub>2</sub> yr<sup>-1</sup> would be sequestered by moso bamboo phytoliths in China. The phytolith C sequestration rate in moso bamboo of China will continue to increase in the following decades due to nationwide bamboo afforestation/reforestation, demonstrating the potential of bamboo in regulating terrestrial C balance. Management practices such as afforestation of bamboo in granodiorite area and granodiorite powder amendment may further enhance phytolith C sequestration through bamboo plants.

With the swift development of modern society, the concentration of atmospheric carbon dioxide (CO<sub>2</sub>) has increased rapidly, resulting in global warming<sup>1–3</sup>. The International Panel on Climate Change (IPCC) warned that the increasing CO<sub>2</sub> emission would cause a series of climate change problems resulting in various adverse influences on the environment and human health<sup>4</sup>. It was estimated that the total emissions of global atmospheric CO<sub>2</sub> had increased to 3.11 × 10<sup>11</sup> t by 2010<sup>5</sup>. Therefore, it is increasingly important to explore feasible technologies to sequester CO<sub>2</sub> and reduce the concentration of atmospheric CO<sub>2</sub> in the near future<sup>6–7</sup>.

Phytoliths, also known as “opal phytoliths”<sup>8</sup>, are a type of amorphous silica which deposits in the cell wall, cell lumen and intercellular spaces during plant growth<sup>7,9–10</sup>. During their formation, some organic C can be occluded within phytoliths (PhytOC)<sup>11–14</sup>. Phytoliths can be stored steadily in soils and sediments after decomposition of plant residues<sup>12,15–17</sup>. For example, phytoliths are stable even under some extreme circumstances such as volcanic explosions, forest fires and earthquakes<sup>15,18–20</sup>. Thus, phytoliths play an important role in the long-term C sink of terrestrial ecosystems<sup>12,21–22</sup>, which may contribute to 82% of the total soil organic C pool in some sediments after 2000 years of decomposition<sup>6</sup>.

Recent studies have indicated that PhytOC production flux of some plants shows a decreasing trend: bamboo (0.70 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>)<sup>6</sup> > sugarcane (0.36 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>)<sup>23</sup> > wheat (0.25 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>)<sup>24</sup> > rice (0.13 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>)<sup>13</sup> > millet (0.03 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>)<sup>25</sup>. Moreover, it has been suggested that if all of the potentially arable land was used to grow bamboo or other crops with a PhytOC production flux of 0.7 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>, 1.5 × 10<sup>9</sup> t CO<sub>2</sub> yr<sup>-1</sup> would be sequestered as PhytOC, approximately 11% of the CO<sub>2</sub> increase in atmosphere<sup>6</sup>.

Bamboo, a typical Si-accumulator, has a global area of 22 × 10<sup>6</sup> ha and is increasing at a rate of 3% annually<sup>26–27</sup>. It is widely distributed in China (approximately 7.2 × 10<sup>6</sup> ha), mainly in Zhejiang, Fujian, and Jiangxi provinces<sup>27</sup>. More than two-thirds of bamboo distribution areas are dominated by moso bamboo in China<sup>28</sup>. At present, the potential of C bio-sequestration within phytoliths of some bamboo species has already been investigated<sup>6</sup>. However, the mechanisms and influencing factors for the production and accumulation of phytoliths and PhytOC within moso bamboo ecosystems have not yet been reported. Moso bamboo is the most commonly used species in the production of bamboo wood. Aerial parts have all been removed, and most of the remaining in the soil are bamboo leaves. In this study, we investigated the concentration of phytolith and PhytOC in moso bamboo leaves with different parent materials. The purposes of this study are to provide scientific references for

Table 1 | Characteristics of top soils with different parent materials<sup>a)</sup>

Sample site	Location	Parent materials	MAT	MAP	pH <sup>b)</sup>	SOC (g kg <sup>-1</sup> )	Avail-Si (mg kg <sup>-1</sup> )	Phytolith (g kg <sup>-1</sup> )	PhytOC (g kg <sup>-1</sup> )
Qingshan, Lin'an	30°13'N, 119°46'E	Shale	9–16	1300–1700	5.2a	13.4cd	65.3b	14.1b	0.2c
Chuanba, An'ji	30°27'N, 119°41'E	Granodiorite	12–16	1100–1900	4.3c	29.3b	50.3c	23.2a	0.5a
Qiaoying, Shaoxing	29°23'N, 121°11'E	Granite	13–17	1300–1500	4.4c	32.9a	67.3b	10.8b	0.4b
Dashiju, Shaoxing	29°28'N, 120°59'E	Basalt	13–18	1400–1700	4.5b	17.3c	237.5a	10.2b	0.3b

a) Soils of the sampling sites are classified as Ferralsols according to FAO soil classification system.

b) Different letters of the same column indicate that the value difference between parent materials is significant.

the regulation of phytolith C sink and to improve understanding of the role of bamboo phytoliths in the terrestrial C cycle.

## Results

Soil pH ranged from 4.3 to 5.2 in four sites (Table 1). The contents of soil organic C varied from 13.4 g kg<sup>-1</sup> to 32.9 g kg<sup>-1</sup> among four parent materials. Available Si content showed a decreasing trend of basalt > granite > shale > granodiorite. Soil phytolith content ranged from 10.2 g kg<sup>-1</sup> to 23.2 g kg<sup>-1</sup>. Soil PhytOC content ranged from 0.2 g kg<sup>-1</sup> to 0.5 g kg<sup>-1</sup> and the ratio between soil PhytOC and SOC ranged from 1.3% to 1.9% (Table 1).

The variations in average contents of SiO<sub>2</sub> (51.8 g kg<sup>-1</sup>–62.6 g kg<sup>-1</sup>) and phytoliths (50.8 g kg<sup>-1</sup>–57.6 g kg<sup>-1</sup>), and C contents of phytoliths (13.5 g kg<sup>-1</sup>–15.2 g kg<sup>-1</sup>) within the leaf samples of different ages were not obvious (Table 2). However, there were distinctive variations in SiO<sub>2</sub> (56.1 g kg<sup>-1</sup>–103.7 g kg<sup>-1</sup>) and phytolith contents (50.8 g kg<sup>-1</sup>–99.1 g kg<sup>-1</sup>) in leaves of bamboo grown in soils of different parent materials (Table 3).

The phytolith content within the same part (leaves from top, middle, or bottom) of bamboos of different ages varied little, while phytolith content in an individual of a given age decreased in the order: top > middle > bottom (Figure 1). This difference was significant in QS-1-year and QS-5-year, but less pronounced in QS-3-year (Figure 1A). Compared with the top and middle leaves, the bottom leaves had the highest C content in phytoliths of QS-1-year, but in the other two years the trend of C content was the same as that of phytolith content (Figure 1B). QS-1-year and QS-3-year showed significant differences in C content among different parts of the leaves (Figure 1B).

There was no significant difference of leaf phytolith contents among different parts of leaves for moso bamboo developed on granite and basalt. However, the variations within the three parts in moso bamboo developed on shale and granodiorite were striking. The phytolith content decreased from top to bottom leaves for moso bamboo developed on different bedrocks except granodiorite (Figure 2A). Generally, the mean phytolith content showed a decreasing order of granite (99.1 g kg<sup>-1</sup>) > granodiorite (85.1 g kg<sup>-1</sup>) > basalt (67.3 g kg<sup>-1</sup>) > shale (50.8 g kg<sup>-1</sup>) (Table 3, Figure 2A). The variation in PhytOC was greatest between bottom leaves rather than between middle and top leaves. The content of C in phytoliths among different parts of the leaves within moso bamboo developed on shale and granite decreased from top to bottom part, and the mean content among different lithologies were in the order of granodiorite > basalt > granite > shale (Table 3, Figure 2B).

## Discussion

### Mechanisms of C occlusion within phytoliths of moso bamboo.

Dramatic differences exist in phytolith and PhytOC content within moso bamboo of different age and soil parent material. The general decreasing trend of phytolith contents from top to bottom leaves (Figures 1 and 2) is mainly the result of reduced transpiration and SiO<sub>2</sub> deposition flux from top to bottom leaves<sup>10</sup>. It was reported that SiO<sub>2</sub> content in leaves would increase with bamboo age<sup>29</sup>. The strong positive correlation between the phytolith and SiO<sub>2</sub> content (R<sup>2</sup>=0.80, P<0.01) (Figure 3A) indicates that the accumulation of phytoliths in bamboo would increase with bamboo age. Nevertheless, phytolith content in leaves of 5-year-bamboo (QS-5-year) was lower than 1-year-bamboo (QS-1-year) and 3-year-bamboo (QS-3-year) (Table 2), which can be explained by the regeneration of bamboo leaves. The turnover period of leaf regeneration is 1–2 years and the content of phytolith in younger leaves is generally lower than that in older leaves. Furthermore, lithology has an important effect on SiO<sub>2</sub> and phytolith content, which may be caused by the variation of bioavailable Si of soils developed on different parent rocks<sup>30</sup> (Table 3), leading to the differences in Si absorption from soil solution and phytolith accumulation in leaves.

There is a positive correlation between PhytOC content in leaves and phytolith content within moso bamboo (R<sup>2</sup> = 0.67, P < 0.01) (Figure 3B). In addition, a strong positive correlation exists between the PhytOC content in leaves and the C content in phytoliths (R<sup>2</sup> = 0.47, P < 0.05) (Figure 3C). However, the result of this study differs from those found in previous studies among bamboo<sup>6</sup>, wheat<sup>24</sup> and millet<sup>25</sup>. This study indicates that the phytolith carbon sequestration depends not only on the efficiency and ability of C encapsulation by the phytoliths<sup>6</sup>, but also on the quantity of phytoliths in moso bamboo. Thus, all mechanisms of enhancing the content of Si in moso bamboo in order to increase the content of phytolith and PhytOC should be taken into consideration. Many previous studies have demonstrated that the application of Si-rich organic mulches (including rice husks and rice straw)<sup>31–32</sup> and silicon fertilizers<sup>33–34</sup> could result in significant enhancement of Si and phytolith content. Furthermore, factors such as location and disease resistance<sup>24–25,35–36</sup> also contribute to the accumulation of Si in plants, influencing the sequestration of phytoliths and PhytOC.

According to the published data, the leaf litter production in moso bamboo is approximately 3.05–6.11 t ha<sup>-1</sup> yr<sup>-1</sup><sup>37</sup>. Combined with the area of moso bamboo (4.8 × 10<sup>6</sup> ha) and dry weight PhytOC content (Tables 2 and 3), this study estimates that the mean of C flux within phytolith is 6.7–39.2 g CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>. It suggests that older

Table 2 | The contents of SiO<sub>2</sub>, phytolith and PhytOC in leaves of moso bamboo with different ages (Dry weight basis)

Sample age	SiO <sub>2</sub> in leaves (g kg <sup>-1</sup> )	Phytolith in leaves (g kg <sup>-1</sup> )	PhytOC in phytolith (g kg <sup>-1</sup> )	PhytOC in leaves (g kg <sup>-1</sup> )	Estimated PhytOC fluxes (kg CO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup> )
QS-1-year	62.6 ± 21.1	52.4 ± 23.2	15.2 ± 6.6	0.7 ± 0.6	1.0–29.8
QS-3-year	51.8 ± 1.5	57.6 ± 7.5	13.5 ± 4.7	0.8 ± 0.4	4.7–26.5
QS-5-year	56.1 ± 9.7	50.8 ± 14.7	13.6 ± 2.4	0.7 ± 0.3	4.3–23.5

Data are mean ± s.d (n=9).


**Table 3 | The contents of SiO<sub>2</sub>, phytolith and PhytOC in leaves of moso bamboo on different parent materials (Dry weight basis)**

Parent materials	SiO <sub>2</sub> in leaves (g kg <sup>-1</sup> )	Phytolith in leaves (g kg <sup>-1</sup> )	PhytOC in phytolith (g kg <sup>-1</sup> )	PhytOC in leaves (g kg <sup>-1</sup> )	Estimated PhytOC fluxes (kg CO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup> )
Shale	56.1 ± 9.7	50.8 ± 14.7	13.6 ± 2.4	0.7 ± 0.3	4.3–23.5
Granodiorite	73.9 ± 8.1	85.1 ± 15.1	22.7 ± 6.2	2.0 ± 0.9	12.2–64.8
Granite	103.7 ± 8.6	99.1 ± 8.6	15.5 ± 5.3	1.6 ± 0.7	10.0–50.0
Basalt	76.4 ± 22.5	67.3 ± 11.4	19.4 ± 4.8	1.3 ± 0.5	8.2–40.4

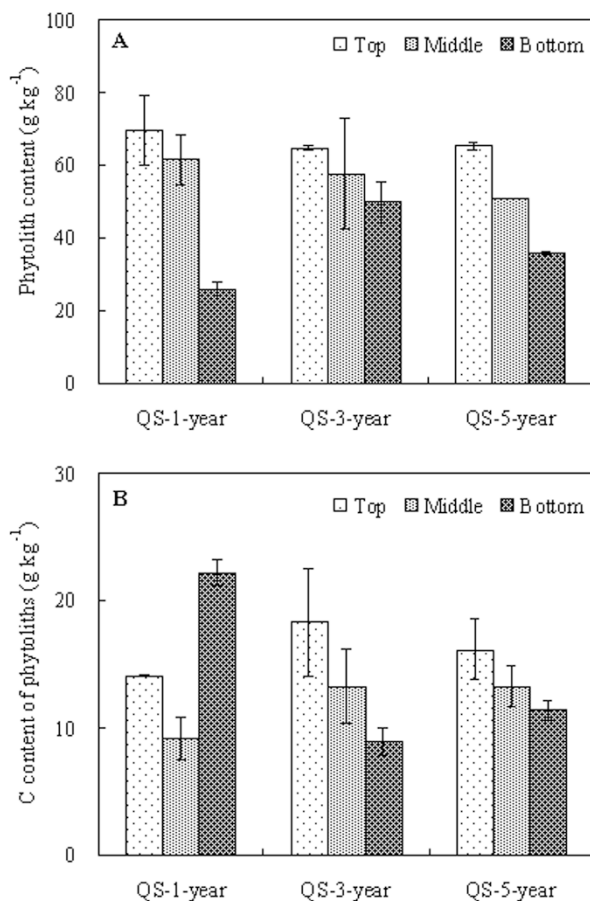
Data are mean ± s.d (n=9).

bamboo contains higher PhytOC than younger bamboo. Average PhytOC fluxes in different lithologies of moso bamboo stands decrease in the order: granodiorite (64.8 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>) > granite (50.0 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>) > basalt (40.4 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>) > shale (23.5 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>). The yielding ability of PhytOC through bamboo leaves in granodiorite is notably higher than other lithologies, which can provide a basis for the subsequent bamboo applications. It has also been demonstrated that management practices such as amendment of rock powder (e.g. granodiorite) may enhance phytolith C sequestration in bamboo forests. Besides ages and lithologies, the ANPP of litter fall must be taken into consideration when refers to the PhytOC flux in moso bamboo. Generally, the litter of moso bamboo is produced all year round, whose quantity changes with season and growth characteristics. Moreover, management practices can influence the amount of bamboo litter. For example, the yield of litter under extensive management is higher than that under intensive management<sup>37</sup>. Relative to other parent materials, the highest

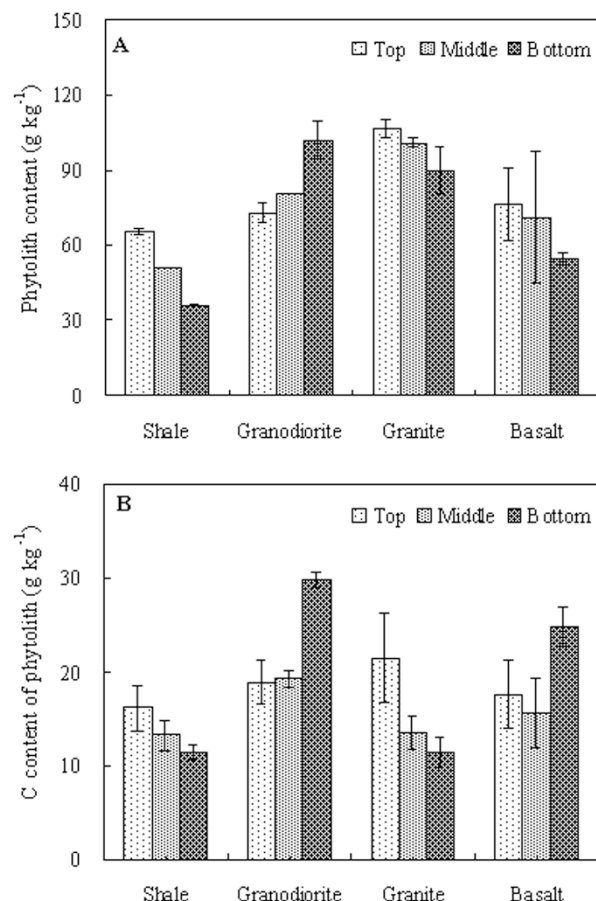
contents of phytoliths and PhytOC in the soils developed on granodiorite (Table 1) is generally the result of highest leaf-litter phytolith production flux (Table 3). Thus, it is possible to estimate phytolith carbon sink of bamboo from data of leaf-litter phytolith production flux in bamboo.

**Potential of phytolith carbon sequestration in moso bamboo.** Relative to other forms of organic C, the PhytOC produced in terrestrial plants is very stable and can accumulate in soil for thousands of years after plant litter decomposition<sup>12,15–16</sup>. Forest phytoliths are stable for thousands of years<sup>6,38</sup>.

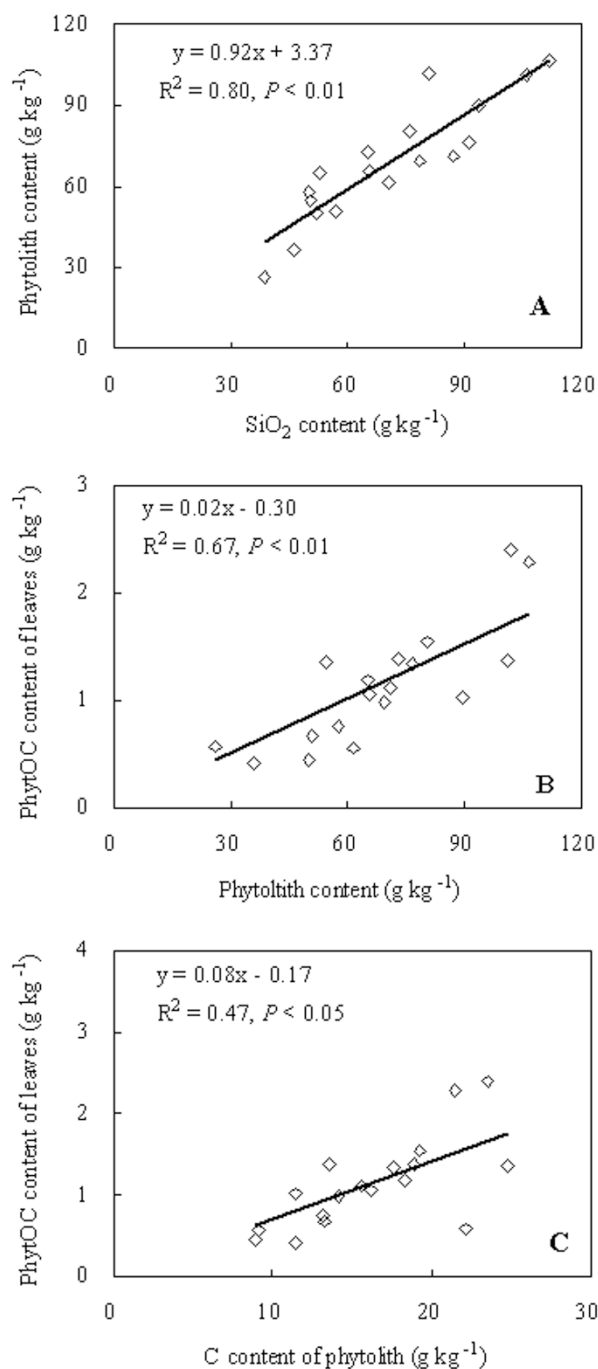
As an efficient phytolith producer, bamboo (mainly moso bamboo) is dominantly distributed in tropical and subtropical regions, accounting for 1.5%–2.0% of the forest area in the world. From 1950 to 2005, the area of bamboo forests in China increased from 165 × 10<sup>4</sup> ha to 483 × 10<sup>4</sup> ha, with an annual growth of 12.6 × 10<sup>4</sup> ha<sup>27</sup>. As the PhytOC flux ranges from 1.0 kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> to 64.8 kg CO<sub>2</sub>



**Figure 1 | Phytolith content (A) and C content of phytoliths (B) for different parts of the leaves within moso bamboo among different ages. Error bars are standard deviations (n=3).**



**Figure 2 | Phytolith content (A) and C content of phytoliths (B) for different parts of the leaves within moso bamboo among different parent materials. Error bars are standard deviations (n=3).**



**Figure 3** | Correlations between (A) content in leaves of phytolith and  $\text{SiO}_2$ , (B) content in leaves of PhytOC and phytolith, and (C) PhytOC content of leaves and C content of phytolith.

$\text{ha}^{-1} \text{yr}^{-1}$ ,  $4.7 \times 10^6$ – $310.8 \times 10^6 \text{ kg CO}_2 \text{ yr}^{-1}$  would be sequestered by moso bamboo phytoliths. Given that the potential area of bamboo stands in China will have doubled ( $9.6 \times 10^6 \text{ ha}$ ) by 2050 as a result of natural expansion, bamboo afforestation and reforestation<sup>39</sup>, and assuming the same flux ( $64.8 \text{ kg CO}_2 \text{ ha}^{-1} \text{yr}^{-1}$ ) of PhytOC, at least  $621.6 \times 10^6 \text{ kg CO}_2 \text{ yr}^{-1}$  from atmosphere would be sequestered in moso bamboo phytoliths in China.

To sum up, PhytOC production flux of moso bamboo on four lithologies varies significantly from 1.0 to  $64.8 \text{ kg CO}_2 \text{ ha}^{-1} \text{yr}^{-1}$  and decreases in the following order: granodiorite > granite > basalt > shale. Moso bamboos possess a great potential to occlude  $\text{CO}_2$  because of their significantly high-phytolith content, fast growth,

rapid reproduction and easy regeneration. It will make great contribution to reducing the concentration of carbon dioxide in atmosphere by selectively managing plants such as moso bamboo that have a strong ability of yielding PhytOC.

## Methods

**Experimental sites.** The study area is located in Zhejiang province, China and has a subtropical monsoon climate, four distinct seasons, ample sunshine and abundant rainfall. The distribution of precipitation is uneven, with an average of 980–2000  $\text{mm yr}^{-1}$ . The annual frost-free period is up to 234 d, and the annual average temperature is 9–18°C. In this study, the sites for moso bamboo investigation were selected from Qingshan, Chuanba, Qiaoying and Dashiju in Zhejiang Province, China (for details, see Table 1).

**Experimental design and Analyses of the phytolith in samples.** Moso bamboo plants with different ages (1, 3, 5 year) in Qingshan, one age (5 year) in Chuanba, one age (5 year) in Qiaoying and one age (5 year) in Dashiju (three replicates) were selected in 2011. Three replicates were from three plots (10 m × 10 m). Mature leaf samples were collected from the top (0–1 m distance from bamboo crown), middle (2–3 m distance from bamboo crown) and bottom (3–4 m distance from bamboo crown) of bamboo. For each plot, eight soil sub-samples from surface layer (0–20 cm) were collected and mixed to composite a soil sample. Each plant and soil sample was about 200 g and 1000 g, respectively.

Soil samples were air-dried and used to analyze soil pH, soil organic C (SOC), total Si and available Si with methods of Song<sup>40</sup>. Soil phytoliths were isolated followed by the method described by Li et al.<sup>14</sup>. Soil samples were deflocculated within  $\text{Na}_4\text{P}_2\text{O}_7$  solution, treated with  $\text{H}_2\text{O}_2$  and cold HCl, and then separated in  $\text{ZnBr}_2$  heavy liquid. The extraction of every soil samples were repeated three times to gain more phytoliths. All plant samples were mixed, rinsed with ultrapure water, oven-dried at 75°C for 48 h. Plant phytoliths were extracted with microwave digestion method described by Parr et al.<sup>41</sup>. Possible extraneous organic materials of phytoliths were removed and examined with 0.8 mol  $\text{L}^{-1}$  potassium dichromate<sup>6,13</sup>. Phytoliths were also checked with an optical microscope (Olympus CX31, Japan) to make sure the extraneous organic materials of phytolith had been removed thoroughly<sup>42</sup>. The phytolith samples were oven-dried at 75°C to a constant weight. The phytoliths were treated with 4 mol  $\text{L}^{-1}$  hydrogen fluoride (HF) at 45°C for 60 minutes to dissolve phytolith-Si<sup>13,43</sup>. The C content of phytoliths was determined with the method of potassium dichromate after HF treatment<sup>40</sup>.

**Data calculations and statistics.** All data in this study were obtained from the average of three replicates. A one-way analysis of variation (ANOVA) was carried out on the data obtained from the present study, and means were compared using Duncan's Multiple Range Test ( $P < 0.05$ ). The statistical analyses were using the SPSS 13.0 for windows, SPSS Inc., Chicago, USA.

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## Author contributions

Z.S. designed the study and supervised the project. P.J., G.Z. and Z.S. selected the sampling sites. B.L., Z.S. and Z.L. carried out the sampling. B.L. and Z.L. performed the experimental work. B.L., H.W. and Z.S. analyzed the data. All authors discussed the results and contributed to the manuscript.

## Additional information

**Competing financial interests:** The authors declare no competing financial interests.

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