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Research paper

Towards producing pure phytolith concentrates from plants that are suitable for carbon isotopic analysis

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ABSTRACT

Phytoliths are micrometric particles of amorphous silica that form inside or between the cells of higher plant tissues throughout the life of a plant. Phytolith morphological assemblages extracted from sediments and buried soils are increasingly used as proxies of grassland diversity and tree cover density. When found in significant amounts in archeological sites they can be used for identifying food habits, cultural and agricultural practices. Phytoliths can contain small amounts of C occluded in their structure (phytC). It is generally assumed that the source of this phytC is atmospheric CO_2 that was fixed by the plant via photosynthesis. Isotopic analyses of phytoliths ($\delta^{13}C$, ^{14}C) were thus expected to inform respectively on the photosynthetic pathway or on the age of the mineralized host plants. However recent ^{14}C analyses of phytC from phytolith concentrates extracted from soils and harvested grasses yielded unexpected ^{14}C ages of several hundreds to kyr old. These ^{14}C phytC results raised the question of a possible source of refractory/old soil organic matter component taken up by roots, which can be attached or occluded in phytoliths. Simultaneously these results highlighted the need for setting standardized protocols leading to concentrates entirely devoid of organic residues, as well as for a robust method for checking phytolith purity. The goal of this work was thus to develop protocols for extracting phytoliths from plants, leading to 100% phytolith purity, as required for phytC analyses. Protocol 1 utilizes a multi-step process of dry ashing and acid digestion, while protocol 2 also uses acid digestion as well as a separate alkali immersion step which removes surface layers. Phytolith concentrate purity was gauged in a semi-quantitative fashion through the use of SEM–EDS analysis. This quality check for phytolith purity can reveal small C particulate contamination of phytolith concentrates that may considerably bias isotopic and quantitative analyses of phytC. Results indicate that the two protocols were able to entirely remove small C particulate contamination. Protocol 1 produced phytolith concentrates with well defined morphologies suitable for both morphological and isotopic analyses. However measurement of C yields showed that protocol 1 probably induced C leakage, leading to lower recovery. Protocol 2 is faster, leads to higher C yield but may lead to a beginning of dissolution. With these protocols on hand, sources of phytC can be properly investigated.

1. Introduction

Phytoliths are micrometric particles of amorphous silica (ASi) that form inside or between the cells of higher plant tissues throughout the life of a plant. Silicon (Si) is taken up by the roots in its dissolved form, translocated in the sap, and deposited in the cells where it can take the shape of the host cell. The concentration of phytoliths ranges from less than 0.01% of dry weight in many gymnosperms and dicotyledon angiosperms to more than 8% of dry weight in Poaceae, Arecaceae, and Equisetaceae (e.g. Geis, 1973; Bozarth, 1992; Webb and Longstaffe, 2002). With plant decay, phytoliths that are preserved in oxidizing environments are either incorporated into soils or exported to sediments via regional watersheds. Phytolith morphological assemblages extracted from sediments and buried soils are increasingly used as proxies of grassland diversity and tree cover density (e.g. Blinnikov et al., 2002; Strömberg, 2002; Boyd et al., 2005; Bremond et al., 2005a, b; 2008a,b; Piperno, 2006; Lentfer and Torrence, 2007; Neumann et al., 2009; Messager et al., 2010; Prasad et al., 2011). When found in significant amounts in archeological sites they can be used for identifying food habits, cultural and agricultural practices (e.g. Delhon et al., 2008; Li et al., 2010; Yost and Blinnikov, 2011). In parallel, phytoliths in plants, soils, and rivers were quantified for investigating the

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biogeochemical cycle of Si, which itself is coupled to the global C cycle (e.g. Blecker et al., 2006; Struyf et al., 2009; Alexandre et al., 2011; Cornelis et al., 2011).

Phytoliths can contain small amounts of C occluded in their structure (phytC), which is thought to range from 0.1 to 2% of phytolith dry weight (Wilding, 1967; Prychid et al., 2003). Raman spectroscopy and GC–MS analyses of phytC evidenced the presence of aliphatic compounds and lignins (Perry et al., 1987; Smith and Anderson, 2001), as well as aromatic hydrocarbon and graphite or coal when phytoliths were burnt (Pironon et al., 2001). PCR associated with protein staining detected the presence of glycoproteins but could not find evidence of any DNA (Elbaum et al., 2009).

It is generally assumed that the source of this phytC is atmospheric CO_2 that was fixed by the plant via photosynthesis (Wilding, 1967; Kelly et al., 1991; Raven et al., 1999; Piperno, 2006; Carter, 2009). From this basis, the assumption that phytC may be a terrestrial sink of C in the global C cycle was recently suggested (Parr and Sullivan, 2005; Jansson et al., 2010). In parallel, carbon isotopic studies have investigated the potential of phytC $\delta^{13}C$ signatures for providing information about photosynthetic pathways (Kelly et al., 1991; Smith and White, 2004; Webb and Longstaffe, 2010; Strömberg and McInerney, 2011) or deriving a paleo-atmospheric CO_2 record (Carter, 2009). However, isotopic calibration studies of phytoliths from grasses showed that the difference between $\delta^{13}C_{tissue}$ and $\delta^{13}C_{phytC}$ values is not constant from a plant to another (Smith and White, 2004; Webb and Longstaffe, 2010) and that changes in $\delta^{13}C_{phytC}$ values are not related to expected variation in the $\delta^{13}C$ values of atmospheric CO_2 (Webb and Longstaffe, 2010).

Few studies have used ^{14}C ages of phytolith concentrates from soils and archeological sediments as chronological indicators (Piperno and Becker, 1996; Piperno and Stothert, 2003; McMichael et al., 2012). One of them found a modern or post-bomb age for phytolith concentrates extracted from superficial soil (Piperno and Becker, 1996). Thousand-year ages were also reported for topsoil phytoliths (McMichael et al., 2012). This was justified by to the long mean residence time of phytoliths in soils. Other studies failed in matching ^{14}C phytC values with expected or independent chronologies (Wilding, 1967; Kelly et al., 1991; McClaran and Umlauf, 2000; Prior et al., 2005; Rieser et al., 2007; Boaretto, 2009). Encountered difficulties were thought to be associated with stratigraphic inversions, preferential oxidation of younger phytoliths or mostly with ineffectiveness of phytolith extraction procedures (Wilding, 1967; Kelly et al., 1991; Prior et al., 2005; Rieser et al., 2007; Boaretto, 2009). Neither the phytolith chemical procedural blank assessment nor the reproducibility and accuracy checks on ^{14}C of large pools of phytC were ever attempted to corroborate or explain the ^{14}C results obtained. A recent study (Santos et al., 2010a) evaluated the background of phytolith chemical extractions and the reproducibility and accuracy of ^{14}C phytC on phytolith concentrates extracted from soils and harvested grasses. Surprisingly, the phytC from harvested grasses yielded unexpected ^{14}C ages of several kyr old (though bulk material from the same plants gave contemporary ^{14}C values), when using an established protocol (Kelly, 1990; Kelly et al., 1991). This case is supported by phytC ^{14}C-AMS data obtained from harvested bamboo leaves and underlying litter layers that were expected to reproduce contemporaneous atmospheric ^{14}C values (Sullivan and Parr, 2008, 2013). As recently discussed in Santos et al. (2012a,b) the dataset shows varying depletions of at least 5 pMC (percent modern carbon) relative to the values expected when taking into account the southern hemisphere bomb radiocarbon peak and its recent decreasing trend in the atmosphere (Santos et al., 2012b). The minimum 5 pMC offset is equivalent to 400 years, reflecting incorporation of a substantial amount of "old" carbon in the phytolith concentrates (Santos et al., 2012b). The offset is maximum for the harvested leaves (which yielded an age of 3.5 ka BP), and undisturbed green litter with minimal contact

Table 1
Main steps of published protocols originally set up for phytolith extraction from plants. These protocols are commonly used for morphological identification purposes; some of them have been used for phytC analyses.

Protocols	Original references	Used for phytC analyses
Wet oxidation		
Main oxidizing agent: H_2SO_4/H_2O_2		
Rinsing of plant material with HCl. Boiling samples in 70% ethanol, washing and drying. Oxidation with concentrated H_2SO_4 at 70°C. Addition of H_2O_2 until solution is clear at room temperature. Rinsing with distilled water.	Geis,1973, 1978 Kelly, 1990	Pironon et al., 2001 Smith and Anderson, 2001 Krull et al., 2003 Smith and White, 2004 Carter, 2009 Santos et al., 2010a Webb and Longstaffe, 2010
Main oxidizing agent: $HNO_3/KClO_3$		
Rinsing of plant material. Oxidation with concentrated HNO_3 and $KClO_3$ at 100°C. Removal of carbonates using HCl at room temperature. Rinsing with distilled water.	Rovner, 1972 Pearsall, 1989 Piperno, 2006	
Main oxidizing agent: $HNO_3/HClO_4$		
Rinsing of plant material with HCl. Two oxidation steps with a 1:1 HNO_3-$HClO_4$ mixture at 80°C. Addition of H_2O_2 at 80°C. Rinsing with distilled water.	Rovner, 1971	Elbaum et al., 2009 Santos et al., 2010a
Microwave digestion		
Oxidation with HNO_3, H_2O_2 and HCl in closed digestion tubes. Microwave irradiation for 30 min. Sieving at 250 µm Rinsing in ethanol	Parr et al., 2001b Parr, 2002	Krull et al., 2003 Parr et al., 2010 Parr and Sullivan, 2005, 2011
Ashing		
Rinsing of plant material with distilled water. Heating crucibles in muffle furnace at 500°C for 6h. Remove from crucibles to test tubes. Oxidation with HCl at 70°C for 20 min/rinsing. Oxidation with H_2O_2 or HNO_3 at 70°C for 20 min. Rinsing with distilled water.	Parr et al., 2001a	