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A review of carbon isotopes of phytoliths: implications for phytolith-occluded carbon sources

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Abstract

Purpose Phytolith-occluded carbon (PhytOC) is mainly derived from the products of photosynthesis, which can be preserved in soils and sediments for hundreds-to-thousands of years due to the resilient nature of the amorphous phytolith silica. Therefore, stable and radioactive carbon (C) isotopes of phytoliths can be effectively utilized in paleoecological and archeological research. However, there still exists debate about the applicability of C isotopes of phytoliths, as a “two-pool” hypothesis to characterize PhytOC sources has been proposed, whereby a component of the PhytOC is derived from soil organic matter (SOM) absorbed through plant roots. Therefore, it is necessary to review this topic to better understand the source of PhytOC.

Materials and method We introduce the stable and radioactive C isotopic compositions of PhytOC, present the impacts of different extraction methods on the study of PhytOC, and discuss the implications of these factors for determining the sources of PhytOC.

Results and discussion Based on this review, we suggest that organic matter synthesized by photosynthesis is the main source of PhytOC. However, it is important to make clear whether and how SOM-derived C present in phytoliths influence the controversial “too-old” skew and isotopic fractionation.

Conclusions Though the two-pool hypothesis has been proved by many researches, the carbon isotopes of phytoliths still have potential in paleoecology and archeology, because the main source is photosynthesis and many previous studies put forward the availability of these parameters. This review also shows that phytolith C isotopes may vary with different organic C compounds within phytoliths, which needs further study at the molecular scale. Different phytolith extraction methods can influence ^{14}C dating results.

Keywords C_3 and C_4 plants · δ^{13}C · ^{14}C dating · Phytolith extraction method

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1 Introduction

Phytoliths are non-crystalline silica minerals ($SiO_2 \cdot nH_2O$) which are formed by the biomineralization of silica in plant tissues (Jones et al. 1963; Currie 2007). In addition to the main components of SiO_2 (between 80 and 97%) and H_2O (between 5 and 15%), phytoliths also contain small quantities of K, Ca, Na, Mg, Fe, Mn, Al, and organic carbon (C) (Jones et al., 1963; Bartoli and Wilding 1980; Li et al. 2014). During the process of phytolith formation, some cell organelles, nuclei, chloroplasts, and mitochondria are enclosed, and phytolith-occluded carbon (PhytOC) is formed (Kelly et al. 1991; Krull et al. 2003; Elbaum et al. 2009; Carter, 2009; Watling et al. 2011; Song et al., 2016a). Once phytoliths have formed, they usually contain some PhytOC (between 0.2 and 5.8%) (Parr and Sullivan 2005; Parr and Sullivan 2011; Song et al. 2013a; Song et al. 2013b; Zuo et al. 2014; Guo et al. 2015; Zhang et al. 2019; Hodson 2019), and after plant death and decomposition, phytoliths can be preserved in soils and sediments for hundreds-to-thousands of years due to the stability of the silica structure (Parr and Sullivan 2005; Blecker et al. 2006; Song et al. 2016a). The mean residence time of phytoliths in terrestrial soil and sediment ecosystems ranges between 150 and 6500 years (Jones and Beavers 1964; Wilding, 1967; Bartoli and Wilding 1980; Blecker et al. 2006; Pan et al. 2017; Zhang et al. 2017). For example, the phytoliths in a 0 to 63 cm portion of a well-drained Brunizem soil in west-central Ohio developed over 14,000 years stored up to 22.43 t phytoliths/ha (Wilding, 1967). The accumulation of phytoliths in another Brunizem soil in Illinois took around 5000 years (Jones and Beavers 1964). Based on the long residence time and large quantity of phytoliths in terrestrial soils and sediments, these sedimentary phytoliths can be used to identify the origin of crop domestication. For example, through identifying phytolith remains, Zuo et al. (2017) found that rice domestication began at the beginning of the Holocene in China.

During the process of growth, plants synthesize most organic matter (> 97%) by photosynthesis using atmospheric CO_2 (Ford et al. 2007; Song et al. 2016a). The organic C within plants and PhytOC (Wilding, 1967; Piperno 1990; McClaran and Umlauf 2000; Carter, 2009; Basu et al. 2015) are therefore derived from recent photosynthesis. Due to different photosynthetic pathways, C isotopic ratios ($\delta^{13}C$) of C_3 and C_4 plants are distinctly different (C_3: $\delta^{13}C = -27‰$ and C_4: $\delta^{13}C = -13‰$) (Cerling et al. 1989; Collister et al. 1994; Farquhar et al. 2003; Basu et al. 2015). Therefore, the stable C isotopic ratios of plants ($\delta^{13}C_{plant}$) (Cerling et al. 1993, 1997; Quade and Cerling 1995; Latorre et al. 1997; Freeman and Colarusso 2001) and phytoliths ($\delta^{13}C_{phyt}$) (McClaran and Umlauf 2000; Cater et al. 2009; Basu et al. 2015) can be used to recognize the different photosynthetic pathways and reflect the C isotopic signatures of atmospheric CO_2. The radioactive C isotope found in phytoliths ($^{14}C_{phyt}$) can be used for archeological-scale dating (Wilding, 1967; Piperno 1990; Piperno, 2015, 2016; Zuo et al. 2016, 2017). However, the applicability PhytOC is debated, as ^{14}C dating of modern phytolith materials yielded results of the "too-old" skew (Sullivan et al. 2008; Santos et al., 2010a, Santos et al., 2012a, Santos et al., 2018). To explain these results of phytoliths, a "two pool" hypothesis has been proposed, which suggested that some PhytOC is derived from atmospheric CO_2 by photosynthesis and the rest from soil organic matter (SOM) (Santos et al. 2010a, Santos et al., 2012a, Santos et al., 2018; Alexandre et al. 2015; Reyerson et al. 2016). Reyerson et al. (2016) compared 200 isotopic results clearly demonstrating the two-pool hypothesis. Outcomes from this two-pool hypothesis result in limitations to the use of phytoliths in the field of paleoenvironment and chronology (Santos et al. 2010a; Santos et al., 2012a; Piperno, 2015, Piperno, 2016; Zuo et al. 2016, 2017).

As described earlier, the analysis of stable and radioactive C isotopes of phytoliths can indicate whether PhytOC is mainly derived from atmospheric CO_2. In this review, we introduce the features of phytolith $\delta^{13}C$ values and ^{14}C dating, and present their implications, especially for identifying the source of PhytOC. Furthermore, we summarize the different extraction methods and their possible impacts on the measurement results of phytolith C isotopes, considering that some extraction methods may be too intense and can destroy structures of phytoliths. Finally, we discuss whether the too-old skew exists in phytoliths, and if the source of PhytOC is derived principally from the products of photosynthesis.

2 Stable C isotopes of phytoliths

2.1 Quantitative relationship between plant $\delta^{13}C_{phyt}$ and $\delta^{13}C_{atm}$

A number of researchers have shown a quantitative relationship between $\delta^{13}C_{phyt}$ and $\delta^{13}C_{atm}$ (Kelly et al. 1991; Smith and White 2004; Elbaum et al. 2009; Carter 2009; Watling et al. 2011). Carter (2009) collected leaf samples of several C_3 plants (three grasses, two ferns, and ten trees) in New Zealand. This research established a quantitative model to calculate the $\delta^{13}C_{atm}$ with $\delta^{13}C_{phyt}$ values, using three principle equations: (1) $\delta^{13}C_{atm} = \delta^{13}C_{grass} + 20.22‰$ (Gröcke 2002); (2) $\delta^{13}C_{grass} = \delta^{13}C_{phyt} + 3.0‰$; (3) $\delta^{13}C_{atm} = \delta^{13}C_{phyt} + 20.22‰ + 3.0‰$. The first equation showed the C isotopic fractionation for photosynthesis in C_3 plants. The value of "3.0‰" in the second and third equations indicated the difference between $\delta^{13}C_{plant}$ and $\delta^{13}C_{phyt}$ ($\Delta_{grass\text{-}phyt}$). These $\delta^{13}C_{atm}$ values calculated by Eq. 1 with $\delta^{13}C_{phyt}$ were consistent with the local real atmospheric $\delta^{13}C$ (Carter 2009).

Although the quantitative relationship between plant $\delta^{13}C_{phyt}$ and $\delta^{13}C_{atm}$ values has been established for C_3 plants, further study is needed to show the role of chemical composition and $\delta^{13}C$ of different organic compounds within phytoliths. Elbaum et al. (2009) measured the $\delta^{13}C_{phyt}$ values of different fractions and found that the values of complete phytoliths, soluble fractions (sugars and/or proteins), and insoluble fractions (lipids) were − 27.3‰, − 30.4‰, and − 24.1‰, respectively. The PhytOC is mixture of many organic compounds, such as carbohydrates, lipids, fragments of proteins (Elbaum et al., 2009), and the chemical compositions of PhytOC may vary due to the different deposition locations in plant tissues.

2.2 Differences in $\delta^{13}C_{phyt}$ values between C_3 and C_4 plants

Previous results on the stable C isotopic composition of phytoliths and bulk plants/plant tissues of C_3 and C_4 plants are summarized in Fig. 1. Kelly et al. (1991) measured the $\delta^{13}C$ in phytoliths from North American grasslands with C_3 grasses ranging between − 30.00 and − 27.70‰ and C_4 grasses between − 23.70 and − 19.90‰. Similarly, Smith and White (2004) showed that $\delta^{13}C$ values varied between − 34.36 and − 26.69‰ for C_3 grass phytoliths and between − 25.70 and − 17.22‰ for C_4 grass phytoliths, while Basu et al. (2015) found corresponding variation between − 31.80 and − 26.90‰ and between − 28.60 and − 22.60‰, respectively. The significant differences between C_3 and C_4 species (− 29.17 and − 23.96‰, respectively) make it possible to reconstruct the C_3/C_4 ratio for paleograssland (Smith and White 2004; Basu et al. 2015).

There are nevertheless some problems with the use of $\delta^{13}C_{phyt}$ for paleovegetation construction. Compared with the $\delta^{13}C$ of bulk plant/plant tissue, $\delta^{13}C_{phyt}$ tends to be lower (Kelly et al. 1991; Smith and White 2004; Basu et al. 2015) (Fig. 1). This decrease in $\delta^{13}C$ for C_4 plant phytoliths is more pronounced than for C_3 plant phytoliths, with average values decreasing by 10.23 and 3.2‰, respectively (Smith and White 2004; Basu et al. 2015). This phenomenon has been interpreted as a reflection of the relative enrichment of some C constituents with low $\delta^{13}C$ values during the formation of phytoliths. For example, the greater difference in $\delta^{13}C$ between phytoliths and plants in C_4 plants might result from the greater lipid content of C_4 plants compared to C_3 plants

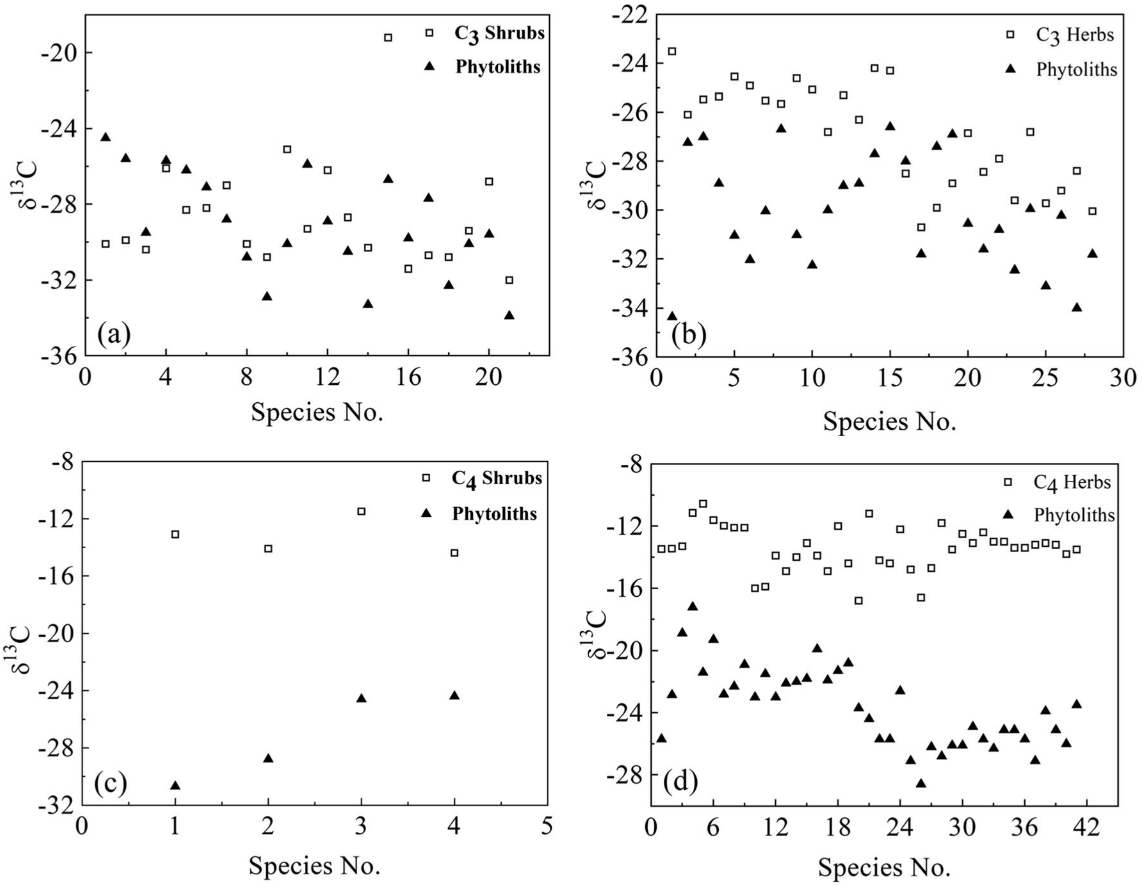

Fig. 1 Stable C isotopic values between plants and phytoliths. **a** C_3 shrubs. **b** C_3 herbs. **c** C_4 shrubs. **d** C_4 herbs. Data are derived from Kelly et al. (1991), Smith et al. (2004), Carter (2009), Webb and Longstaffe (2010), and Basu et al. (2015)

(Smith and Anderson 2001; Smith and White 2004). However, the isotopic composition of carbohydrates within PhytOC has not yet been determined (Carter 2009; Watling et al. 2011), thus limiting further evaluation.

2.3 Stable C isotopes of soil phytoliths

Numerous studies have used δ^{13}C values of SOM for C_3/C_4 evolution research (Quade et al. 1989; Sanyal et al. 2004, 2010; Agrawal et al. 2012; Basu et al. 2015; Li et al. 2010). However, when using the δ^{13}C values of SOM to reconstruct the biogeography of C_3 and C_4 paleograssland, it cannot distinguish C_3 grasses from trees/shrubs because trees and most of the shrubs are also C_3 plants (Sage et al., 1999, Gillon and Yakir 2001). Therefore, this approach has limitation for research on paleograssland reconstruction (Smith and White 2004). Though many trees and shrubs can also produce phytoliths, grass phytoliths are the dominant phytolith assemblage in grassland (Strömberg, 2004). Therefore, $\delta^{13}C_{phyt}$ also has provided a method to reconstruct the paleoecology of C_3 and C_4 grasses (McClaran And Umlauf 2000; Smith and White 2004; Song et al., 2016b).

McClaran And Umlauf (2000) measured the δ^{13}C of phytoliths and SOM in soil profiles to reconstruct the composition of C_3/C_4 plants in desert grassland over the last 8000 years in Arizona, USA. Their study showed that $\delta^{13}C_{phyt}$ reflected C_3/C_4 grass dynamics, while SOM δ^{13}C values indicated the evolution of total vegetation types. Similarly, Lü et al. (2000) studied the $\delta^{13}C_{phyt}$ of surface soils from China and the variation was consistent with the spatial distribution of C_3 and C_4 plants (Kelly et al. 1991; Lü et al. 2000).

However, some other studies have shown that δ^{13}C of fresh plant phytoliths (ranging from − 27.7 to − 19.6‰) are inconsistent with the $\delta^{13}C_{phyt}$ values derived from soil (from − 27.8 to −16.2‰) (Kelly 1989; Fredlund 1993; Kelly et al. 1998; Lü et al. 2000; Mcclaran and Umlauf, 2000). Smith and White (2004) showed that this inconsistency might be due to taphonomic effects prior to the pedogenic processes in the soil, which can be explained by: (1) Phytoliths are mainly from grass roots and their $\delta^{13}C_{phyt}$ values are more positive than that of leaves and steams; (2) phytolith assemblages with poor lipids may have higher preservation potential; and (3) the modern living plant phytoliths represent a very short moment in time, but soil phytoliths are the average mixture of all phytoliths at a given soil depth. That is why surface or top soil phytoliths were dated to hundreds and even thousands of years old. Thus, $\delta^{13}C_{phyt}$ values of soil are positive comparing to fresh plants. Therefore, Smith and White (2004) corrected the end-members of grass $\delta^{13}C_{phyt}$ values ($C_3 = -26.8‰$, $C_4 = -15.3‰$).

3 Radiocarbon of phytoliths

The C in phytoliths is not influenced by other forms of C and C turnover in soil, which ensure the reliability of $^{14}C_{phyt}$ dating (Wilding 1967; Santos et al. 2010a; Piperno 2015). Wilding (1967) was the first to use $^{14}C_{phyt}$ of soil phytoliths to study the chronological characteristics. More recently, $^{14}C_{phyt}$ dating has been used in many fields, such as paleoecology, the study of ancient agriculture and archeology (Piperno 1990; McClaran and Umlauf 2000; Piperno, 2015; Zuo et al. 2017).

3.1 ^{14}C isotopes of soil or sediment phytoliths

Theoretically, $^{14}C_{phyt}$ ages would increase with increasing soil depth (Piperno and Stothert 2003), but the reverse of this has also been reported (Fig. 2a, b). Kelly et al. (1991) analyzed $^{14}C_{phyt}$ ages from three archeological sites, with two showing decreasing age with soil depth. This can be partly explained by the migration of phytoliths due to geological and biological processes (e.g., water transport) (Kelly et al. 1991). Meanwhile, phytoliths with various morphologies may have different preservation and migration potential in soils. Furthermore, phytoliths in soils persist for considerably longer periods than most other organic-derived matter; thus, even uppermost soil horizons may contain an unspecified number of phytoliths incorporated over hundreds of years (Bremond et al. 2004; Piperno 2006; McMichael et al. 2012). The relationship between $^{14}C_{phyt}$ and ^{14}C of other materials from soil or sediment also differs (Figs. 2c, d and 3). Piperno and Stothert (2003) measured the age of phytoliths and associated bulk sediment from the Valdivia ancient sites and found that the ages of soil phytoliths reflected the chronological sequence of SOM. However, McClaran and Umlauf (2000) showed that the $^{14}C_{phyt}$ age might be generally older than associated SOM or other materials (Fig. 2d). They considered that this difference could be attributed to two factors. Firstly, phytoliths are less influenced by water transport or percolation, and that their migration rate is relatively low due to their relatively large size in soil. This difference causes different ^{14}C ages between phytoliths and SOM in soil profiles, with the phytolith age being relatively older (Piperno 1988; McClaran and Umlauf 2000). Secondly, young organic matter mixes with the original SOM in soil profiles through percolation or rhizodeposition (Wang et al. 1996). Meanwhile, the pedogenesis of SOM (e.g., microbial respiration) results in a ^{14}C age much younger than expected in soil (Wang et al. 1996; McClaran and Umlauf 2000; Piperno 2015) (Table 1).

In addition, some studies have compared the dating of phytoliths and other materials to show the potential for phytolith C dating at archeological sites (Zuo et al. 2016, 2017; Asscher et al. 2017). Zuo et al. (2016) studied the ^{14}C ages of phytoliths and other archeological samples (chars, plant

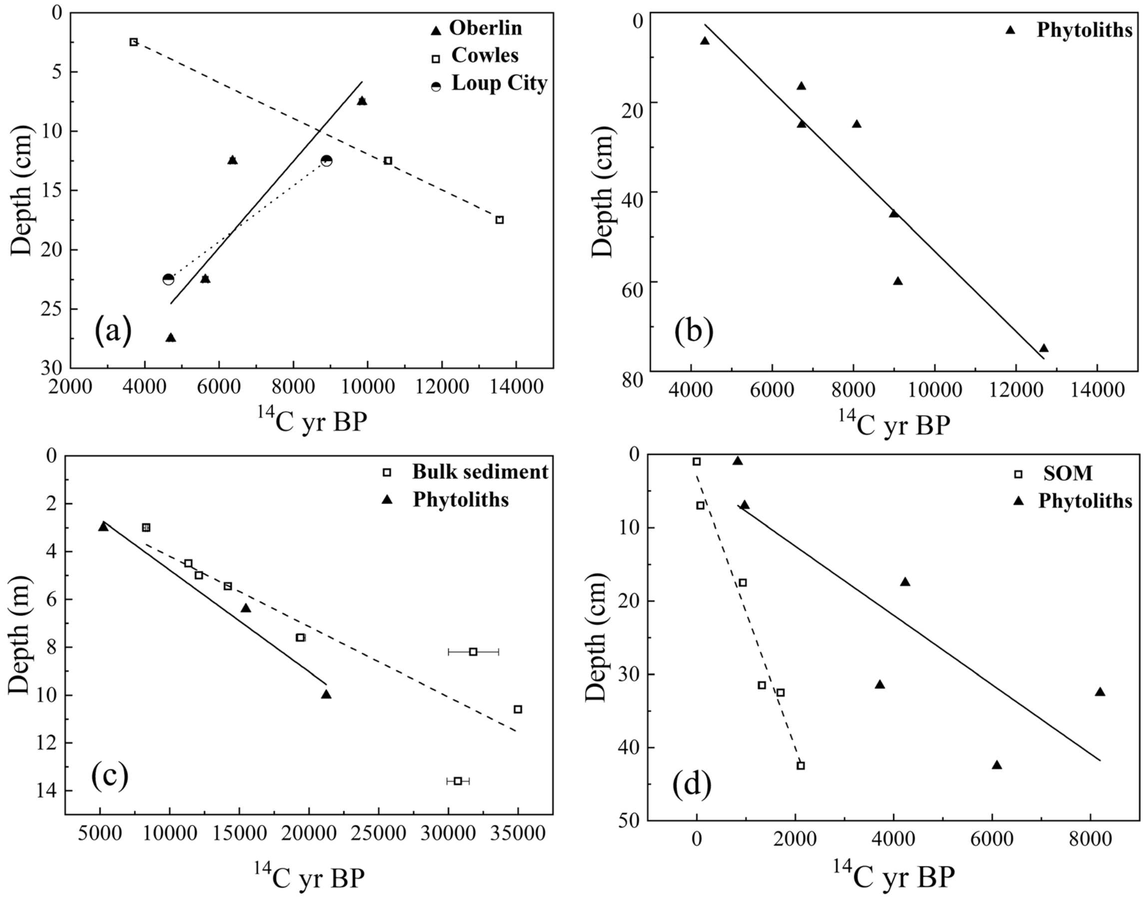

Fig. 2 Radiocarbon dating ages with soil (**a**, **b**, **d**) or sedimentary (**c**) depth. **a** Data are derived from Kelly et al. (1991), and Oberlin, Cowles and Loup city indicate the three different sample sites. **b** Data are derived from Piperno and Stothert (2003). **c** Data are derived from Piperno (2006). **d** Data are derived from McClaran and Umlauf (2000)

remains, and seeds) from six independent sites in China, showing that the ^{14}C ages of phytoliths were mostly consistent with other materials. Furthermore, Asscher et al. (2017) extracted insoluble organic C within phytoliths and showed that the phytolith ^{14}C ages were stratigraphically consistent with the ages of charred seeds and the chronological features of Iron Age sediments (Fig. 3). All of these results have added weight to the thesis that $^{14}C_{phyt}$ dating is applicable for archeological studies. Zuo et al. (2017) validated the $^{14}C_{phyt}$ dating method using a direct comparison with the age of seeds and charcoal in the Huxi archeological site (Shangshan, China). This study revealed that $^{14}C_{phyt}$ was younger than the organic materials in pottery (between 11,348 cal year B.P. and 8608 cal year B.P., respectively), and refuted the “old C.” Meanwhile, we can find that only nine of the 22 pairs of radiocarbons on paired samples of phytoliths and charcoal/plant remains are statistically consistent at 95% confidence (Fig. 3). However, most of the 13 pairs of ^{14}C ages are slightly different (< 500 years), except for the two results with differences more than 1000 years. On the one hand, in soil and sediment environments, some of the phytoliths might be produced from previous plants, demonstrating diverse and long deposition times for phytoliths (Madella and Lancelotti 2012; Shillito 2013). Therefore, the $^{14}C_{phyt}$ ages of soil or sediment phytolith could only represent the yielded time and phytoliths have different ages from that of charcoal at the same soil profile or sediment depth (Smith and White 2004; Zuo et al. 2016). On the other hand, the same

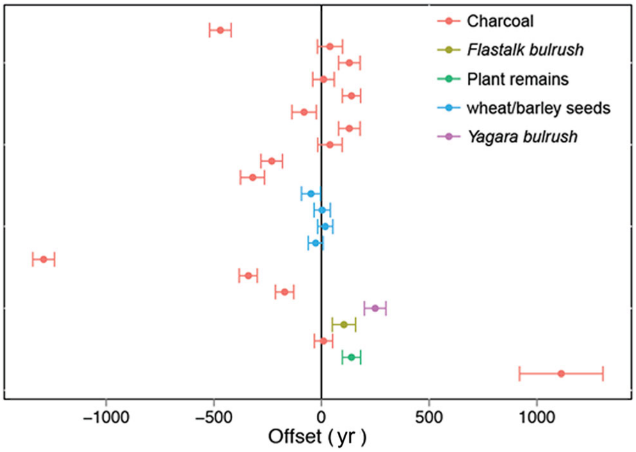

Fig. 3 Offsets between radiocarbon ages on phytoliths and other material from the same sample (data derived from Table 1)

Table 1 Comparison of ^{14}C age between phytoliths and other samples

Sample number	Dating materials	pMC	^{14}C yr B.P.	References
MSG70	Charcoal	62.16 ± 0.65	3820 ± 85	Santos et al. (2010a)
	Phytoliths	71.39 ± 1.52	2707 ± 174	
	Phytoliths (OM removed)	72.68 ± 0.76	2563 ± 85	
Kandara	Phytoliths	94.75 ± 0.23	433 ± 20	
	Phytoliths (OM removed)	76.39 ± 3.33	2163 ± 359	
HX	Plant remains	37.77 ± 0.14	7820 ± 30	Zuo et al. (2016)
	Phytoliths	38.44 ± 0.14	7680 ± 30	
WLXP	Char	45.30 ± 0.16	6360 ± 30	
	Phytoliths	45.36 ± 0.16	6350 ± 30	
TLS	*Flatstalk bulrush*	47.11 ± 0.26	6045 ± 45	
	Phytoliths	47.73 ± 0.17	5940 ± 30	
TLS	*Yagara bulrush* seed	50.87 ± 0.25	5430 ± 40	
	Phytoliths	52.47 ± 0.19	5180 ± 30	
XLF	Char	55.12 ± 0.20	4800 ± 30	
	Phytoliths	52.93 ± 0.19	5110 ± 30	
YC	Char	53.86 ± 0.20	4970 ± 30	
	Phytoliths	51.63 ± 0.19	5310 ± 30	
YY	Char	57.32 ± 0.21	4470 ± 30	
	Phytoliths	48.82 ± 0.24	5760 ± 40	
BS12	Olive pit	69.78 ± 0.23	2891 ± 27	Asscher et al. (2017)
	Olive pit	69.28 ± 0.25	2948 ± 29	
BS18	Barley seeds	69.83 ± 0.24	2884 ± 27	
BS19	Olive pit	69.56 ± 0.24	2916 ± 27	
BS21	Wheat seeds and barley seed	69.75 ± 0.16	2894 ± 20	
BS23	Wheat and barley seeds	69.31 ± 0.25	2945 ± 30	
	Phytoliths	69.08 ± 0.15	2971 ± 17	
		69.47 ± 0.17	2927 ± 19	
		69.35 ± 0.20	2941 ± 23	
		68.89 ± 0.27	2993 ± 32	
BS24	Olive pit	69.63 ± 0.19	2906 ± 23	
	Olive fragment	69.87 ± 0.27	2880 ± 31	
BS25	Phytoliths	69.15 ± 0.14	2964 ± 16	
BS27	Legume fragments	69.70 ± 0.25	2900 ± 30	
	Olive pit	69.28 ± 0.25	2950 ± 30	
HHS	Phytoliths	36.35 ± 0.18	8130 ± 40	Zuo et al. (2017)
	Phytoliths	36.76 ± 0.14	8040 ± 30	
	Char	37.82 ± 0.19	7810 ± 40	
	Char	36.17 ± 0.18	8170 ± 40	
	Nutshell	36.71 ± 0.18	8050 ± 40	
HX-8	Phytoliths	37.52 ± 0.19	7870 ± 40	
	Char	37.92 ± 0.19	7790 ± 40	
HX-7	Phytoliths	38.44 ± 0.14	7680 ± 30	
	Char	37.78 ± 0.14	7820 ± 30	
	Char	38.39 ± 0.19	7690 ± 40	
HX-6	Phytoliths	39.17 ± 0.15	7530 ± 30	
	Char	38.54 ± 0.19	7660 ± 40	
	Char	38.97 ± 0.24	7570 ± 50	
HX-5	Phytoliths	40.91 ± 0.23	7180 ± 40	
	Char	43.73 ± 0.22	6710 ± 40	

Sample number and origin data are obtained from two sample sites (Santos et al. 2010a), six different archeological sites (Zuo et al. 2016), different archeological layers (Asscher et al. 2017), and two archeological sites (Zuo et al. 2017). The percent modern carbon (pMC) of plants, influenced by the atmospheric bomb testing during 1955 to 1963, is greater than 100. The values of pMC and ^{14}C ages were converted on http://calib.qub.ac.uk/CALIBomb/

thickness of soil and sediment accumulate over various periods, and phytolith deposition takes place over a number of years due to their differing depositional processes in soils or sediments. When sampling a relatively "thick" soil profile or sediment depth of 5–10 cm, a difference of hundreds of years between the ^{14}C ages of phytoliths and other materials is generally acceptable (Madella and Lancelotti 2012; Piperno 2015; Zuo et al. 2016, 2017). For the discrepancy of thousands of years between phytoliths and other materials, however, the post-depositional processes of phytoliths in stratigraphic blocks are one of the possible explanations (Wallis 2001). Phytoliths are subjected to translocation, stratigraphic mixing processes, and bioturbation after deposition. Heavy translocation and extreme bioturbation may produce a stratigraphic block with varying chronology and composition even in the same soil or sediment profiles (Piperno and Becker 1996; Shillito 2013; Zuo et al. 2016, 2017).

3.2 ^{14}C isotopes of modern plant phytoliths

In order to verify the reliability of phytolith ^{14}C dating, some studies have been carried out using modern plant samples (Sullivan et al. 2008; Sullivan and Parr 2013; Santos et al., 2012b; Piperno, 2015). Sullivan et al. (2008) analyzed the ^{14}C$_{phyt}$ ages of 12 plant samples from mature bamboo leaves and different litter layers. The results of this study showed that ten were modern, or close to modern (Table 2). Santos et al. (2010a, 2012a) showed that phytoliths from harvested grass materials were much older than at the time of collection (Table 2), but the organic tissues of these grasses showed modern ^{14}C ages as expected. The two-pool explanation for these contradictory results held that plants could acquire organic C and nitrogen from SOM via mutualistic mycorrhizal fungi (Nasholm et al. 1998; Talbot et al. 2008; Talbot and Treseder 2010; Santos et al., 2012b) or proteolytic enzymes and endocytosis (Paungfoo-Lonhienne et al. 2008). These SOM might preferentially deposit into phytolith bodies (Santos et al., 2012b; Alexandre et al. 2015; Reyerson et al. 2016) and eventually lead to a deviation of ^{14}C$_{phyt}$ results. Reyerson et al. (2016) carried out grass cultivation experiments and measured comparative isotope values (^{14}C and δ^{13}C) of PhytOC, original plants, atmospheric CO$_2$, and soil SOM and found that the isotopes of SOM affected the C isotopic values of phytoliths. These experiments indicated that the ^{14}C offsets between PhytOC and stems and leaves pointed toward the ^{14}C signal of SOM. Meanwhile, C isotopic values of each plant tissue imply that some C can be absorbed by the roots from soil, then transported toward the stems and leaves, and finally occluded into phytoliths. Reyerson et al. (2016) also suggested that the C from SOM

Table 2 Radiocarbon ages of PhytOC from modern plant samples

Sample type	Collection dates (AD)	Sample description	pMC	^{14}C yr B.P.	Reference
Phytoliths from bamboo leaves	2008	Mature	61.9	3510	Sullivan and Parr (2013)
	2008	Recently fallen	76.9	1855	
	2008	0.5 cm depth	93.6	250	
	2008	1.5 cm depth	100.6	modern	
	2008	2.5 cm depth	95.8	70	
	2008	3.5 cm depth	99.0	modern	
	2008	4.5 cm depth	97.4	modern	
	2008	5.6 cm depth	98.4	modern	
	2008	6.4 cm depth	95.8	60	
	2008	8.0 cm depth	95.5	85	
	2008	10.5 cm depth	100.6	modern	
	2008	13.5 cm depth	101.6	modern	
Phytoliths or organic tissues from grasses	2007	Phytoliths	77.91 ± 0.43 75.00 ± 1.84 73.07 ± 6.01	2005 ± 452,310 ± 2,002,520 ± 690	Santos et al. (2012a)
	2008	Phytoliths	36.75 ± 2.47	8040 ± 560	
	2008	Organic tissue	104.75 ± 0.49	modern	
	2008	Phytoliths	53.66 ± 0.92	5000 ± 140	
	2008	Organic tissue	105.31 ± 0.11	modern	
Phytoliths from neotropical plants	1991–1997	*Zea mays*	103.3 ± 0.3	modern	Piperno, 2015, 2016)
	1991–1997	*Zea mays*	106.7 ± 0.3	modern	
	1995	*Cucurbita ecuadorensis*	114.2 ± 0.3	modern	
	1995	*Cucurbita ficifolia*	114.4 ± 0.4	modern	
	1995	*Cucurbita ficifolia*	101.8 ± 0.4	modern	
	1964	*Hirtella americana*	81.53 ± 0.3	1640 ± 30	
	2013	*Hirtella americana*	101.1 ± 0.4	modern	
	1981	*Socratea durissima*	123.3 ± 0.5	modern	

Bamboo leaf samples in Sullivan et al. (2008) were collected from the mature leaves of bamboos and the leaves in different litter layers, and the data of pMC and ^{14}C ages were digitized with the Getdata Graph Digitizer from Sullivan and Parr (2013) was more resistant. Meanwhile, the Stimulated Raman Scattering (SRS) results showed that different soils lead to different organic C within phytoliths, which may further support the two-pool hypothesis (Gallagher et al. 2015).

To confirm the application of $^{14}C_{phyt}$ dating, Piperno (2015) determined the $^{14}C_{phyt}$ ages of modern Neotropical plants. This study showed that the results of most samples agreed with their collection ages, except one sample which was incidentally treated with organic chemicals (containing ^{14}C-dead C) (Table 2). These contrasting results confound the reliability of ^{14}C aging of phytoliths, as the too-old skew is occasionally observed. However, the results presented in this study may lack methodological rigor, such as the absence of a suitable blank, which may cause some modern C contamination during phytolith extraction procedures (Santos et al. 2010a, Santos et al., 2018).

4 The impacts of different methods on phytolith C isotopic measurement

4.1 Phytolith extraction and purity examination

Appropriate methods for phytolith extraction and purity examination are necessary to ensure the accuracy of C isotopic measurement. Traditional and new proposed methodologies for the extraction and analysis of phytoliths from plants and soils are displayed in Fig. 4; Table 3 and Fig. 5; Table 4, respectively. According to the procedures of plant phytolith extraction, the microwave digestion method followed by Walkley-Black digestion is quick and can keep phytoliths intact (Parr et al. 2001a, b, Parr and Sullivan, 2014). The wet oxidation method associated with heavy liquid floatation is a more traditional approach for soil phytolith extraction, while the microwave technique followed by filtration provides a timesaving alternative (Parr and Boyd 2002; Parr and Sullivan 2005). The extraction results can be influenced by the characteristics of the phytoliths (e.g., variable deposition environments of soils, or different plants), which may necessitate a variety of extraction times and different dose of reagents (Piperno 2015). It is therefore important to characterize samples thoroughly before the extraction of phytoliths.

The purity of extracted phytoliths has been examined by a series of methods, such as Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy equipped with energy-dispersive spectrometer (SEM–EDS), and nanoscale secondary ion mass spectrometry (NanoSIMS) (Table 3; Table 4) (Watling et al. 2011; Corbineau et al. 2013; Alexandre et al. 2015). The utilization of these methods can not only detect the composition of organic C but can also reveal elemental composition and provide information on the surface characteristics of phytoliths.

4.2 Influence of methods on the determination of phytolith age

To compare the impact of different degrees of oxidation on PhytOC measurements, Parr and Sullivan (2014) studied the effects of two different extraction methods (microwave digestion and wet oxidation with H_2SO_4 and H_2O_2) on plant PhytOC (sorghum and sugarcane). They found that microwave extraction had 50 times more PhytOC content than wet oxidation. Upon further examination using SEM, the authors showed that wet oxidation degraded the phytolith structure. Based on the variable resistance to oxidation, two different models have been proposed to conceptualize the spatial distribution of organic C within phytoliths (Fig. 6) (Parr and

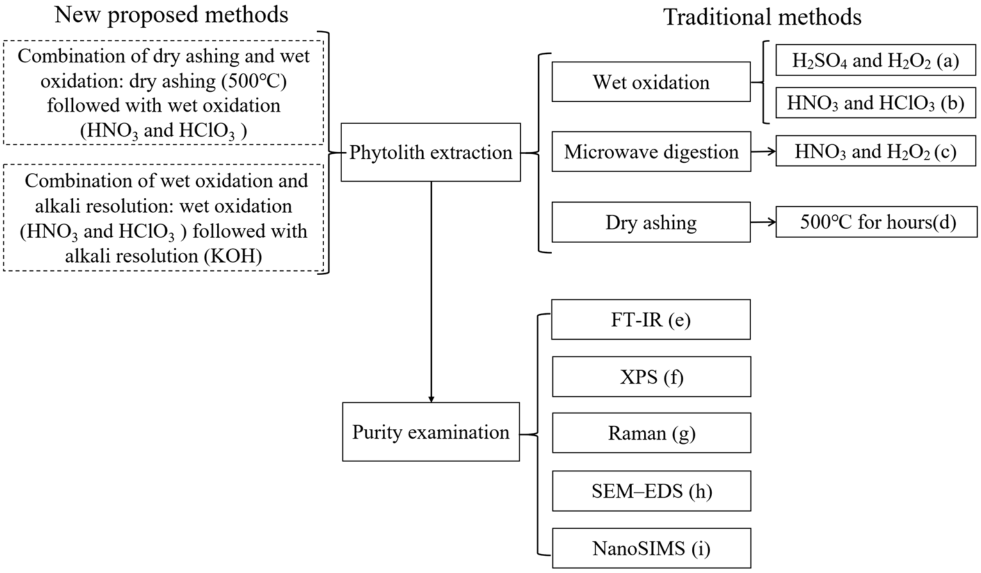

Fig. 4 Main steps of different protocols for plant phytoliths before C isotopic measurement. Traditional methods: **a** Parr and Sullivan (2014), Smith and White (2004), Santos et al. (2010a), and Zou and Lu (2011); **b** Piperno (2015); **c** Parr and Sullivan (2014); **d** Parr et al. (2001). New proposed methods: Corbineau et al. (2013). **e**–**g** Watling et al. (2011); **h** Corbineau et al. (2013); **i** Alexandre et al. (2015)

Table 3 A summary of published experimental/analytical procedures for extracting and examining plant phytoliths prior to C isotope analysis

Method	Main procedures		References
Wet oxidation	H_2SO_4 and H_2O_2	Digest dry plant material with H_2SO_4 in 80 °C water bath until dissolved. Slowly add H_2O_2 until solution is clear.	Parr and Sullivan (2014)
		After extracting with H_2SO_4 and H_2O_2, further oxidize samples with H_2SO_4 and H_2O_2.	Smith and White (2004)
		After extracting with H_2SO_4 and H_2O_2, further clean samples with HNO_3 and $HClO_4$ at least 3–4 times.	Santos et al. (2010a)
		Prior to the digestion with H_2SO_4 and H_2O_2, digest dry plant material with HNO_3, HCl and HNO_3 step by step.	Zou and Lü (2011)
	HNO_3 and $HClO_3$	Add concentrated HNO_3 and digest dry plant materials in boiling water bath, add $KClO_3$ to enhance digestion. Further clean with HNO_3 and $HClO_3$.	Piperno (2015)
Microwave digestion and Walkley-Black digestion	HNO_3 and H_2O_2	Digest dry plant materials with HNO_3, H_2O_2 and HCl by microwave digestion system. Gradually rise digest temperature from 0 °C to 170 °C during 19 min, hold at 170 °C for 29 min.	Parr and Sullivan (2014)
Dry ashing	High temperature	Heat dry plant materials at 500 °C for 6 h in muffle furnace. Clean samples with HCl and H_2O_2.	Parr et al. (2001a, b)
SEM–EDS	The pictures of SEM-EDS can present the microstructure of phytolith surface and semi-quantitatively analyze the element content of Ca, C and Si. The absence of Ca and C:Si < 0.5 indicates the purity of phytoliths.		Corbineau et al. (2013)
Raman, FT-IR and XPS	These technologies can present the chemical structure of phytoliths and analyze the compound composition of phytoliths.		Watling et al. (2011)

Sullivan 2014; Alexandre et al. 2015). These models posit that there may be two forms of phytoliths: solid phytoliths in which PhytOC is mainly occluded within the silica matrix and cavate phytoliths in which PhytOC may be occluded in a silica wall and cavity. Alexandre et al. (2015) showed that the inner voids were open to the surface. The PhytOC within cavate phytoliths is less stable compared to those in the solid phytoliths (Parr and Sullivan 2014; Alexandre et al. 2015). This was supported by research from Watling et al. (2011) who showed that phytoliths remained intact after microwave digestion, but rapid acid oxidation caused the silicate network to be transformed into a SiO_3 ring structure. Further, XPS and FT-IR analyses demonstrated that PhytOC extracted by these two methods exhibited different chemical spectra. PhytOC following microwave extraction was identified as cellulose and lignin, while acid digestion being mostly long-chain hydrocarbons (Watling et al. 2011; Parr and Sullivan 2014). The percentages of soil C in phytoliths would increase with increased oxidation (Reyerson et al. 2016; Santos et al. 2018). Therefore, it is possible that some extraction methods are over-vigorous and digest some parts of cavate PhytOC. Future work needs to investigate the effects on isotopic measurements (Yin et al. 2014; Zuo et al., 2018).

Though the phytolith dating results published by Piperno (2015) and Zuo et al. (2016, 2017) showed that both plant and soil phytoliths could be confidently dated using rigorous phytolith isolation techniques, direct evidence to verify the source of PhytOC recovered following different extraction procedures is still lacking. However, Santos et al. (2018) thought the studies by Zuo et al. (2017) should consider the blanks and standards of ^{14}C samples. Chemical reagents used for the isolation of phytoliths may contain organic compounds

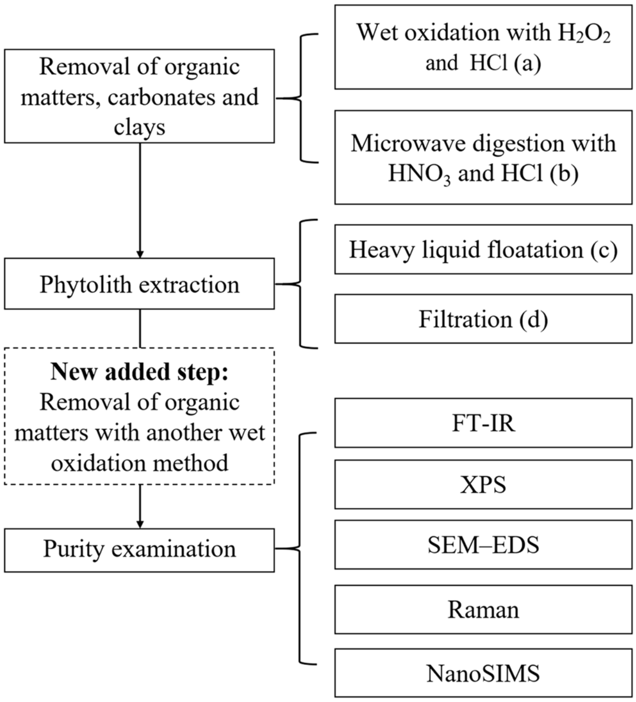

Fig. 5 Main methods for extraction and examination of soil phytoliths before C isotopic measurement. **a** Smith and White (2004); **b**–**d** Parr and Sullivan (2005). New added step is referenced from Santos et al. (2010a, b)

Table 4 A summary of published experimental/analytical procedures for extracting and examining soil phytoliths prior to C isotope analysis

Methods	Main procedures	References
Wet oxidation	(1) Oxidize soil samples with 30% H_2O_2 in 70 °C hot water bath to remove organics. (2) Cates are removed by acidification with 30% HCl in 70 °C hot water. (3) Diffuse samples with 5% sodium pyrophosphate and sieving under 2 μm to remove clays.	Smith and White (2004)
Microwave digestion	Digest soil samples with 3 mL HNO_3 and 3 mL HCl under digestion mode: 0 to 120 °C during 8 min, held at 120 °C for 8 min.	Parr and Sullivan (2005)
Heavy liquid floatation	Separate the supernatant (phytoliths) from the mineral fraction via heavy floatation liquid (2.35 g/ml). Further select phytoliths (subnatant) with heavy floatation liquid at 1.95 g/ml and decant the supernatant.	Parr and Sullivan (2005)
Filtration	Select the phytoliths with size between 0.5 and 250 μm using filters.	Parr and Sullivan (2005)
SEM–EDS	The picture of SEM-EDS can present the microstructure of phytolith surface and semi-quantitatively analyze element content of O and Si. The mass fraction of Si and O over 90%, Si:O ≈ 0.5 and the absence of Ca indicate the purity of phytolith.	Zuo et al. (2016)
FT-IR	Analyze the compound composition of phytolith.	Asscher et al. (2017)
NanoSIMS	Investigate the distribution of different organic compounds within phytoliths at the nanoscale.	Alexandre et al. (2015)

which are not detected using phytolith purity assessment, including SEM-EDX which is recommended by Corbineau et al. (2013) and Santos et al. (2018). Thus, Santos et al. (2012b) utilized SiO_2 to conduct a background correction, possibly leading to bias of phytolith age.

5 Implications of $\delta^{13}C_{phyt}$ and $^{14}C_{phyt}$ dating for PhytOC sources

This review highlights the inconsistency in the reporting of PhytOC sources from a stable and radioactive C isotopic perspective. Although the stable isotope alone is not a direct indication for the source of C in phytoliths for the change of $\delta^{13}C$ from atmospheric CO_2 into plant tissues (Hildebrandt et al. 2015), the relationship between ^{13}C values of phytoliths, atmospheric CO_2, and C_3/C_4 plants indicates that PhytOC is mainly derived from photosynthetic products (Kelly et al. 1991; Smith and White 2004; Carter 2009; Basu et al. 2015). Furthermore, the $^{14}C_{phyt}$ dating results from both archeological contexts and modern plants indicate that phytolith ages are not significantly biased by extraneous C and can be used to establish reasonable chronological sequences in archeological research (Piperno and Stothert 2003; Piperno 2015; Zuo et al. 2016, 2017; Asscher et al. 2017).

However, the ^{14}C ages from parts of modern plant phytoliths can, on occasions, give results older than is likely (Table 2). A hypothesis has been recently put forward by

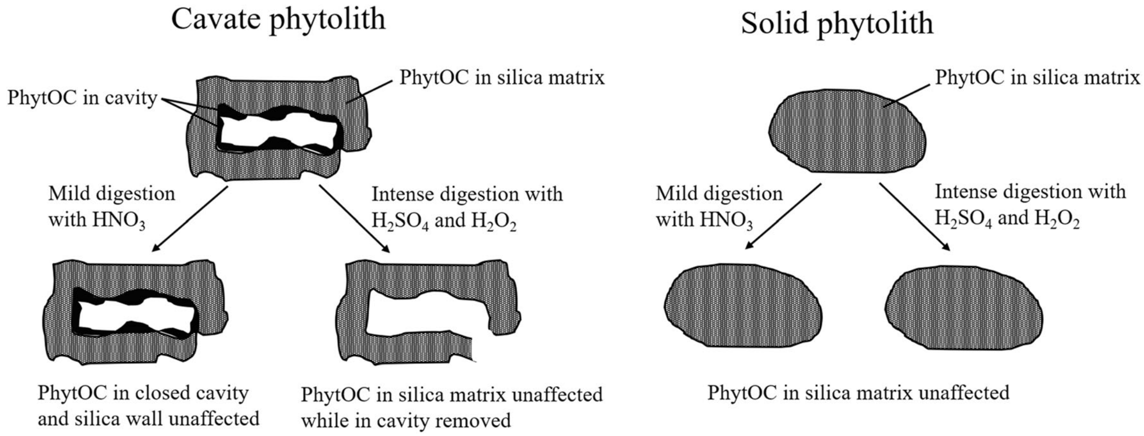

Fig. 6 Conceptual model of spatial distribution of organic C in cavate (**a**) and solid (**b**) phytoliths. Figures are modified from Parr and Sullivan (2014), Song et al. (2016a, b)

Santos et al. (2010a, b, 2012a, b), which includes root-plant uptake, transport. and reallocation of SOM into plants and phytoliths. Moreover, Alexandre et al. (2016) cultured the grass *Festuca arundinacea* in silicon-rich hydroponic solution with ^{13}C- and ^{15}N-labeled amino acids and found that the roots, stems, leaves, and phytoliths took parts of ^{13}C and ^{15}N values characteristic of supplemented amino acids.

In addition, Santos et al. (2012a) showed that the ^{14}C dating ages of modern grass tissues were consistent with the time of collection, while the $^{14}C_{phyt}$ ages from the same materials was much older. The author explained that the fraction of organic C from soil was too small (between 0.1 and 0.2% of all plant organic C) to affect ^{14}C ages of bulk plant, while the absorbed SOM would be preferentially obtained by phytoliths, resulting in the $^{14}C_{phyt}$ age being too old. There are two possible hypotheses for the fixation within phytoliths. The first hypothesis is that some SOM may be associated with Si when absorbed by the roots, or form organosilicate complexes when transported in stems and leaves, which are preferentially occluded into phytoliths. Secondly, organisms cannot metabolize this part of the SOM-derived C and selectively fix them during the precipitation of silica. Nonetheless, Alexandre et al. (2016) measured the relative content of PhytOC from nutrient solution and found that the proportion of organic C derived from nutrient solution in phytoliths (0.15%) was similar to that in the bulk grasses (0.1–0.2%). Additionally, the roots that contain the low Si content also contain the highest amount of C from nutrient solution, which is inconsistent with the associated relation between SOM-derived C and Si. These results indicated that phytoliths did not selectively capture C from the soil before the abundant photosynthetic products. Further investigations for characterizing PhytOC at the molecular level are necessary to examine these hypotheses and quantify the contribution of the SOM-derived C and too-old skew of $^{14}C_{phyt}$ ages.

6 Conclusions and perspectives

The stable C isotopic composition of plant and soil phytoliths can be used to distinguish C_3 and C_4 plants, while the C isotopic composition of atmospheric CO_2 can be calculated from the $\delta^{13}C_{phyt}$ values of C_3 plants. Phytoliths have the potential for ^{14}C dating in archeology. Both stable and radioactive C isotopes indicate that the main source of PhytOC is from atmospheric CO_2. This review also underlines the processes by which SOM-derived C within phytoliths causes the too-old skew. Different methods can cause the age of $^{14}C_{phyt}$ to be exaggerated. This review suggests that the following issues need to be resolved:

1. Although there were some applications of phytolith stable C isotopes, further work is still required to study C isotopic fractions from the different organic compounds (molecular scale) to derive a more representative C isotopic model for the process of phytolith formation.
2. The analysis of PhytOC source should eliminate the influence of different experiment methods. Thus, it is necessary to quantify the effects of different experiment processes on phytolith C isotopes and build a set of standard protocols for phytolith extraction and subsequent analysis.

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