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Effects of Silicon Fertilization on Soil Chemical Properties and Phytolith Formation of *Phyllostachys pubescens*

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ABSTRACT

Silicon is benefit to *Gramineae* plants in growth and resistance to various stresses. However, the effect of silicon fertilizer application on *Phyllostachys pubescens* is still not investigated yet. *Phyllostachys pubescens* Mazel ex J. Houz is one kind of *Gramineae* plants which distributes in a large area. In this study, a field experiment with five Si fertilizer application rates (0, 125, 250, 375, and 500 kg ha⁻¹) was setup in a *Phyllostachys pubescens* forest in China to examine the effects of Si fertilizer on bamboo Si and phytolith accumulation in fresh leaf and leaf litter. Results showed that Si application increased soil available Si content in deep layers. Si content of leaf-litter increased with the increasing level of Si fertilizer application rate, with the value ranging from 114.3 g kg⁻¹ to 172.7 g kg⁻¹, however, no significant difference was observed in fresh leaf, with the value ranging from 84.0 g kg⁻¹ to 115.0 g kg⁻¹. The phytolith contents of leaf-litter and fresh leaf were consistent with the Si contents, the phytolith content in leaf-litter of T4 (500 kg ha⁻¹) was 48.4% higher than the control, suggesting *Phyllostachys pubescens* exhibited an increasing carbon sink in phytolith when Si fertilizer applied, which is an effective way to increase long-term soil organic carbon storage in *Phyllostachys pubescens* forests with a suitable Si fertilization.

1. Introduction

Silicon (Si) is the one of the most abundant elements both on the surface of the Earth's crust and in the soils^[1, 2]. Si was not necessary for plant growth, it was beneficial for stimulating the plants's resistance to abiotic (e.g., metal toxicity, salinity, drought, extreme temperature, *et al.*) or biotic (e.g., plant diseases and insect pests) stresses^[3-7]. The silicon widely reported,

particularly *Gramineae* plants such as rice and sugarcane and some cyperaceous plants^[1, 3, 8-10]. Bamboo distributes widespread plants belonging to *Gramineae* and considered as Si accumulators, with Si concentrations ranging from 3 to 410 mg g⁻¹ SiO₂ dry matter (DM) and forming a significant organic silicon pool^[11, 12].

Bamboo comprises approximately 1,500 species and 87 genera in the *Bambusoideae* subfamily worldwide^[13]. Bamboos are widely distributed in temperate, tropical

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and subtropical regions and widely used for producing paper, foods (edible shoots), building material and even in medicines^[14]. Bamboos have many advantages like high resistance, high growth rate. In China, with 500 species in 48 genera, has a highly rich bamboo flora^[15]. Bamboo plantations are an important part of forest ecosystems, especially in Southern China. The total bamboo plantation area was 6 million hectares (M ha) until 2009, which accounted for about 3% of the total forest area in China^[16]. *Phyllostachys pubescens* are one of the most important portion of bamboo forests, accounting for approximately 70% of the total bamboo forest area in China.

In China, *Phyllostachys pubescens* is important not only as a bio-resource but also for agriculture^[17, 18]. However, the rapid development of bamboo industry associated with high application of chemical fertilizers, which inevitably resulted in land degradation and ecological destruction^[17]. Therefore, in order to sustain the bamboo sustainable development, the bamboo forest management should emphasize nutrient cycling and balance, soil property^[19-23].

Silicon fertilizer has been widely used in rice cultivation. Ando *et al.* found that the canopy structure of the rice plants can be improved by Si fertilizer amendment^[24]. Song *et al.* considered that Si fertilizer was able to sustain rice production in a long-term farming^[25]. Moreover, Si fertilizer application was shown to reduce human health risk by reducing arsenic (As) and cadmium (Cd) uptake in shoots and roots^[26, 27].

Silicon has a positive effect on bamboo growth^[28]. Si might support the vegetative reproduction and rapid growth. Meanwhile, bamboo could use more silicon to strengthen cell walls.^[18] Recently, Rong *et al.* suggested silicon fertilizer could promote the growth of *Phyllostachys violascens* and enhance the ability of soil to supply available silicon^[29]. In addition, Si contributes to regulating atmospheric CO₂ by occluding carbon within phytoliths (PhytOC), the silicified features that deposit within bamboo tissues^[30-32]. The PhytOC is highly resistant against decomposition and may accumulate in soils with pH values that range from 3.5 to 9.8 for several thousand years^[33-34]. Accumulation of PhytOC in plants and soils has been considered to be an effective way to increase the long-term organic C storage^[30, 35-36].

However, the effect and function of silicon amendment to *Phyllostachys pubescens* bamboo forests are poorly documented so far. Therefore, this study was conducted to investigate the influence of Si fertilizer application on soil basic properties of *Phyllostachys pubescens* bamboo forest, Si accumulation in leaf and leaf-litter and its implication on soil organic carbon storage.

2. Materials and Methods

2.1 Study Site Description

The study site was conducted in Guzhu Village of Shuikou Township (31°12'N, 119°81'E), Changxing County, Zhejiang Province, China. The area belongs to a monsoonal subtropical climate region with a mean annual temperature of 15.6 °C and an average annual rainfall of 1,309 mm. Low mountain and hilly landform dominates this region. The soil type of experimental site was classified as “red soil” in the Chinese system of soil classification, equivalent to Ferralsols in the food and agriculture organization (FAO) soil classification system.

2.2 Experimental Design and Sampling

2.2.1 Experimental Design

The chemical composition of silicon fertilizer used in this study includes soluble SiO₂ (> 50%) and K (3.0% ~ 5.0%). The experiment consisted of five treatments arranged in a randomized block design with three replicates per treatment (one block for one replicate). The five treatments were 0 (Control), 125 (T1), 250 (T2), 375 (T3), and 500 (T4) kg ha⁻¹ silicon fertilizer, respectively. This study was carried out at June 2012.

2.2.2 Bamboo Leaf Sample Collection

Fresh leaves were sampled from three new bamboos according to mean diameter at breast-height (DBH) in each block in January 2014. Leaves of *Phyllostachys pubescens* usually refresh once every 2 years. The fresh leaves sampled were the leaves on the bamboo after 2 years since silicon fertilizer applied. The leaf samples were transported to the laboratory, washed using an ultrasonic bath (<1 min), rinsed twice in deionized water, oven dried at 65 °C and ground to a fine powder (0.25 mm) with a mill prior to chemical analyses.

2.2.3 Leaf-litter Sample Collection

Three leaf-litter traps of 50 cm × 50 cm in size were randomly placed on the *Phyllostachys pubescens* forest floor of each block at June 2012. Litter samples from each trap were collected in January 2014. The leaf-litter sampled just was the leaf on the bamboo when silicon fertilizer applied in June 2012. The litter samples were transported to the laboratory, washed using an ultrasonic bath (<1 min), rinsed twice in deionized water, oven dried at 65 °C and ground to a fine powder (0.25 mm) with a mill prior to chemical analyses.

2.2.4 Soil Sample Collection

Within each block, soils in bamboo growth fields were also sampled in January 2014. Soil samples were collected from the 0–10 cm, 10–20 cm, and 20–40 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. Then, the soil samples were air-dried and sieved through 2 mm for further analysis.

2.3 Analysis of Samples

2.3.1 Analysis of Soil Chemical Properties

Soil pH was analyzed using a pH meter in a 1:2.5 (w/v) soil/water extract. Soil organic matter (SOM) was determined through the wet-combustion method with 133 mM $K_2Cr_2O_7$ and concentrated H_2SO_4 at 220–230 °C. 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. NH_4^+-N and NO_3^--N were analyzed through KCl extraction–colorimetry method. Soil available silicon (Si) was determined using the $HOAc-NaOAc$ (pH=4.0) extraction–colorimetric molybdenum blue method. All methods described above followed by Lu^[37].

2.3.2 Analysis of Silicon of Leaf and Litter

The silicon content in plants was determined according to Elliott *et al.*^[38]. In short, 100 mg of plant samples mixed with 3 mL of 50% (w/v) NaOH in a polyethylene tube. Then, these tubes were covered with loose plastic cover and autoclaved at 125 °C for 1 h and analyzed by the colorimetric silicon molybdenum blue method.

2.3.3 Analysis of Phytolith of Leaf and Litter

The phytolith extraction method was according to Zuo and Lu (2010)^[39]. The detailed steps are as follows: 1 g of oven-dried sample was added into a tube, then 5 mL HNO_3 added into the tube and the tube was heated at 80°C, then the tube was transferred to centrifuge at 5000 r/min for 10 min twice. After the supernatant was transferred to a beaker, 5 mL of 10% HCl was added into the previous tube and heated at 80°C for 30 min, then the tube was centrifuged at 5000 r/min for 10 min and supernatant was decanted. The 5 mL of HNO_3 was added into the tube and the tube was heated until all organic material was removed, then the tube was centrifuged and supernatant was decanted. After 5 mL of H_2SO_4 was added into the tube, the tube was heated at least 1 h and cooled indoor. Then, the tube was reheated with 30% H_2O_2 until the liquid in tube was clear. The tube was centrifuged 4 times at 5000

r/min for 10 min, then phytoliths were oven-dried in the tube at 70°C for 24 h.

2.4 Statistical Analysis

Data were presented as the means of 3 triplicates \pm S.D. A one-way analysis of variation (ANOVA) was used to assess the effects of silicon fertilizer on *Phyllostachys pubescens* forest. Each silicon fertilizer gradient was treated as a block, and the statistical significance of the differences in effects of the silicon fertilizer on soil pH, the contents of nutrients, and organic matter and available silicon of the soil as well as silicon, and phytolith contents of leaf and leaf-litter was determined. An alpha level of 0.05 for significance was used in all statistical analysis. The statistical analysis was performed with the SPSS software version 20 (IBM, Chicago, IL, USA).

3. Results

3.1 Effect of Si Fertilizer on Soil pH

Soil pH increased with the increasing level of Si fertilizer application rate (T1 to T4) from 4.6 to 5.37, 4.63 to 5.87 and 4.76 to 6.08 in the 0-10 cm, 10-20 cm, and 20-40 cm soil layers, respectively (Table 1). In the layers of 0-10 cm and 10-20 cm, soil pH was higher under the T4 compared with the control, but no significant difference in other treatments. In the layer of 20-40 cm, soil pH has a significant difference among the treatments of T3, T4 and control. In the profile of the control, soil pH decreased with increasing depth from 4.75 to 4.67. However, soil pH increased with the increasing depth from 4.60 to 4.76 and from 5.37 to 6.08 in the profile T1 and T4, respectively.

3.2 Effect of Si Fertilizer on Soil Organic Matter

The soil organic matter (SOM) contents decreased with the increasing soil depth in each treatment (Table 1). In each soil layer, SOM contents decreased with the increasing level of Si fertilizer input. In the 0-10 cm, the SOM content was 28.6%, and 61.8% higher under the control than the T3 and T4, respectively (Table 1). Similarly, in the 10-20 cm, the SOM content was 20.5%, 36.9%, and 60.2% higher under the control than the T2, T3 and T4, respectively (Table 1), and the SOM content was 60.1% higher under the control than the T4 in 20-40 cm (Table 1).

3.3 Effect of Si Fertilizer on Soil Available Nutrients

Soil NH_4^+-N content had no significant difference among the soil layers and different Si fertilizer treatments (Table 1). However, soil NO_3^--N content trended to decrease

with the increasing soil depth while there was no significant influence among different Si fertilizer treatments either (Table 1). Soil available P content decreased with the increasing soil depth in different treatments (Table 1). Soil available P content with Si fertilizer applied was lower compared with the control except for the soil layer of 0-10 cm in T4. Soil available K content showed no significant difference among the different treatments (Table 1). However, soil available K content decreased with the increasing soil depth.

Table 1. Selected soil chemical properties in *Phylllostachys pubescens* forests under different treatments ^a.

Soil profile	Treatment	pH	SOM (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
0-10cm	Control	4.75 b	28.82 a	30.58 a	9.74 a	5.27 b	43.00 a
	T 1	4.60 b	29.66 a	31.34 a	10.40 a	2.64 c	40.67 a
	T 2	4.65 b	29.90 a	22.40 a	11.94 a	2.27 c	26.00 b
	T 3	4.92 b	22.40 b	30.58 a	8.91 a	2.50 c	41.33 a
	T 4	5.37 a	17.82 b	21.56 a	9.89 a	7.92 a	49.33 a
10-20cm	Control	4.73 b	28.25 a	34.40 a	8.71 a	3.74 a	39.00 a
	T 1	4.63 b	27.97 a	30.12 a	8.12 a	2.16 ab	34.00 a
	T 2	4.87 b	23.40 b	31.50 a	7.99 a	1.93 ab	23.00 a
	T 3	5.09 b	20.62 b	28.59 a	6.48 a	1.32 b	40.00 a
	T 4	5.87 a	17.55 b	18.27 a	8.75 a	3.47 a	43.33 a
20-40cm	Control	4.67 c	25.32 a	33.71 a	7.64 a	2.83 a	32.67 a
	T 1	4.76 bc	20.02 ab	26.22 a	4.64 a	0.64 a	30.00 a
	T 2	4.78 bc	20.97 ab	27.14 a	6.99 a	1.06 a	23.33 a
	T 3	5.04 b	18.39 ab	35.78 a	4.79 a	0.87 a	34.00 a
	T 4	6.08 a	15.80 b	22.32 a	6.21 a	1.71 a	44.67 a

Note: ^aMeans with different letters indicate significant differences under different treatments for each parameter within each soil layer at P < 0.05 level.

3.4 Effect of Si Fertilizer on Soil Available Si Content

The distribution of soil available Si content in soil profile was varied among different Si fertilizer treatments (Figure 1). Under the control, soil available Si content decreased with the increasing soil layers. However, soil available Si content increased with the increasing soil layers when Si fertilizer applied (Figure 1). Soil available Si content was higher under the Si fertilizer treatments than that of the control (Figure 1).

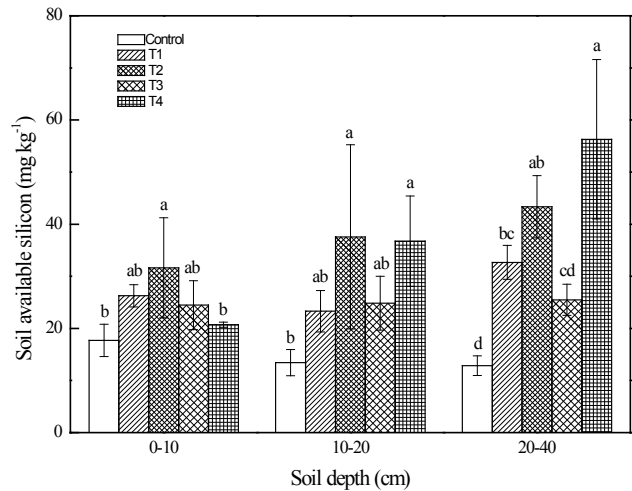


Figure 1. Soil available Si in the different soil layers under different treatments

Note: He five treatments Control, 0 kg ha⁻¹ silicon fertilizer; T1, 125 kg ha⁻¹ silicon fertilizer; T2, 250 kg ha⁻¹ silicon fertilizer; T3, 375 kg ha⁻¹ silicon fertilizer; T4, 500 kg ha⁻¹ silicon fertilizer. Error bars are standard deviations (n = 3); different lowercase letters indicate significant differences at P = 0.05 level based on the Duncan test (the same hereinafter).

3.5 Effect of Si fertilizer on Si content in leaf and leaf-litter

No significant difference of Si content in leaf was observed among the different treatments (Figure 2). The Si content in leaf ranged from 84.0 g kg⁻¹ to 115.0 g kg⁻¹ (Figure 2).

Si content in leaf-litter increased with the increasing level of Si fertilizer application rate (Figure 3). Si content in leaf-litter ranged from 114.3 g kg⁻¹ to 172.7 g kg⁻¹ (Figure 3). Comparatively, Si content of the T2, T3, and T4 was 19.8%, 23.6%, and 51.1% higher than that of the control, respectively (Figure 3).

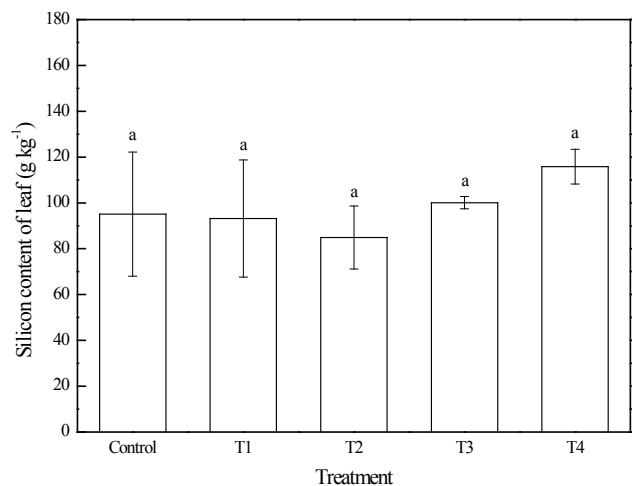


Figure 2. Si content in leaf under different treatments

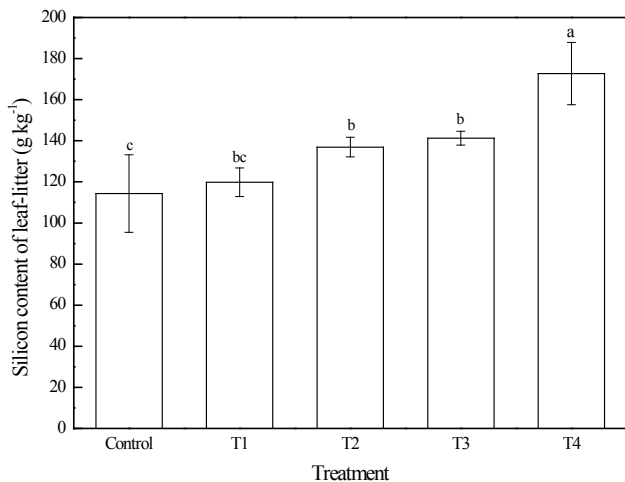


Figure 3. Si content in leaf-litter under different treatments

3.6. Effect of Si Fertilizer on Phytolith Content in Leaf and Leaf-litter

The phytolith content of fresh leaf ranged from 71.8 g kg⁻¹ to 103.4 g kg⁻¹ (Figure 4). However, there was no significant difference of phytolith content in leaf observed among the different treatments. The phytolith content of leaf-litter increased with the increasing level of Si fertilizer application rate (Figure 5). It ranged from 114.9 g kg⁻¹ to 170.5 g kg⁻¹ (Figure 5). Moreover, the phytolith content of the T3 and T4 was 13.5% and 48.4% higher than the control, respectively (Figure 5).

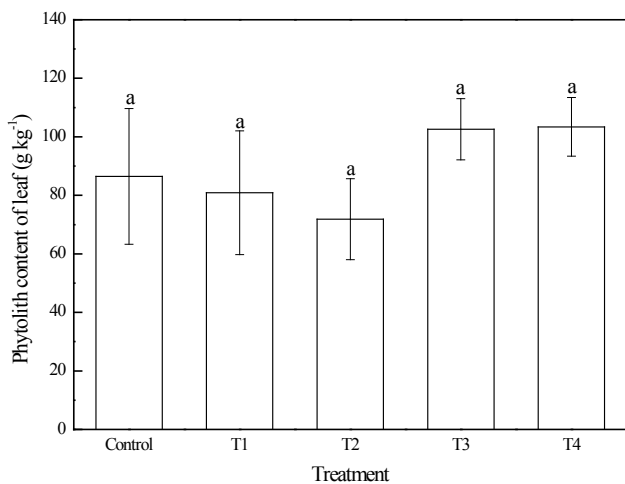


Figure 4. Phytolith content of leaf under different treatments

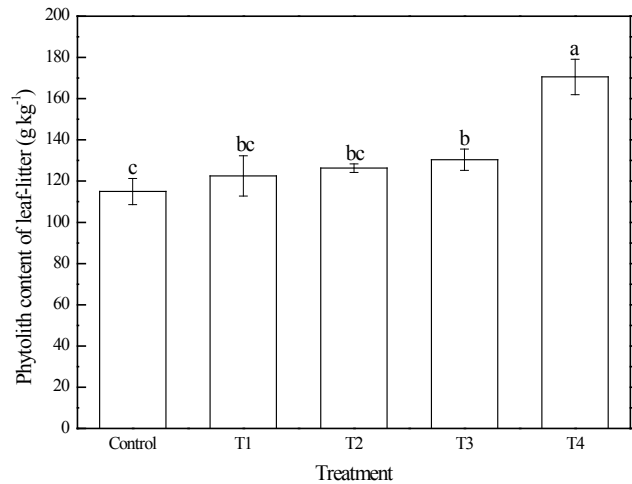


Figure 5. Phytolith content of leaf-litter under different treatments

4. Discussion

Soil available Si and soil pH were increased with the Si fertilizer application (Figure1, Table 1), which was beneficial for the growth of plants especially in acid soils [40, 41]. In soil, most of Si is held in the crystalline structure of sand, silt and clay size particles. Plants take up Si from the soil solution as silicic acid (H₄SiO₄) as soil particles weather and release Si into the soil solution [42]. Although the total Si content of mineral soils can be very large, the amount of soluble Si available for crop uptake may be limited [43, 44]. The critical value of soil available Si for rice in acid soils of Southern China is 95-100 mg kg⁻¹ (HOAc - NaOAc, pH=4.0), and it will play a significant role when silicon fertilizer is applied below this value [45]. In our study, the background value of soil available Si was 15.03 – 20.70 mg kg⁻¹ (0 – 40cm), so the use of fertilizer containing water-soluble silicon is very profitable. The increase in pH after single application of silicate fertilizer has also been observed by Smyth and Sanchez [46], the was possibly due to the OH⁻ release from colloidal surfaces during the adsorption of silicate [47].

In our study, the vertical distribution of SOM appeared to differ in all treatments, with the top surface soils (0–10 cm) showed relatively higher SOM content compared to the 10–20 cm and 20-40 cm depth layer, which was similar to former studies, soil organic matter storage was associated with root biomass and litter decomposition, both of them decreased with soil depth, which decreased the organic carbon inputs [48, 49], a result of corresponding decrease of organic matter storage via root biomass and litter decomposition, which are the main pathways of organic carbon inputs [50,51]. However, the SOM decreased as

Si fertilizer rate increased in this study. As we mentioned before, soil pH was an important property related to soil characteristics which may affect the enzymes activities and the availability of nutrients^[52]. The enzymes activities influenced by different pH may be attributed to different SOM decomposition rate which promoted SOM loss after Si fertilizer. The content of soil NO_3^- -N, available P and available K decreased as soil depth increased in different treatments (Table 1), because the content of SOM decreased with the increasing soil depth and its mineralization decreased as well.

Bamboo is a silicon accumulated plant. The silicon content in bamboo leaves was higher than other organs and increased with bamboo age^[18]. Ding *et al.* supported the idea that silicon enters bamboo as water-soluble monosilicic acid, which is transported subsequently through the root, stem, branch and leaves and polymerized and precipitated to form amorphous silica (phytolith) at tissue sites of these organs^[12]. In leaf, Si mainly deposited in the epidermis, and studies found that the highest concentration of Si was in silica cells^[28,52]. In our study, Si concentrations in leaves were 84 to 115 mg g^{-1} , which is similar to the results reported in several previous studies^[12,28]. There is no significant difference in leaf Si content among the different treatments, however, Si content in leaf litter significantly increased with the Si fertilizer application rate (from 114.3 to 172.7 mg g^{-1}). This may be contributed to the difference of Si accumulation period between the fresh leaf and leaf-litter. The fresh leaf sample was collected from the bamboo stick, while the leaf-litter was collected from the soil surface. As known, most of Si deposits in leaves which cannot translocate to other organs again^[12]. Besides, Motomura *et al.* reported that in bamboo leaves silica is continuously accumulated in the tissues throughout their life^[52]. The time for leaf-litter accumulated Si is much longer than fresh leaf, which still needs a period time to accumulate Si, so leaf-litter better reflects the discrepancy after different Si fertilizer treatments. In addition, the soil available Si may be not enough for leaf uptake to reflect the discrepancy between different treatments after Si fertilizer application for one year. Therefore, the application of water-soluble silicon fertilizer was beneficial for increasing the contents of silica in bamboo, and thus increase the yield of bamboo.

In this study, the phytolith contents in leaf and leaf-litter were 7.18%–10.34% and 11.49%–17.05%, respectively, which is similar to the results reported in several previous studies^[30,31]. The phytolith contents in leaf showed no significant differences among the different treatments, but increased with the increasing level of Si fertilizer application rate in leaf-litter, which were consis-

tent to the Si contents. Some researches show that there is a strong positive correlation between the phytolith and Si contents of biomass^[30,31,53]. Therefore, the Si in leaf-litter may be beneficial for the forestry for a long time. After leaves fall from the bamboo, phytoliths contribute to the pool of amorphous Si in the upper soil layers and constitute an important component of soil systems^[54]. Phytoliths released into the soil by physically and chemically weathered, and the dissolved silicon can be recycled within the forest ecosystem through the plant re-absorption^[55-59], or be redeposited in soils^[52, 60-62]. Consequently, bamboos play an important role in silicon cycle of terrestrial environment. We suggest that silicon fertilizer should be applied before the remove of branches and leaves of *Phyllostachys pubescens*, and a rate of 500 kg ha^{-1} together with other fertilizers will be recommended for a practical management.

5. Conclusion

Si fertilizer application increased soil available Si content. Si content of leaf-litter increased with the increasing level of Si fertilizer application rate, however, no significant difference was observed in fresh leaf. This finding implied that it needs a period time to accumulate Si for *Phyllostachys pubescens*. The phytolith contents of leaf-litter and fresh leaf were consistent with the Si contents, in leaf-litter the phytolith content of T4 (500 kg ha^{-1}) was 48.4% higher than the control. This result suggested that *Phyllostachys pubescens* exhibited the deposit of phytolith with Si fertilizer amendment, which is an effective way to increase long-term soil organic carbon storage in *Phyllostachys pubescens* forests.

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