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Carbon sequestration within millet phytoliths from dry-farming of crops in China

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Phytoliths are noncrystalline minerals that form inside cells and cell walls of different parts of plants. Organic carbon in living cells can be occluded in phytoliths during plant growth. It has been documented that the occluded carbon within phytoliths is an important long-term terrestrial carbon reservoir that has a major role in the global carbon cycle. Common millet and foxtail millet have become typical dry-farming crops in China since the Neolithic Age. The study of carbon conservation within phytoliths in these crops could provide insights into anthropogenic influences on the carbon cycle. In this study, we analyzed the carbon content in phytoliths of common millet and foxtail millet. The results indicated that (1) common millet and foxtail millet contained $0.136\% \pm 0.070\%$ and $0.129\% \pm 0.085\%$ phytolith-occluded carbon (PhytOC) on a dry mass basis, respectively; (2) based on the mean annual production of common millet and foxtail millet in the last 10 years, the phytolith occluded carbon accumulation rate of common millet and foxtail millet was approximately 0.023 ± 0.015 and 0.020 ± 0.010 t CO_2 ha^{-1} a^{-1}, respectively; (3) assuming a similar phytolith occluded carbon accumulation rate as for common millet (the highest accumulation rate was 0.038 t CO_2 ha^{-1} a^{-1}), this could result in the sequestration of 2.37×10^6 t CO_2 per year for the 62.4×10^6 ha dry-farming crops in China. Although there was a decline in the annual production rate and planting area of foxtail millet during 1949 to 2008, the total phytolith carbon sequestration rate was 7×10^6 t CO_2 within the 60-year period. However, phytolith occluded carbon has not yet been fully considered as a global carbon sink. Also, this carbon fraction is probably one of the best candidates for the missing carbon sink.

phytoliths, carbon sequestration, dry-farming, phytolith occluded carbon, PhytOC

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The carbon cycle plays a central role in global change and its importance has already been recognized by the International Geosphere-Biosphere Programme (IGBP) [1–4]. There is an imbalanced budget (or some missing carbon) in the carbon cycle which has been known for nearly half-century, however, the mechanism for (and distribution of) this carbon has not yet been identified completely [5–8]. Many previous studies have suggested that terrestrial ecosystems in the mid- and high-latitudes of the Northern Hemisphere might be functioning as a significant carbon sink [9–11]. The pedosphere, a part of terrestrial ecosystems, contains a large amount of carbon, that is about 1550 Pg carbon in soil inorganic matter and 950 Pg carbon in soil organic matter. A minor variation in such big carbon pool may have a significant effect on the carbon sink [12]. Consequently, the soil was recognized as a probable candidate for the location of the missing carbon sink [13], and thus a greater understanding of its carbon pools has increased our knowledge of the importance of this part of the carbon cycle.

Phytoliths, also called silica phytoliths, are noncrystalline minerals that deposit inside cells and cell walls of different parts of plants when soluble silica is absorbed by the roots [14,15]. Phytoliths can occlude some organic carbon (phytolith occluded carbon (PhytOC)) incorporated during plant

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growth [16]. When plants die and decay, phytoliths are released into the soil and sediment. PhytOC can remain in the soil for a long time even being present in Tertiary [17] and Late Cretaceous sediments [18]. Recent studies indicate that PhytOC from bamboo vegetation globally could sequester 15.6×10^6 t of CO_2 per year. These authors argued that if all 4.1×10^9 ha of potentially arable land was used to grow bamboo, the global potential for phytolith carbon sequestration was 1.5×10^9 t CO_2 a^{-1}. This carbon sequestration rate could therefore account for 11% of the current increase in atmospheric CO_2 [19]. PhytOC is a recalcitrant fraction of soil carbon [20] which has drawn particular attention from many researchers in the study of the terrestrial carbon cycle [21].

Early studies have analyzed the physical and chemical properties of phytoliths and found that they can occlude carbon to levels ranging from 0.2% to 5.8% [22–25]. Current studies on phytoliths have mainly concentrated on the paleoclimate [26–29], archaeobotany [30–32], radiocarbon AMS measurements [23,24], carbon isotopes [33–35] and plant taxonomy [36,37]. However, far fewer studies have examined the PhytOC content of different plants. Although there already have been some studies on the PhytOC content of bamboo and sugarcane [19,38], to date, very little is known about other higher silicon accumulating plants, such as *Sorghum bicolor*, *Oryza sativa*, *Panicum miliaceum* and *Setaria italica*.

Previous research has claimed that the carbon sequestered by crops in the form of PhytOC was considered as zero because the litter decayed and decomposed quickly [39]. As China has a long farming history and a large area of arable land, it is necessary to further explore the importance of PhytOC which may help to identify agricultural carbon sinks in the past and re-evaluate the carbon sink potential of current agricultural land. This study aims to (1) examine the PhytOC content of foxtail millet and common millet, (2) estimate the PhytOC accumulation rate of foxtail millet and common millet, and (3) estimate the amount of carbon sequestered by PhytOC from dry-farming crops in China.

1 Materials and methods

In this study, eight millet species, including foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*), were collected from Beijing, Gansu and Liaoning provinces during the harvest season (Table 1). The root of the millet was discarded and rest of the plant such as the stem leaf and spike were placed in a beaker for further treatment. All of the millet samples were rinsed twice in distilled water and placed in an ultrasonic bath for 20 min, and then dried at 70°C for 24 h. Four parallel samples were selected from each species of millet.

The method for phytolith extraction was described in detail in previous work [15,40]. In this study, a revision to the wet oxidation method was made that aimed to digest the organic matter more completely. The detailed steps are as follows: (1) Weigh about 1 g dry sample into a tube (to the nearest 0.01 mg); (2) add 5 mL HNO_3 into the tube and heat in a water bath at 80°C until reaction stops, then centrifuge 2 times at 3000 r/min for 5 min and decant supernatant; (3) add 10% HCl into a tube and heat in a water bath at 80°C for 30 min, then centrifuge at 3000 r/min for 5 min and decant; (4) add 5 mL HNO_3 into the tube and heat to ensure removal of all organic material, then centrifuge and decant; (5) add 5 mL H_2SO_4 into the tube and heat in a water bath for at least 1 h; (6) cool to room temperature, and reheat in a water bath, and add 30% H_2O_2 slowly until the liquid clears; and (7) centrifuge 4 times at 3000 r/min for 5 min, then dry phytoliths in the tube at 70°C for 24 h.

Weigh the phytoliths using an analytical balance and check the samples under an optical microscope at 400× magnification to ensure no organic material exists. As Figure 1 shows, all the organic material has been digested. The PhytOC content was determined using an Elemental Analyzer vario EL (Elementar Analysen systeme GmbH, Germany).

2 Results

As shown in Table 2, the millet phytolith content varied from 3.027% to 18.787%. The PhytOC content of millet also show a significant variation which ranged from 0.88% to 4.775%. Common millet yielded a higher mean value of PhytOC content than foxtail millet phytolith content (2.51% vs. 1.920%). The relationship among PhytOC content on a dry weight basis, the occluded carbon of phytoliths and phytolith content was examined. The results indicated that

Table 1 Location and samples for each millet species

Sample code	Millet species (breed)	Location	Samples (N)
GSFM	*Setaria italica* Gansu	Xifeng District, Gansu Province	4
LNFM-A	*Setaria italica* Liaoning A	Suizhong County, Liaoning Province	4
LNFM-B	*Setaria italica* Liaoning B	Suizhong County, Liaoning Province	4
BJFM	*Setaria italica* Beijing	Mengtougou District, Beijing	4
GSCM-A	*Panicum miliaceum* Gansu A	Xifeng District, Gansu Province	4
GSCM-B	*Panicum miliaceum* Gansu B	Xifeng District, Gansu Province	4
LNCM	*Panicum miliaceum* Liaoning	Suizhong County, Liaoning Province	4
BJCM	*Panicum miliaceum* Beijing	Mengtougou District, Beijing	4

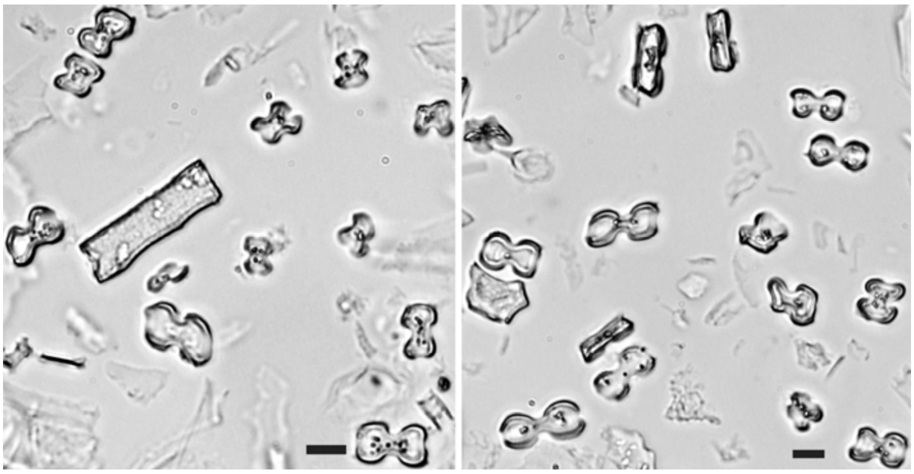

Figure 1 Phytoliths extracted from the millet samples (Both are from foxtail millet and black bar represents 10 μm).

Table 2 Dry weight, plant phytolith content, percentage phytolith content, percentage of PhytOC, and percentage PhytOC content on a dry weight basis

Sample code	Dry weight (g)	Phytolith content in plant (g)	Percentage of phytolith content (%)	Percentage of PhytOC in phytoliths (%)	Percentage of PhytOC content/dry weight (%)
GSFM-1	1.079	0.049	4.541	4.775	0.217
GSFM-2	1.075	0.058	5.395	2.085	0.112
GSFM-3	1.051	0.063	5.994	2.59	0.155
GSFM-4	1.040	0.042	4.038	4.605	0.186
LNFM A-1	1.035	0.065	6.280	1.03	0.065
LNFM A-2	1.014	0.065	6.410	1.17	0.075
LNFM A-3	1.010	0.060	5.941	1.005	0.060
LNFM A-4	1.033	0.065	6.292	0.97	0.061
LNFM B-1	1.050	0.061	5.810	1.06	0.062
LNFM B-2	1.015	0.076	7.488	0.98	0.073
LNFM B-3	1.012	0.063	6.225	1.12	0.070
LNFM B-4	1.037	0.106	10.222	1.15	0.118
BJFM-1	1.058	0.094	8.885	0.94	0.084
BJFM-2	1.004	0.142	14.143	0.88	0.124
BJFM-3	1.059	0.138	13.031	1.85	0.241
BJFM-4	1.073	0.086	8.015	4.515	0.362
Mean±SD		0.077 ± 0.029	7.419 ± 2.860	1.920 ± 1.427	0.129 ± 0.085
GSCM A-1	1.054	0.050	4.744	2.92	0.139
GSCM A-2	1.019	0.048	4.711	4.62	0.218
GSCM A-3	1.017	0.055	5.408	3.665	0.198
GSCM A-4	1.014	0.043	4.241	3.05	0.129
GSCM B-1	1.037	0.044	4.243	3.86	0.164
GSCM B-2	1.033	0.038	3.679	3.39	0.125
GSCM B-3	1.041	0.042	4.035	2.95	0.119
GSCM B-4	1.014	0.041	4.043	4.635	0.187
LNCM-1	1.022	0.192	18.787	1.45	0.272
LNCM-2	1.009	0.147	14.569	1.495	0.218
LNCM-3	1.015	0.058	5.714	1.815	0.104
LNCM-4	1.002	0.088	8.782	1.245	0.109
BJCM-1	1.024	0.031	3.027	1.345	0.041
BJCM-2	1.034	0.043	4.159	1.295	0.054
BJCM-3	1.020	0.034	3.333	1.215	0.040
BJCM-4	1.036	0.049	4.730	1.21	0.057
Mean±SD		0.063 ± 0.044	6.138 ± 4.384	2.51 ± 1.265	0.136 ± 0.070

PhytOC content on a dry weight basis is correlated with the occluded carbon of phytoliths ($P < 0.01$, $R^2 = 0.434$).

3 Discussion

3.1 Phytoliths and PhytOC

Plants have different abilities to yield phytoliths. In general, angiosperms accumulate more phytoliths in their shoots than gymnosperms. Within the angiosperms, the *Poaceae* accumulate more phytoliths than other monocot species [41]. Whether different plants yield differing amounts of PhytOC, and what factors control this in each remain to be found. To answer the former question is beyond the scope of this paper. However, our analysis indicates that there is no significant correlation between phytolith yield and percentage PhytOC content on a dry weight basis in eight species of millet ($P = 0.071$, $R^2 = 0.105$), but the percentage PhytOC content on a dry weight basis is correlated with the occluded carbon of phytoliths ($P < 0.01$, $R^2 = 0.434$). Studies on wheat phytoliths have also demonstrated that there was no relationship between phytolith yield and percentage PhytOC content on a dry weight basis ($P = 0.047$, $R^2 = 0.075$) and a strong correlation also existed between percentage PhytOC content on a dry weight basis and the occluded carbon content of the phytoliths themselves [42]. These results all show that percentage PhytOC content on a dry weight basis is not determined by phytolith yield, but by the efficiency of carbon trapping during the phytolith's deposition in plant [19].

In other work, phytoliths could not be decomposed by using conventional methods of measuring soil carbon such as dry combustion and wet oxidation [43,44]. The occluded carbon in phytoliths therefore could not be determined and thus this carbon fraction was not included as a part of the total soil organic carbon pool. Also, previous studies have determined PhytOC using electron probes [45]— it is not an appropriate method for measuring PhytOC because the results only represent a small area bombarded by an electron beam. This is a likely reason why some data shows PhytOC can vary by up to 30% for some plants. In this study PhytOC was determined by elemental analyzer which combusted samples at 1000°C. Phytoliths then decomposed and released the occluded carbon.

3.2 Carbon sequestrated by PhytOC of millet

Common millet and foxtail millet are important crops in arid and semi-arid regions of eastern Asia [46]. They were the earliest domesticated crops in the middle reaches of the Yellow River [47] and are still staple foods for these regions of Northern China. These two species of millets usually have an overlapping distribution because of similar physiological characters [48]. The total planting area for foxtail millet was 838900 ha in 2007 [49]. Common millet has an annual production of 1.5 million tonnes and the planting area is 1×10^6 ha [50,51].

Dry biomass is a key factor for estimating phytolith carbon sequestration rate. However, not many studies have obtained the dry biomass of foxtail millet and common millet, and only grain production data are available. The ratio of grain to straw (grain/straw) has been widely applied to estimate the above-ground biomass of crops [52,53]. In this study, we used this ratio to estimate the straw weight and then obtain dry biomass weight.

Based on the harvest data of crops from 300 agricultural experimental stations in China, the mean value of grain/straw for foxtail millet which was found to be 0.62 [54]. We analyzed 442 species of common millets from China and found that the mean value of grain/straw was 0.58 [55]. The mean annual production of foxtail millet and common millet were 1.83 t ha^{-1} a^{-1} and 1.5 t ha^{-1} a^{-1} during 1999 to 2008, respectively [49,51]. These results show that the PhytOC accumulation rate of foxtail millet was 0.023 ± 0.015 t ha^{-1} a^{-1}. In contrast to Foxtail millet, the PhytOC of common millet could sequester carbon at a rate of 0.020 ± 0.010 t ha^{-1} a^{-1}. Compared with sugarcane, the phytolith carbon sequestration rate (0.12–0.36 t CO_2 ha^{-1} a^{-1}), of millets was rather low. This could be attributed to (1) millet phytoliths encapsulating less carbon than sugarcane and (2) millets yielding less dry biomass than sugarcane.

Recently, soil phytoliths from a typical stratigraphy of volcanic ash and paleosols at the Numundo sites in Papua New Guinea were examined [16]. Here it was found that the proportion of PhytOC to total carbon increased dramatically from less than 10% in the youngest layers to 82% in the older layers (Figure 2), which means that the accumulation of PhytOC in soil is a long-term sequestration. Assuming the highest PhytOC to dry biomass ratio (0.214%) and using the annual production of foxtail millet during 1949 to 2008 (http://www.sannong.gov.cn), we can estimate the highest PhytOC sequestration in the last 60 years (Figure 3). The results showed that 7×10^6 t CO_2 are likely to have been sequestered and this would be sufficient to offset CO_2 emission released by the combustion of 2.69×10^6 t standard coal. The PhytOC sequestration of foxtail millet generally declined during 1949 to 2008 in conjunction with changes in its planting area. However, other PhytOC crops such as wheat can capture more CO_2 from the atmosphere and securely sequester it for a long time too.

Dry farming occurs on 48% of the 130×10^6 ha of arable land in China [56,57]. If all dry farming crops have a similar PhytOC accumulation rate as millets, this would lead to 2.37 $\times10^6$ t CO_2 being sequestered per year. Although phytoliths can occlude some organic carbon [58], the significant contribution of PhytOC to the carbon cycle has been ignored during the last half-century. Fully understanding the mechanisms of how Phytoliths occlude carbon and the PhytOC content in different plants would enable us to accurately estimate global phytolith accumulation rate.

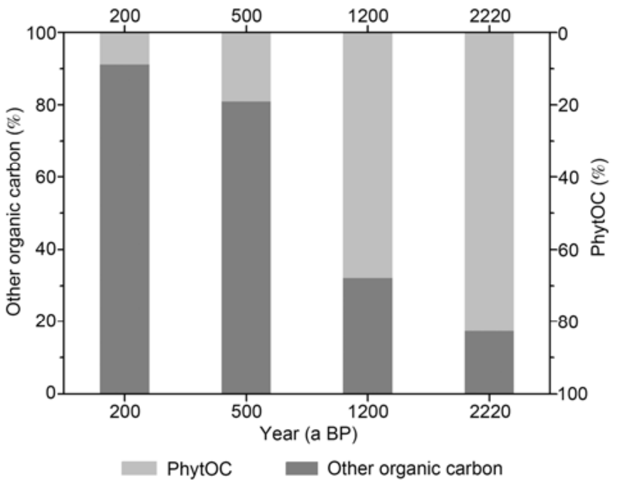

Figure 2 Comparison of soil PhytOC to soil organic carbon in Numundo, Papua New Guinea [16].

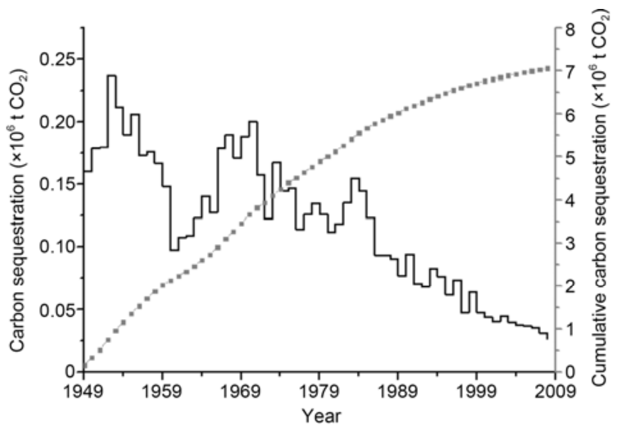

Figure 3 PhytOC accumulation rate of foxtail millet during 1949 to 2008.

4 Conclusions

Previous studies have argued that crops contribute less to carbon sink because they readily decomposed and quickly release CO_2 back to the atmosphere. However, our study shows crops could sequester a substantial amount of carbon through the PhytOC fraction. The PhytOC carbon sequestration rate in Chinese common millet and foxtail millet were approximately 0.020 ± 0.010 and 0.023 ± 0.015 t CO_2 ha^{-1} a^{-1}, respectively. Assuming a similar PhytOC accumulation rate as common millet for other crops, unirrigated Chinese farming systems could sequester 2.37×10^6 t CO_2 per year. From 1949 to 2008, about 7×10^6 t CO_2 may have been sequestered by the PhytOC pool of foxtail millet. Agricultural activities have a complicated interaction with the global carbon balance. However, current studies on terrestrial ecosystem carbon sinks pay little attention to carbon sequestration by the PhytOC pool which has been poorly integrated into our current understanding of the carbon cycle. The potential of PhytOC sequestration provides a new approach for enhancing the soil organic carbon sink. More studies on the PhytOC of different crops are needed and this carbon fraction should now be incorporated in future long-term global carbon sequestration estimates.

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