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# When the carbon being dated is not what you think it is: Insights from phytolith carbon research

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ABSTRACT

For proper interpretation of radiocarbon ($^{14}$C) age results, the carbon fraction being dated must be identified beforehand, ideally as a single homogeneous entity that best represents the event being studied. Radiocarbon dating of fossil phytoliths (biosilica formed in living higher-plants) has been used in a number of archaeology and paleoenvironmental studies. More precisely, the carbon occlusion (phytC) has been $^{14}$C dated. This method relies on the phytC being photosynthetic in origin, so that its $^{14}$C signature is similar to that of the host plant. However, we have recently presented overwhelming evidence that phytC in modern plants is made up of a mixture of carbon photosynthesized by the plant (from atmospheric $CO_2$) and soil carbon comprised of multiple $^{14}$C signatures (ages). The discussion presented here is based on our assessments of phytC $^{14}$C signatures, their chemical nature, location, origin and fate as well as the current state of knowledge on plant cell silica interactions with biomolecules. Finally, regardless of the fact that there are cases where fossil phytC $^{14}$C results appear to match expected values, the impossibility of establishing *a priori* either the amount of the soil carbon contribution to phytC or the mean $^{14}$C age of its occluded mixed pool precludes the use of phytoliths as a reliable $^{14}$C dating tool.



## 1. Introduction

Phytoliths are micrometric hydrated silica particles that form inside and outside the cells of living plant organs. When they form in the cells, they take the shape of the cells and are assigned a taxonomic value (e.g. Piperno, 2006). After plant death, phytoliths can either dissolve and take part in the silicon cycle (e.g. Alexandre et al., 1994; Alexandre et al., 1999; Oleschko et al., 2004; Borrelli et al., 2010; White et al., 2012; Cornelis et al., 2014; Opalinska and Cowling, 2015) or be incorporated and preserved in soils, sediments or archaeological deposits (Cabanes et al., 2011; Gao et al., 2018). In the latter cases, the phytolith morphological assemblages can be used as paleoenvironmental or archaeological indicators, provided that the soil or sediment sequence is chronologically constrained and that taphonomic processes are taken into account (e.g. Nogué et al., 2017; Woodburn et al., 2017; Yost et al., 2018 for the most recent reconstructions).

Phytoliths trap trace elements, including carbon (phytC) in their silica structure (Exley, 2015). The amounts of phytC reported by scholars vary, but are typically below 2%. Hodson et al. (2008) suggested that variations are likely due to differences in the extraction methods and analytical techniques employed. Since high purity phytolith extracts are difficult to obtain, this assessment is probably correct even when only one protocol is being used. Assuming that this phytC is of photosynthetic origin, phytC $^{14}$C extracted from living vegetation should have a $^{14}$C signature similar to that of the host-plant, which in turn should reflect the $^{14}$C signature of the ambient atmospheric $CO_2$ (atm-$CO_2$). This would imply that direct $^{14}$C dating of fossil phytC $^{14}$C offers the potential to determine a calendar age since the C was encapsulated within the biosilica precipitate. Silica precipitates in cells over a period of a few hours (Kumar and Elbaum, 2017). While bulk phytolith assemblages extracted from plants reflect silica deposition throughout the life of the plant, bulk phytoliths extracted from soils, sediments or archaeological deposit are expected to reflect longer time spans (10s–100s of years), depending on accumulation rates and residence times.

The earliest attempt at direct fossil phytC $^{14}$C dating appeared in an investigation of phytoliths accumulated in an Ohio soil

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developed in a riverine terrace (Wilding, 1967). Although the measured phytolith age was over 10,000 years older than the expected age, this article has been frequently cited as a proof-of-concept of the reliability of phytC $^{14}C$ dating (e.g. Kelly et al., 1991; Sullivan et al., 2008; Sullivan and Parr, 2013; Carter, 2009; Piperno, 2006, 2016a; Zuo et al., 2017). In an attempt to reconstruct the American Great Plains paleo-vegetation, Kelly et al. (1991) measured $\delta^{13}C$ and $^{14}C$ in fossil phytoliths. However, over 60% of these $^{14}C$ phytolith chronologies were biased-old (or inverted) by more than 3000 years (Santos, 2009). Kelly et al. (1991) acknowledged the phytC $^{14}C$ discrepancies and attributed them to remobilization effects. However, this work has also been cited in the literature as a proof of the reliability of phytolith $^{14}C$ dating (Carter, 2009, for example). The interpretation of phytC $\delta^{13}C$ data obtained from C3 and C4 plants have also been considered problematic. Webb and Longstaffe (2010) determined that C3 and C4 phytC $\delta^{13}C$ data can overlap if preferential occlusion of plant molecular $^{13}C$-depleted compounds occurs.

In order to investigate whether phytoliths were indeed a proxy of plant C and atm-$CO_2$, phytoliths from living vegetation harvested from different locations were extracted in distinct laboratories using conventional protocols and then measured by $^{14}C$ accelerator mass spectrometry (AMS) (Santos et al., 2010a). Whereas the data were expected to reflect contemporary atmospheric $^{14}CO_2$ levels from after the onset of thermonuclear testing in the middle 1950's (Hua et al., 2013), they showed systematic shifts towards several thousand years (Table 1).

Other post-bomb chronology studies have also presented ambiguous $^{14}C$ data. For instance, phytC $^{14}C$ dating of contemporary samples failed to adequately reproduce atmospheric values, e.g. the $^{14}C$-signatures of the bomb-pulse calendar years of harvesting. When trying to reproduce the bomb-peak using phytoliths extracted from mature bamboo and litter from samples collected on or before 2008, Sullivan et al., 2008, Sullivan and Parr, 2013) obtained ages thousands of years old $^{14}C$ for the most recent material (1.9 and 3.5 kyrs BP), while the litter phytC $^{14}C$ results yielded signatures that were mostly from or before the early 1950's. Even if the litter samples were not well characterized by direct isotopic measurements of their bulk organics, their $^{14}C$ profiles should be somewhat elevated due to post-bomb labeling (Carrasco et al., 2006). Still, the litter phytC $^{14}C$ data has been reported as "modern" (e.g. "post-bomb" by default - Sullivan and Parr (2013)). However, this does not necessarily constitute correctness. The application of post-bomb chronologies requires careful assessment of the results and the use of global $^{14}C$ atmospheric datasets (Hua et al., 2013) if the precise $^{14}C$ signature of the plant-host is unknown.

In another study, phytC $^{14}C$ results from Neotropical plants collected over multiple calendar years after 1950 were reported (Piperno, 2016a). However, the data did not match the $^{14}C$ bomb atmospheric inventories as expected. A modelling framework presented in Santos et al. (2016) indicates that such decadal to centennial phytoliths $^{14}C$ offsets are better resolved when plotted against best-fit curves calculated by applying different values of soil carbon (soil-C) turnover rates coupled with the temporal atm-$CO_2$ data after 1950. Although some of those phytolith $^{14}C$ mismatches were explained by local and regional variations in atm-$CO_2$ emissions (Piperno, 2016b), no direct $^{14}C$ measurements of the collected plants have yet been reported to corroborate this assessment. Such large variations are extremely unlikely in regions with very low anthropogenic $CO_2$ emissions (Hansen and Sato, 2016). Moreover, large discrepancies should have been apparent for other Neotropical biomass archives across the region (Dezzeo et al., 2003; Santos et al., 2016; Baker et al., 2017). Regardless, validation experiments using poorly or improperly characterized materials are pointless. A reanalysis of the data presented in Sullivan et al. (2008), Sullivan and Parr (2013) and Piperno (2016a) were presented in Santos et al. (2012a) and Santos et al. (2016), respectively. Additional present-day phytolith cross-validation studies have also shown phytC $^{14}C$ anomalies (Yin et al., 2014; Reyerson et al., 2016; Asscher et al., 2017). Evidence was produced directly from phytolith extracts and plant-host pairs, and will be discussed in detail later.

After examining previous "too-old" or "age inversions" findings (Wilding, 1967, Kelly et al., 1991, Rieser et al., 2007, for example) misconstrued in the literature as demonstration of phytolith $^{14}C$ dating accuracy and reliability, and from the results shown in Table 1, we concluded that phytC had an unknown confounder that can bias its $^{14}C$ age(s) and that was not properly assessed in earlier investigations. The results shown in Table 1 are based on the intra- and inter-laboratory investigations conducted by Santos et al. (2010a). A hypothesis was developed that phytC may include carbon that differs from the host-plant photosynthetic carbon. Specifically, it was hypothesized that soil-C acquired by plant roots may contribute to phytC and bias the phytC $^{14}C$ results towards unexpected values (Santos et al., 2012b). This hypothesis was based on previous evidence of direct root uptake from the rhizosphere by higher plants and upward translocation of organic compounds such as sugars, amino acids, organic acids, fatty acids, urea, quaternary ammonium compounds, as well as other nitrogenous substances. This evidence have been accumulating in the literature since the late 1950's (see findings and references compiled in Jones et al., 2009, Paungfoo-Lonhienne et al., 2008, 2010, 2012, Warren, 2013, Pinton et al., 2016, Zhalnina et al., 2018).

**Table 1**
Averaged fraction modern $^{14}C$ ($Fm^{14}C$) values and uncalibrated $^{14}C$ ages of phytoliths extracted from modern grasses clippings at different locations. Ages reported herein are uncalibrated years B.P. (years before present). Present-day corresponds to $^{14}C$ results that matched the expected ambient $^{14}CO_2$ signatures of the harvesting year. Individual uncertainties can be attributed to counting statistics, spectrometer isotopic fractionation, and scatter of results from primary and secondary standards, and most importantly, background corrections attained from chemical extraction blanks. The complete dataset, including blank determinations was reported in Santos et al. (2010a).

| Sample location | Sample type | $Fm^{14}C$[a] | $^{14}C$ age[b] |
|---|---|---|---|
| Crop field - | Grass clipping | $1.0490 \pm 0.0020$ (n = 2) | Present-day |
| CEREGE, France | Phytolith extracts | $0.7790 \pm 0.0041$ | $2280 \pm 260$ yrs BP |
| | | $0.7505 \pm 0.0178$ | |
| | | $0.7306 \pm 0.0620$ | |
| Rural area - | Grass clipping | $1.0605 \pm 0.0011$ (n = 2) | Present-day |
| Minnesota, USA | Phytolith extracts | $0.5370 \pm 0.0090$ | $5000 \pm 140$ yrs BP |
| Rural area - | Grass clipping | $1.0546 \pm 0.0050$ (n = 2) | Present-day |
| Madison, USA | Phytolith extracts | $0.3677 \pm 0.0254$ | $8040 \pm 560$ yrs BP |

[a] n represents the number of individual measurements performed on grasses. Clipping indicates a small section cut off of a mature stem or leaf of about 2–3 cm maximum used as reference for the host plant $^{14}C$ signature, after a light chemical cleaning and measurement.
[b] Where applicable, numerical results are reported as average ± standard deviation.