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Long-term intensive management increased carbon occluded in phytolith (PhytOC) in bamboo forest soils

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Carbon (C) occluded in phytolith (PhytOC) is highly stable at millennium scale and its accumulation in soils can help increase long-term C sequestration. Here, we report that soil PhytOC storage significantly increased with increasing duration under intensive management (mulching and fertilization) in Lei bamboo (*Phyllostachys praecox*) plantations. The PhytOC storage in 0–40 cm soil layer in bamboo plantations increased by 217 Mg C ha^{-1}, 20 years after being converted from paddy fields. The PhytOC accumulated at 79 kg C ha^{-1} yr^{-1}, a rate far exceeding the global mean long-term soil C accumulation rate of 24 kg C ha^{-1} yr^{-1} reported in the literature. Approximately 86% of the increased PhytOC came from the large amount of mulch applied. Our data clearly demonstrate the decadal scale management effect on PhytOC accumulation, suggesting that heavy mulching is a potential method for increasing long-term organic C storage in soils for mitigating global climate change.

The global soil organic carbon (SOC) storage is estimated at 1500 Pg, which is larger than the sum of the atmospheric (500 Pg) and biotic C pools (800 Pg)[1]. Therefore, SOC storage is an important global C sink[2]. The SOC has different stabilities with the mean residence time ranging from a few days for the labile fractions to thousands of years for the recalcitrant fractions[3]. Therefore, increasing both the total amount and the stability of SOC stored in ecosystems will have significant implications for mitigating global climate change. Mechanisms for long-term SOC sequestration include physical protection of chemically recalcitrant organic matter within organo-mineral complexes[4], formation of charcoal[5], and organic C occluded in phytolith (PhytOC)[6].

Phytolith, also called plant opal, is a kind of noncrystalline mineral that deposits in the intra- and extra-cellular structures of different plant tissues after the absorption of soluble silica by plant roots in the form of monosilicic acid ($Si(OH)_4$)[6,7]. Some organic C can be firmly occluded during the formation of phytolith in plant tissues[6]. When plants die and subsequently decompose, the phytolith is released into soils and sediments. Because phytolith characteristically is highly resistant to decomposition[6,8–10], the PhytOC is much more stable than other organic C fractions in soils or sediments[6,11]. For example, Parr and Sullivan[6] found that 82% of the total C was PhytOC in 2000-year old topsoils, which was buried 2.00–2.10 m below the current soil surface in Numundo oil palm (*Elaeis guineensis*) plantations. It has been estimated that PhytOC makes up between 15 and 37% of the estimated global accumulation rate (24 kg C ha^{-1} yr^{-1}) of the soil C with long-term stability[6], suggesting that PhytOC accumulation has a significant role to play in long-term terrestrial C sequestration[11,12].

The accumulation rate of soil PhytOC mainly depends on its biogeochemical stability and the amount of influx of external phytolith[6,11,12]. The storage of soil phytolith can be decreased due to the chemical dissolution of phytolith[13] or its leaching from soils into rivers[14], which can consequently decrease soil PhytOC storage. The influxes of external phytolith are mainly from plant residues, litter-fall, and degradation of mulching materials[6,12]. It has been extensively reported that management practices, such as fertilization, mulching and tillage, would significantly affect soil SOC storage and concentrations of labile organic C fractions[15–17]. However, little information is available about the effect of management practices on soil PhytOC storage. Theoretically, fertilization would improve the absorption of soluble Si by plant roots, and consequently increase the formation of phytolith in plant tissues, which will indirectly increase soil PhytOC storage through increased litter-fall. In addition, mulching with materials with high phytolith concentration would result in the accumulation of PhytOC after the labile

C fractions in the plant tissue are decomposed. Therefore, it would be expected that soil PhytOC would accumulate under the combination of fertilization and mulching practices. However, to the best of our knowledge, no field study existed that tests such a hypothesis, resulting in large uncertainty in the development of technology to increase soil PhytOC storage.

Lei bamboo (*Phyllostachys praecox*) is a bamboo species widely distributed in southern China. To increase bamboo shoot production in early spring and to consequently obtain a better price, a common practice is used to place mixed bamboo leaf and rice (*Oryza sativa*) straw/husk over the soil surface in bamboo plantations as mulch material in each fall to maintain the bamboo forest soil at proper temperature and moisture conditions in winter[18]. The income for intensively managed Lei bamboo plantations is about 20 times that for rice production[19]. As a result, farmers have frequently converted paddy fields to bamboo plantations in the last several decades. The bamboo leaf and rice straw used as mulch have high concentrations of phytolith[12,20]. Such intensively managed bamboo plantations provide an opportunity to study the effects of long-term intensive management on soil organic C and PhytOC dynamics.

Results

Here we present evidence that SOC and PhytOC accumulated over a period of 20 years based on the study of a chronosequence of intensively managed Lei bamboo plantations. The SOC concentration and storage in the 0–20 and 20–40 cm soil layers and the phytolith concentration in the 0–20 cm soil layer in the bamboo stands did not change during the first five years, a period in which mulch was not applied, and then they significantly increased with increasing duration under intensive management (Fig. 1 and 2), after heavy mulch application (with a large amount of mulching material applied) began. The phytolith concentration in the 20–40 cm soil layer increased after one year of intensive management, but thereafter it did not change, reflecting the impact of the initial land use conversion from paddy fields to bamboo plantations (Fig. 2). The C concentration in phytolith in the 0–20 and 20–40 cm soil layers did not change along the chronosequence (Fig. 2). The PhytOC concentration and storage in 0–20 cm soil layer in bamboo stands did not change during the first five years of intensive management (before mulch material application) and significantly increased thereafter (Fig. 2). The phytOC concentration and storage in the 20–40 cm soil layer changed in the same way as the phytolith concentration (Fig. 2). Based on the quantity of litter-fall, phytolith concentration in litter-fall, and C concentration in phytolith, we estimated that the soil PhytOC accumulation rate caused by litter-fall was 11.3 kg C ha^{-1} yr^{-1}, which only constituted 14% of the total soil PhytOC accumulation rate (79 kg C ha^{-1} yr^{-1} over a 20-year chronosequence) in this study (Fig. 3). Therefore, it is estimated that approximately 86% of the increased PhytOC came from the large amount of mulch applied. In addition, PhytOC storage was positively correlated with phytolith concentration in the 0–20 cm soil layer (Fig. 4), suggesting that increased PhytOC storage was a result of increased phytolith accumulation rather than increased C concentration in phytolith.

Discussion

Soil PhytOC has a great potential in the long-term biogeochemical sequestration of atmospheric CO_2 due to its high stability[6,11]. Therefore, it would be significant if soil PhytOC storage can be increased through management practices. In previous publications, the potential role of PhytOC on long-term C sequestration was mostly estimated using PhytOC concentration in biomass and biomass production or litter deposition rate[12,20–23]. This is the first field study that examined the dynamic change in soil PhytOC storage using a chronosequence approach. Even though some researchers suggested that there would be opportunities to enhance both short- and long-term C sequestration by cultivating high PhytOC yielding plant species, such as rice, wheat and bamboo[6,12,20,22], Chen and Zhang[24] found that phytolith concentration in soils did not significantly increase with increasing age of rice cultivation in a 1000-year chronosequence. To the best of our knowledge, our result is the first field evidence that it is possible to increase the storage of PhytOC, a soil C fraction with long-term stability, through management practices, highlighting the need to investigate the response of soil stable C pools to anthropogenic activities, as has been advocated in the literature[2,25,26].

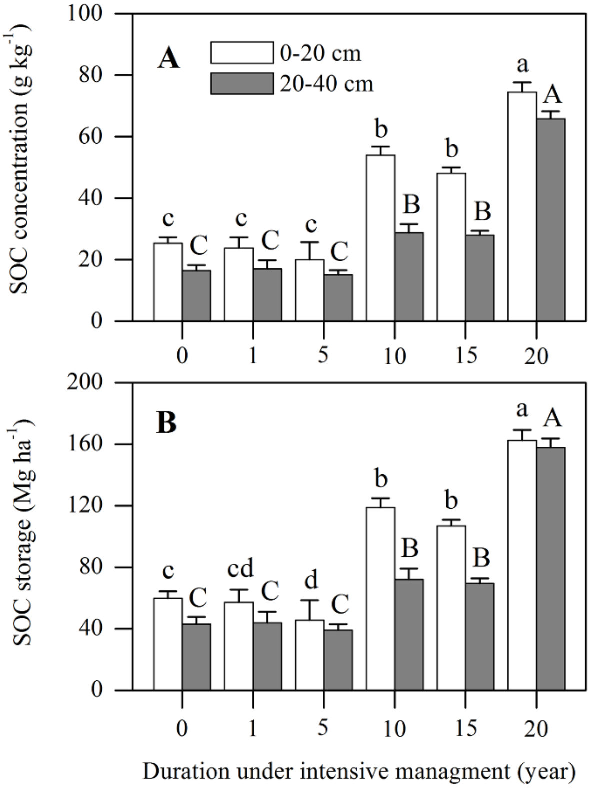

Figure 1 | Concentration (A) and storage (B) of SOC in Lei bamboo plantations with 0, 1, 5, 10, 15, and 20 years of intensive management. Error bars are standard deviations ($n = 3$); different lowercase and uppercase letters indicate significant differences among the stands in the chronosequence in the 0–20 cm and 20–40 cm soil layers, respectively, at $P = 0.05$ level based on the least significant difference (LSD) test.

Elucidating the mechanisms related to the increase of soil PhytOC storage is vital to developing technologies to increase the soil C sequestration. In this study, the soil PhytOC storage increased by intensive management, including fertilization and mulching practices. Fertilization would not likely affect soil PhytOC storage directly, since the phytolith in soils is very stable and would not respond to fertilization[6,11]. The indirect effect of fertilization on soil PhytOC storage is through improving the growth of plants, which increases the absorption of soluble Si by plant roots and consequently more phytolith would be formed, increasing the amount of phytolith input into the soil through litter-fall. However, the soil PhytOC accumulation rate caused by litter-fall only accounted for 14% of the total soil PhytOC accumulation rate (Fig. 3). In addition, we found that soil PhytOC storage did not change during the first five years, a period in which mulch was not applied, and then it significantly increased with increasing duration under intensive management (Fig. 2), after the application of mulch began. Therefore, the dramatic increase in soil PhytOC storage beginning in the sixth year of intensive management was attributable to the practice of organic mulching. Our result clearly demonstrated that addition of materials with high concentrations of phytolith (47.0 and 51.5 mg phytolith

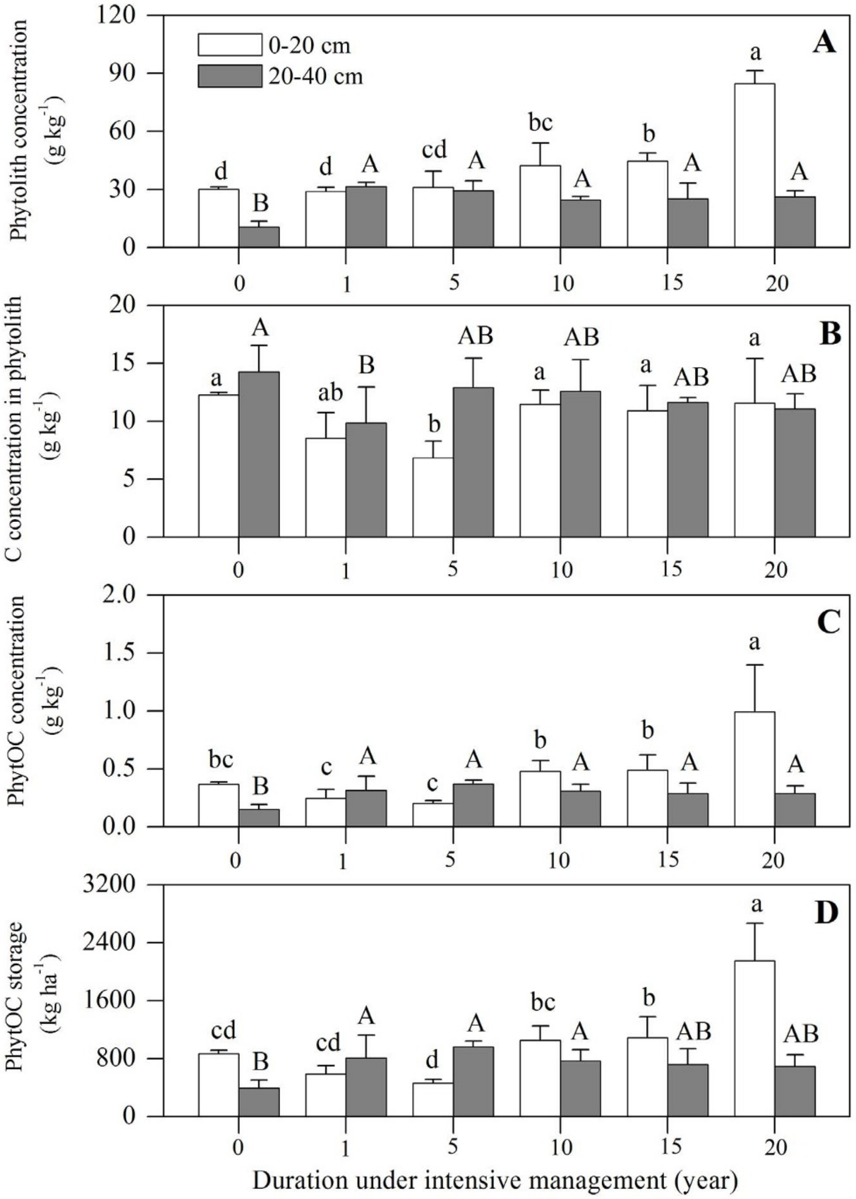

Figure 2 | Soil phytolith concentration (A), C concentration in phytolith (B), soil PhytOC concentration (C), and soil PhytOC storage (D) in Lei bamboo plantations with 0, 1, 5, 10, 15, and 20 years of intensive management. Error bars are standard deviations ($n = 3$); different lowercase and uppercase letters indicate significant differences among the stands in the chronosequence in the 0–20 cm and 20–40 cm soil layers, respectively, at $P = 0.05$ level based on the least significant difference (LSD) test.

g^{-1} for bamboo leaf and rice straw, respectively) enhanced soil PhytOC accumulation in soils. It is worth noting that other factors, such as chemical dissolution of phylith and phytolith leaching through soil layers, would also affect soil PhytOC storage to some extent[13,14], which should be investigated in further studies.

In comparison with paddy fields, the SOC storage in the 0–40 cm layer in bamboo plantations after 20 years of intensive management was increased by 217 Mg C ha^{-1}. Most importantly, the PhytOC storage was increased by 1.58 Mg C ha^{-1} over 20 years or by 79 kg C ha^{-1} yr^{-1}. Although PhytOC accumulation only accounted for 0.73% of the total SOC accumulation after 20 years of intensive management, it still has great significance. Because the C sequestrated through PhytOC is stable for thousands of years[6], while increases in other forms of organic C may only exist in soils for several days or months[3]. In addition, the PhytOC accumulation rate in this study was much higher than that reported for tropical and sub-tropical sites that had rates range between 7.2 and 8.8 kg C ha^{-1} yr^{-1} [6] and that reported for temperate sites with a mean value of 3.6 kg C ha^{-1} yr^{-1} [9,27]. This is the first report on effective PhytOC accumulation in soils of bamboo plantations; this rate is also much higher than the estimated global mean long-term soil C accumulation rate of 24 kg C ha^{-1} yr^{-1} [28], indicating that intensively managed bamboo plantations had an advantage to accumulate the stable C fraction in soils. Assuming that 10% of the current area under rice production in China, approximately 2.96×10^6 ha[29], is converted to Lei bamboo plantations, the potential national annual accumulation rate of PhytOC in soils is calculated to be 0.23 Tg C ha^{-1} yr^{-1}, based on the PhytOC accumulation rate of 79 kg C ha^{-1} yr^{-1}. The high economic return combined with the high C sequestration potential, intensively managed Lei bamboo plantations maybe a win-win alternative to rice production economically and ecologically.

According to the PhytOC concentration in biomass and biomass production data, the potential PhytOC sequestration rates for bamboo, sugarcane, wheat, and millet have been estimated to be 0.70,

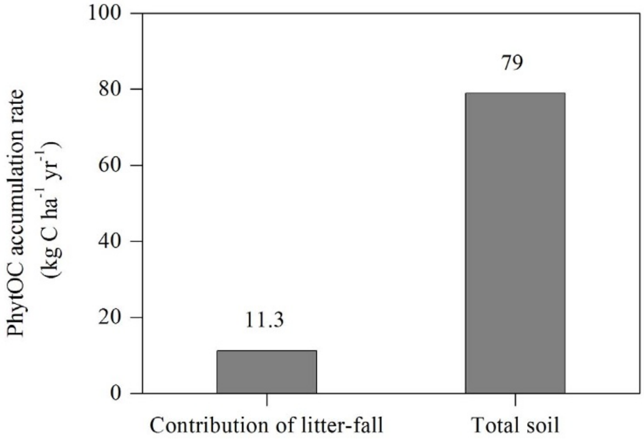

Figure 3 | **Contribution of litter-fall to the total soil PhytOC accumulation rate over a 20-year chronosequence in intensively managed Lei bamboo plantations.**

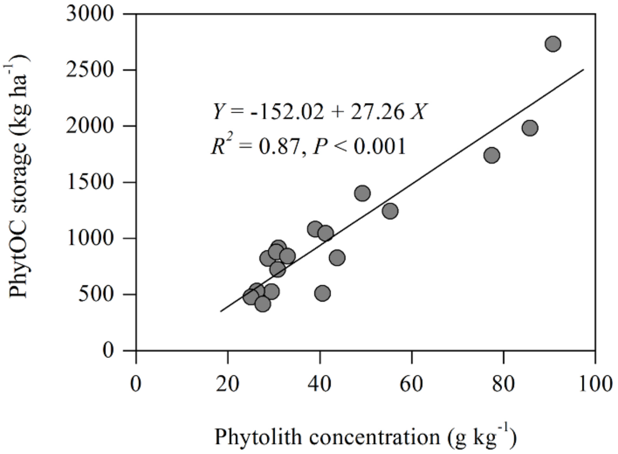

Figure 4 | **Relationship between soil PhytOC storage and phytolith concentration in intensively managed Lei bamboo plantations.**

0.36, 0.25, and 0.03 t CO_2 ha^{-1} yr^{-1}, respectively[12,22,23,30]. Recently, Song et al.[31] estimated the annual phytolith C sink in China's forests to be 1.7 ± 0.4 Tg CO_2 yr^{-1}, with 30% of which contributed by bamboo forests. Therefore, intensively managed bamboo forest ecosystems could be one of the most effective systems to sequester atmospheric CO_2 in the form of PhytOC. The potential PhytOC sequestration rates (0.70 t CO_2 ha^{-1} yr^{-1}) in bamboo forests in Parr et al.[12] was much higher than the soil PhytOC accumulation rate (0.29 t CO_2 ha^{-1} yr^{-1}) found in this study. A possible explanation is that only a small portion of the biomass produced in a bamboo forest enters into the soil through litter-fall (Fig. 3).

As discussed earlier, the high rate of soil PhytOC accumulation in intensively managed bamboo plantations in this study was mainly caused by the mulching practice. As such, the more mulch material applied the greater accumulation of phytolith and PhytOC in the soil. In general, the effect of mulching on soil PhytOC storage largely depends on the application rate of mulching materials, the phytolith concentration in mulching materials, and the C concentration in phytolith. For a given quantity of mulching material applied, increasing the phytolith concentration or the C concentration in phytolith in the mulching materials is another effective way to increasing soil PhytOC storage. The rate of phytolith production in tissues and the C occluded in phytolith varied greatly among different genotypes of rice[20] and bamboo[12], therefore, the higher PhytOC concentration in bamboo leaf or in rice straw could be obtained by selecting higher PhytOC-yielding genotypes. By adding mulching materials with high PhytOC concentrations, the accumulation of PhytOC in Lei bamboo plantation soils maybe further increased. Effectively increasing the long-term storage of organic C in intensively managed systems has significant implications in mitigating climate change and enhancing the ecological services of such ecosystems.

Methods

Experimental site. This study was carried out at Congkeng Village in Shankou Township (30°14′N, 119°42′E), Lin'an City, Zhejiang Province, in southeast China. The experimental area has a monsoonal subtropical climate with four distinct seasons. Between 2000 and 2009, the average annual temperature and average annual precipitation of this site were 15.9°C and 1422 mm, respectively. The site has an average of 239 frost-free days and 1946 day-light hours. The elevation of the site is 100–150 m above sea level and the soils of the experimental area were classified as Anthrosols in the FAO soil classification system[32], equivalent to the Red Soil in the Chinese system of soil classification[33].

About 25 years ago, farmers began to convert paddy fields to Lei bamboo plantations, due to the much higher economic profit for Lei bamboo plantations than for rice production. During the past several decades, the paddy fields were converted to Lei bamboo plantations every two or three years, which allowed us to establish a chronosequence of Lei bamboo plantations with different duration under intensive management. The stocking rate of the bamboo stands was 2045 stems ha^{-1} with a mean diameter at breast height of 3.90 cm. The typical management regime for intensively managed Lei bamboo plantations involves placement of organic residues at the soil surface in November to increase soil temperature (by as much as 4–5°C) and to preserve soil moisture[18]. Typically, mulching involves placing 10–15 cm of rice straw at the soil surface, then 15–20 cm of bamboo leaf is added on top of the rice straw, the annual rate of application is equivalent to 40 Mg ha^{-1} of rice straw and 55 Mg ha^{-1} of bamboo leaf. In April of the following year, the undecomposed mulching materials, mainly bamboo leaf, is removed, mixed with new bamboo leaf and used as the mulching material for next winter. For the bamboo stands, fertilizers are typically applied three times a year: mid May, mid September and mid November. Fertilizers applied included an NPK compound fertilizer ($N{:}P_2O_5{:}K_2O = 15:15:15$, applied at 2.25 t ha^{-1}) and urea (1.125 t ha^{-1}). All of the fertilizers were broadcast applied, followed by tillage to 30–35 cm depth.

Experimental design and soil sampling. A method of substituting space for time was used to establish a chronosequence of Lei bamboo plantations with different duration under intensive management. After evaluation of the available sites and consultation with the farmer, fifteen Lei bamboo stands were selected to represent 5 different duration under intensive management, i.e., 1, 5, 10, 15, and 20 years, with each age with triplicate. All of the Lei bamboo plantations were converted from paddy fields. Three paddy fields adjacent to those chronosequences were selected as the control, and treated as year zero of intensive management. The above bamboo stands and paddy fields were randomly selected in the experimental area and they had similar site conditions, including elevation, soil type, slope gradient and aspect, and thus the distribution of sampling plots followed a completely randomized design. The comparison between the paddy fields and Lei bamboo stands after 1 year of intensive management allowed us to evaluate the land-use change effect. The chronosequence consisting of 1-, 5-, 10-, 15-, and 20-year of intensive management allows us to evaluate the effect of the duration of intensive management.

Within each of the bamboo stands and paddy fields, a 400 m^2 plot was established in June 2011, and thus 18 plots (6 ages time 3 replications) were established for the present study. Within each plot, soil samples were taken from the 0–20 and 20–40 cm layers from five randomly-selected points in each plot, mixed to form a composite sample for each layer. The samples were sealed in plastic bags and shipped to the laboratory. Visible roots were removed and each soil sample was sieved ($<$ 2 mm), homogenised and air-dried. At the time of field sampling, soil bulk density samples were collected using a bulk density corer with a 200 cm^3 volume.

Analyses of SOC, soil and plant phytolith, and C concentration in phytolith. Determination of SOC was by means of the wet digestion method with 133 mmol L^{-1} $K_2Cr_2O_7$ and concentrated H_2SO_4 at 170–180°C[34]. Soil samples for PhytOC determination were manually milled with a mortar and pestle. A microwave digestion method was used in this study to isolate phytolith from plant material[35] and soil samples[36]. This process was followed by a Walkley-Black type digestion[34] to ensure that extraneous organic materials in the samples were completely removed[12]. The phytolith isolated were oven-dried at 75°C for 24 h in a centrifuge tube of known weight. The samples were allowed to cool and then weighed to determine the quantity of phytolith. The C concentration in phytolith was then analysed on a Thermo Finnigan Flash EA 1112 CHNS-O Analyser. Quality control was done by including a soil standard sample (GBW07405) and a plant standard sample (GBW07602) as part of the analysis. Repeated analysis of samples achieved a precision of better than 5% in the measurement of phytolith and better than 8% in the measurement of C concentration in phytolith.

Calculation of PhytOC that came from litter-fall. To calculate the PhytOC accumulation rate in soils caused by litter-fall in bamboo plantations, we conducted a one-year experiment to collect litter-fall monthly. The phytolith concentration in litter-fall and C concentration in phytolith were determined following the methods described above. And then the PhytOC addition rate from litter-fall was calculated by multiplying the litter-fall weight, phytolith concentration and C concentration in phytolith. The difference between the PhytOC measured in the soil and that from litter-fall is considered as the PhytOC from mulching.

Data calculations and statistics. The data presented in this paper were the average of three replicates. Soil phytolith concentration, C concentration in phytolith, soil PhytOC concentration, and soil PhytOC storage were calculated using the following formulas:

$$\text{Soil phytolith concentration}\ \left(\mathrm{g\ kg^{-1}}\right) = \text{phytolith weight (g)/soil weight (kg)} \quad (1)$$

$$\text{C concentration in phytolith}\ \left(\mathrm{g\ kg^{-1}}\right) = \text{C content in phytolith (g)/phytolith weight (kg)} \quad (2)$$

$$\text{Soil PhytOC concentration}\ \left(\mathrm{g\ kg^{-1}}\right) = \text{C content in phytolith (g)/soil weight (kg)} \quad (3)$$

$$\text{Soil PhytOC storage}\ \left(\mathrm{kg\ ha^{-1}}\right) = \text{PhytOC concentration}\ \left(\mathrm{g\ kg^{-1}}\right) \times BD \times th \times 10000 \quad (4)$$

Where BD is the bulk density of the soil layer (Mg $\mathrm{m^{-3}}$), and th is the thickness of the soil layer (m).

Before performing the ANOVA analysis, the normality and homogeneity of variance were tested and data were log-transformed if homogeneity of the variance was not met. A one-way analysis of variance (ANOVA) was conducted to test the duration under intensive management effect on the SOC concentration and storage, phytolith concentration, C concentration in phytolith, and the PhytOC concentration and storage in soils. When the ANOVA analysis indicated a significant treatment effect, the least significant difference (LSD) test was used to separate the means. An alpha level of 0.05 for significance was used in all statistical analyses, unless mentioned otherwise. Linear relationships between phytolith concentration and PhytOC storage in soils were determined. All of the statistical analyses were performed using the SPSS software (SPSS 13.0 for windows, SPSS Inc., Chicago, USA).

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Author contributions

Z.H. and Y.L. performed the experimental work, analyzed the data, and wrote the manuscript. P.J. supervised the project and edited the manuscript. S.X.C. wrote/edited the manuscript. All authors discussed the results and commented on the contents of the manuscript.

Additional information

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