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# Carbon bio-sequestration within the phytoliths of economic bamboo species

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## Abstract

The rates of carbon bio-sequestration within silica phytoliths of the leaf litter of 10 economically important bamboo species indicates that (a) there is considerable variation in the content of carbon occluded within the phytoliths (PhytOC) of the leaves between different bamboo species, (b) this variation does not appear to be directly related to the quantity of silica in the plant but rather the efficiency of carbon encapsulation by the silica. The PhytOC content of the species under the experimental conditions ranged from 1.6% to 4% of the leaf silica weight. The potential phytolith carbon bio-sequestration rates in the leaf-litter component for the bamboos ranged up to 0.7 tonnes of carbon dioxide (CO<sub>2</sub>) equivalents (t-e-CO<sub>2</sub>) ha<sup>-1</sup> yr<sup>-1</sup> for these species. Assuming a median phytolith carbon bio-sequestration yield of 0.36 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>, the global potential for bio-sequestration via phytolith carbon (from bamboo and/or other similar grass crops) is estimated to be ~ 1.5 billion t-e-CO<sub>2</sub> yr<sup>-1</sup>, equivalent to 11% of the current increase in atmospheric CO<sub>2</sub>. The data indicate that the management of vegetation such as bamboo forests to maximize the production of PhytOC has the potential to result in considerable quantities of securely bio-sequestered carbon.

*Keywords:* occluded carbon, organic matter decomposition, phytoc, phytoliths, soil organic carbon, terrestrial carbon sequestration

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## Introduction

Net world carbon dioxide (CO<sub>2</sub>) emissions are projected to increase to 31.10 billion tonnes by the year 2010 (DOE, 2008). Given that increases in atmospheric CO<sub>2</sub> concentration are considered by many to underlie dangerous climate change (IPCC, 2007) there is perhaps an urgent need to develop methods that can securely reduce and sequester carbon emissions. Currently, the sequestration of carbon is largely dependent on existing forestry or hardwood plantations broadly described as 'woody plants'. A more recent approach has been to look at increasing the world's soil carbon stocks: these have previously been estimated to accumulate at ~ 2.4 g C m<sup>-2</sup> yr<sup>-1</sup> (Schlesinger, 1990). However, quantifying soil carbon changes is difficult due to differences in methodologies currently employed and rates of decomposition resulting in both spatial and temporal variability (McKenzie *et al.*, 2000; Skjemstad *et al.*, 2000; García-Oliva & Masera, 2004). Current methods of

carbon quantification in soil include determination of total carbon (TC). TC measures all carbon fractions and is unable to distinguish between the more volatile soil carbon fractions and the stable soil carbon forms.

One inert form of organic carbon that is bio-sequestered within plants (and hence can be measured whilst in standing vegetation) and that accumulates in soil after the decomposition of that vegetation is the phytolith-occluded carbon (PhytOC) fraction (Parr & Sullivan, 2005). Phytoliths also referred to as 'plantstones' or 'plant opal' are found in many plants species but are particularly prolific in grasses such as bamboo species. Piperno (1988) describes three sites of silica deposition within plant tissue: (1) the cell wall deposits, (2) infillings of the cell lumen and (3) in intercellular spaces of the cortex. The cell wall deposits of silica often replicate the morphology of the living cells, while those forming in the lumen do not. Such silicification results in the occlusion of carbon within phytoliths (Jones & Milne, 1963). The silicified epidermal cells of the leaf and stem within all grasses are particularly good at occluding carbon (Parr & Sullivan, 2005). This carbon fraction can be made up of the internal cytoplasmic

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organic cellular material (Wilding *et al.*, 1967) and other materials such as cellulose (Perry *et al.*, 1987) depending on the location of the silicification. Upon maturity the leaf material is deposited onto the soil surface: phytoliths later become incorporated into the soil matrix during decomposition of this organic material.

The occlusion of carbon within phytoliths has been demonstrated to be an important long-term terrestrial carbon fraction (Parr & Sullivan, 2005) representing up to 82% of soil carbon in some buried topsoils after 2000 years of *in situ* decomposition depending on the overlying vegetation type and drainage regime. Moreover, it has been demonstrated that relative to the other soil organic carbon fractions that decompose over a much shorter time scale, the carbon occluded in phytoliths is highly resistant against decomposition (Wilding *et al.*, 1967; Wilding & Drees, 1974; Mulholland & Prior, 1993; Parr & Sullivan, 2005). Our research has demonstrated (using radiocarbon dating of the phytoliths themselves) that phytoliths extracted from palaeosols and peat sediments reach ages of at least 8000 BP (Parr & Sullivan, 2005) and in another study a date of  $13\,300 \pm 450$  BP was acquired (Wilding, 1967). While under some circumstances bioturbation may move them up or down a soil profile, or erosion and dust storms may transport phytolith assemblages over some distance, or they may be burnt in a grass fire, or pass through the digestive system of an animal, the durability and persistence of phytoliths against such processes has been well documented (Baker, 1959, 1961; Baker *et al.*, 1961; Jones & Milne, 1963; Jones & Handreck, 1967; Wilding, 1967; Wilding *et al.*, 1967; Sangster & Parry, 1981; Rovner, 1986; Piperno, 1988; Pearsall, 1989; Humphreys, 1994; Hart & Humphreys, 1997; Parr, 2006; Bowdery, 2007). Moreover, the ability to radiocarbon date the phytoliths themselves demonstrates that they can remain stable sequesters of carbon over millennia despite being subject to the above circumstances. Importantly, Hodson *et al.* (2008) report that for wheat phytoliths the mean carbon to nitrogen ratio is around 41. This indicates that unlike other forms of carbon PhytOC is not locking up significant amounts of nutrients.

Bamboo species are known to be particularly proficient silica accumulators (Drees *et al.*, 1989). Bamboo forests cover approximately 22 million ha worldwide and at least 7.2 million ha are currently growing in China (Jiang, 2004). There has been a significant increase in the use of bamboos for economic purposes such as crafts, charcoal and gas (for fuel), human consumption, housing construction including flooring, panelling, roofing and veneers as well as paper, oil and the production of textiles for the clothing industry which will result in an increase in demand for bamboo plantations (Lobovikov *et al.*, 2007). While the culms are

harvested for these various applications the leaf litter is often overlooked in carbon inventories (Zhou *et al.*, 2008). In this study we examine the PhytOC content in the leaf-litter fraction of 10 economically important clumping bamboo species in China. While PhytOC has been shown to be an important long-term soil carbon fraction (Parr & Sullivan, 2005) and some grass crops such as sugarcane are particularly good at PhytOC production (Sullivan & Parr, 2007; Parr *et al.*, 2009), the potential of bamboo, a known silica accumulating plant, to securely bio-sequester carbon through this process has not been examined previously.

## Materials and methods

### Plant material

In this study bamboo species were used to examine the variability of the yields of plant silica and PhytOC within 10 economically important species and to examine the relationship between these two characteristics. The importance of variability of PhytOC content is that such variability would allow, by the selection of a high PhytOC yielding species or cultivar over a lower yielding species or cultivar, to increase the rate of terrestrial carbon securely sequestered in PhytOC (Parr *et al.*, 2009).

Living leaf samples were collected from 10 different sympodial (clumping) bamboo species of economic importance that had been established for 8 years in trial plots at the Fujian Academy of Forestry Sciences (26°09'N, 119°17'E) in Fuzhou (Fujian, China) (Table 1). Fuzhou, is located in southeast China, it has a warm and wet subtropical monsoon climate, with a frost-free period up to 326 days and annual average temperature

**Table 1** Corresponding sample number for each bamboo species and sample collected at the Fuzhou City, Fujian China site

Sample	Scientific name
1	<i>Dendrocalamopsis basihruta</i> (McClure) Keng F. et W. T. Lin
2	<i>Bambusa pervariabilis</i> McClure
3	<i>Bambusoideae cerosissima</i> McClure
4	<i>Thyrsostachys siamensis</i> (Kurz ex Munro) Gamble
5	<i>Bambusa ienta</i> Chia
6	<i>Phyllostachys pubescens</i> Mazel ex H. de Lehaie
7	<i>Dendrocalamus latiflorus</i> Munro
8	<i>Dendrocalamus minor</i> var. <i>amoenus</i> (Q. H. Dai et C. F. Huang) Hsueh et D. Z. Li)
9	<i>Bambusa multiplex</i> cv. <i>fernleaf</i> R. A. Young
10	<i>Bambusa vulgaris</i> var. <i>striata</i> Gamble

of 19.6 °C, the coldest average (January) temperature of 10.5 °C, the hottest (July) average temperature 28.6 °C, the average humidity 77%, with an average annual rainfall of 1343 mm.

#### *Sampling of plant material*

In most commercial bamboo applications the shoots and culms are harvested for consumption, the production of building materials or textiles. The leaf litter from new growth and the harvested culms is generally returned to the soil. In this study we have focused on the PhytOC component contained within the leaf litter. The accumulation of silica has been found to be greater in plants at maturity than in juvenile plants (Norris & Hackney, 1999; Motomura *et al.*, 2002; Parr & Kerr, 2007), so to ensure valid comparison and to gain maximum accumulation contents, only the mature leaf samples from each bamboo species were collected and analysed (Table 1).

#### *Sampling of soils*

Soil samples were also collected to determine the PhytOC content and to calculate its contribution to the total soil organic carbon (TOC). Soil samples from the 0–20 cm surface soil layer in each bamboo plot were taken. These soil samples were acidic (pH 4.0–5.5).

#### *PhytOC analysis*

The method used in this study for the isolation of phytoliths from duplicate leaf and soil samples is a microwave digestion process described in Parr *et al.* (2001) and Parr (2002). The basic method adopted here is a modified version of a stepped microwave digestion process (Parr *et al.*, 2001) for plants and for soil samples (Parr, 2002). This process was followed by a Walkley–Black type digest (Walkley & Black, 1934) to ensure extraneous organic materials in the samples were removed. This is a similar method to that used in the preparation of phytoliths for radiocarbon dating (Wilding, 1967; Parr & Sullivan, 2005). The presence or absence of extraneous materials in the samples was further checked by well-established methods of optical microscopic examination, including cross-polarized light, which can be used to differentiate plant silica from cellulose, calcium oxalate crystals and starch, etc. (cf. Reichert, 1913; Ward *et al.*, 1979; WHO, 1998; Murphy, 2002). The phytolith isolates were then thoroughly dried at 75 °C for 24 h in a fan-forced oven to remove all moisture content and sealed in pre weighed centrifuge tubes. Samples were then weighed to obtain plant silica yields. The dried phytolith isolates were then com-

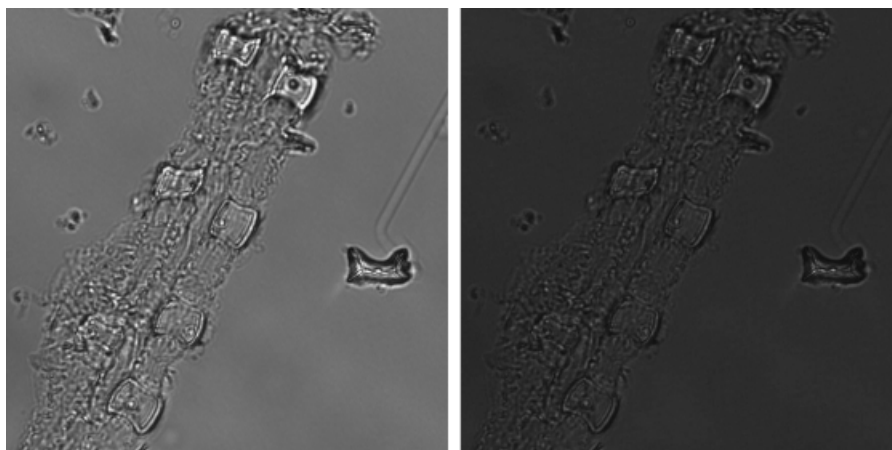
busted in an Vario Macro Elementar CNS analyser (Elementar Analysensysteme GmbH, Donaustrasse, Hanau, Germany) to determine carbon contents. The PhytOC results for duplicate leaf samples were combined and the mean percentage calculated.

Annual leaf-litter deposition rates were not available for the study sites. Published data describing typical yields of two of the bamboo species were used in conjunction with the relative PhytOC yields (% biomass by weight) to provide estimates of the potential annual PhytOC yields in tonnes of CO<sub>2</sub> equivalents (t-e-CO<sub>2</sub>) ha<sup>-1</sup>. Published data of annual leaf-litter deposition rates for the remaining bamboo species consist of highly variable leaf-litter accumulation rates for different geographical locations and species. Estimates of leaf-litter accumulation rates in mature bamboo stands range from 1 to 37 t ha<sup>-1</sup> (Kleinhenz & Midmore, 2001; Peng *et al.*, 2002; He *et al.*, 2003). Using the published data for two species and the potential range based on the above leaf-litter accumulation rates, the PhytOC percentages per hectare were then quantified and compared (Parr & Sullivan, 2004). PhytOC results for soil carbon were normalized to 100% as a percentage of TC. It is realized that for relatively young stands of vegetation that these PhytOC contents may reflect more the PhytOC produced by the preceding vegetation types than that produced by the current bamboo stands but the data do serve to show the proportion of PhytOC in such soil materials. Soil PhytOC data have only been published previously in Parr & Sullivan (2005) for different soil types under different climates and under different vegetation.

## **Results**

The phytolith extraction methods used in this study are designed to completely remove all organic material from the leaf litter of each bamboo species and all available organic material from soils apart from that within the phytolith fraction (Walkley & Black, 1934; Parr *et al.*, 2001; Parr, 2002). The absence of extraneous organic materials in the samples was confirmed by optical microscopic examination (Fig. 1). Both the silica content and the PhytOC content of leaf litter show significant variation between species (Table 2). The phytolith content for the 10 bamboo species varied from between 8% and 28% of the original mass of leaf-litter material (Table 2). There was considerable variation in the phytolith carbon contents of leaf-litter material for each bamboo species, ranging between of 1.60% and 4.02% (Table 2).

The TC content of soil samples taken from the base of each bamboo species ranged from 0.26% to 6.80% (Table 3) and the PhytOC content of the soil samples ranged



**Fig. 1** Bamboo phytoliths extracted by the microwave method followed by a Walkley–Black digest using standard (left) and polarized light (right) light microscopy. The cross-polarized light technique is used here to show the absence of cellulose, calcium oxalate crystals and starch grains, which appear as bright white and blue if present.

**Table 2** Bamboo species, percentage of silica (Si) post digestion to original plant sample dry weight, percentage of phytolith occluded carbon (PhytOC) post digestion in the silica, standard deviation of PhytOC and the estimated range of phytolith occluded carbon (PhytOC) per hectare in tonnes of carbon dioxide equivalents (t-e-CO<sub>2</sub>) (assuming the published range of bamboo leaf litter deposition rates of between 1 and 37 t yr<sup>-1</sup>)

Bamboo species	Si (%)	PhytOC/Si (%)	PhytOC/Si (%) SD	PhytOC/dry leaf biomass (%)	Estimated range of leaf PhytOC yields (t-e-CO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup> )
<i>Dendrocalamopsis basihirsuta</i>	21.27	2.40	0.31	0.51	(0.018–0.691)
<i>Bambusa pervariabilis</i>	28.03	1.60	0.08	0.45	(0.016–0.606)
<i>Bambusoideae cerosissima</i>	9.34	4.02	0.26	0.38	(0.014–0.508)
<i>Thyrsostachys siamensis</i>	21.56	1.83	0.14	0.39	(0.014–0.534)
<i>Bambusa ienta</i>	13.71	3.82	0.62	0.52	(0.019–0.709)
<i>Phyllostachys pubescens</i>	15.82	3.00	0.35	0.47	(0.017–0.637)
<i>Dendrocalamus latiflorus</i>	8.15	2.99	0.01	0.24	(0.008–0.325)
<i>Dendrocalamus minor</i> var. <i>amoenus</i>	13.01	3.18	0.44	0.41	(0.015–0.560)
<i>Bambusa multiplex</i> cv. <i>fernleaf</i>	13.19	3.35	0.45	0.44	(0.016–0.598)
<i>Bambusa vulgaris</i> var. <i>striata</i>	11.33	3.39	0.24	0.38	(0.014–0.520)

from 4% to 100% of the total soil carbon content (Table 3).

## Discussion

Both the silica content and the PhytOC content of the leaf litter show significant variation and there was a relatively strong negative correlation between the two variables (Fig. 2). These results suggest that it is the nature of silica deposition and efficiency by which carbon is encapsulated by silica within the cell walls of the phytoliths rather than the actual quantity of silica up-taken by the plant that determines the relative PhytOC yield.

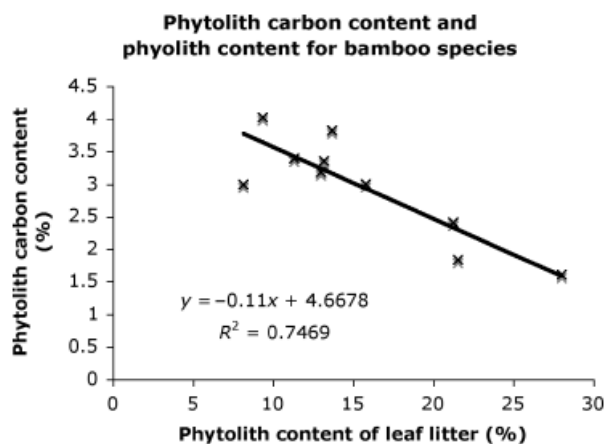
For carbon accounting the methods employed for the measurement of a carbon fraction need to be accurate and relatively easy to apply. The results of this study

indicate that the methods used for the isolation of plant silica phytoliths and the subsequent quantification of the PhytOC fraction meet these criteria because they involve the use of well-established procedures. For example, the extraction of silica from fresh leaf material and soils is a proven, quick and reliable procedure for extracting phytoliths (Parr *et al.*, 2001; Parr, 2002). Verification that no extraneous material remains on the outside of the phytoliths, which may influence the carbon analysis is, also quickly carried out with established microscopy methods that have stood the test of time (cf. Reichert, 1913; Ward *et al.*, 1979; WHO, 1998; Murphy, 2002). The use of a CNS analyser to determine carbon contents is also the current standard procedure for carbon analysis. Another important factor for carbon accounting under Land Use, Land-Use Change and Forestry (IPCC, 2000) guidelines is the ability to show



**Table 3** Total soil carbon, phytolith occluded carbon (PhytOC) and the percentage of PhytOC to: total soil carbon in the surface soil (0–20 cm) underneath the plots of each bamboo species

Soil beneath bamboo	Total soil carbon (%)	Soil PhytOC (%)	Soil PhytOC/total soil carbon (%)
<i>Dendrocalamopsis basihirsuta</i>	1.09	0.174	15.96
<i>Bambusa pervariabilis</i>	3.98	0.297	7.46
<i>Bambusoideae cerosissima</i>	6.80	0.299	4.40
<i>Thyrsostachys siamensis</i>	0.27	0.204	75.56
<i>Bambusa ienta</i>	1.11	0.329	29.64
<i>Phyllostachys pubescens</i>	1.28	0.231	18.05
<i>Dendrocalamus latiflorus</i>	1.85	0.518	28.00
<i>Dendrocalamus minor</i> var. <i>amoenus</i>	0.53	0.520	98.11
<i>Bambusa multiplex</i> cv. <i>fernleaf</i>	0.40	0.404	100.00
<i>Bambusa vulgaris</i> var. <i>striata</i>	2.38	0.515	21.64

**Fig. 2** Phytolith carbon content and phytolith content.

that the carbon being sequestered is additional to that, which would normally be present. The high variability of PhytOC content for the different species used in this study provides some opportunity to increase the amount of bio-sequestered carbon. For example, the PhytOC content of leaf litter for the lowest yielding species contained 1.60% and the highest yielding species 4.02%, a relative ratio of 250% (Table 2). For the two species of which the leaf-litter data per hectare have been determined in a study, *Dendrocalamus latiflorus* and *Phyllostachys pubescens* the PhytOC accumulation rates were 0.030 and 0.102 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Table 2), a relative difference of 340%. The potential range of PhytOC accumulation rates shown in Table 2 indicates that if (1) high PhytOC yielding bamboo species were grown instead of lower PhytOC yielding bamboo species under (2) conditions that are conducive to the production of biomass, then the amount of carbon being securely bio-sequestered (in phytoliths) could be substantially increased. This is also applicable for other grass species such as sugarcane where significant var-

iation in PhytOC yield has been shown across different cultivars (Sullivan & Parr, 2007; Parr *et al.*, 2009).

The use of bamboo may be species specific, for example, *Phyllostachys pubescens* is the most commonly used species in the manufacture of flooring due to its structure and hardness (Yu *et al.*, 2008). Thus there are several factors a land manager would need to take into account (e.g. location, disease resistance, culm production and the end use or application of the bamboo, etc.) before planting a bamboo plantation. If for example a land manager chose to grow *Phyllostachys pubescens* over *Dendrocalamus latiflorus* for the production of flooring there would be, assuming the published leaf-litter deposition rates apply, as a direct result of that decision, an increase of 0.072 t-e-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> of additional securely sequestered carbon in phytoliths each and every year for that plantation. While data on culm production was not available for all species tested in this study published data were available for *Phyllostachys pubescens* and *Dendrocalamus latiflorus*. The published data on culm production for *Phyllostachys pubescens* was 116.5 t ha<sup>-1</sup> (Isagi *et al.*, 1997) while *Dendrocalamus latiflorus* was far less productive at 16.67 t ha<sup>-1</sup> (Lin *et al.*, 2000). Based on these data there would be no compromise on productivity when selecting for high PhytOC producing bamboos. Significantly, previous studies have shown that there is no compromise in product yield when selecting for high PhytOC yielding varieties in agricultural grasses such as sugarcane (Parr *et al.*, 2009).

The wide range of values of PhytOC/total soil carbon in the soil samples in such young plantations reflects the nature of the former vegetation rather than the present but serves to illustrate the considerable ability of soils to accumulate this protected carbon fraction. The ability to isolate and accurately quantify the PhytOC carbon fraction for each bamboo species before its incorporation into soil is a distinct advantage for carbon

accounting purposes because it bypasses many of the potential problems discussed earlier that are associated with the measurement of soil carbon (McKenzie *et al.*, 2000; Skjemstad *et al.*, 2000; García-Oliva & Masera, 2004).

In this paper we have (1) discussed methods of plant and soil carbon quantification and (2) demonstrated that the ability to accurately quantify the PhytOC carbon fraction in the leaf-litter fraction for each bamboo species before incorporation into soils is possible. This latter point provides a distinct advantage for bamboo plantation and/or land managers in general wishing to quantify and trade in soil carbon. The results also show that the quantity of carbon occluded in phytoliths varies considerably between different bamboo species. This indicates that substantial quantities of carbon could be sequestered securely by choosing to grow bamboo species that have high PhytOC yields over those that have low PhytOC yields as well as by maximizing biomass production. Finally, this study has demonstrated that bamboo has considerable potential for the secure bio-sequestration of phytolith carbon in soils. For example, were the 22 million ha of current bamboo forests managed to maximize the production of PhytOC equivalent to that of the maximum rate estimated in this study of  $0.709 \text{ t-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ , this would result in 15.6 million tonnes of securely bio-sequestered carbon (as e-CO<sub>2</sub>) per annum. Extrapolating further, if all 4.1 billion ha of potentially arable land (WRI&IIED, 1986) was used to grow bamboo [or other grass crops with similar capacity to sequester carbon in phytoliths (e.g. Sullivan & Parr, 2007; Parr *et al.*, 2009)], assuming only a median phytolith carbon bio-sequestration yield of  $0.36 \text{ t-e-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ , the global potential for phytolith carbon bio-sequestration is  $\sim 1.5$  billion t-e-CO<sub>2</sub> yr<sup>-1</sup>. This rate of carbon bio-sequestration would effectively reduce global CO<sub>2</sub> emissions by a rate equivalent to  $\sim 11\%$  of the current increase in atmospheric CO<sub>2</sub> (Solomon *et al.*, 2007). Thus the selection of specific species of vegetation like bamboo with high PhytOC yields and the appropriate management of that vegetation to maximize biomass production could substantially increase the secure terrestrial sequestration of carbon.

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