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Comment on: “Possible source of ancient carbon in phytolith concentrates from harvested grasses” by G. M. Santos et al. (2012)

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Abstract

Santos et al. (2012) address the important issue that ^{14}C dating of the carbon occluded in silica phytoliths (PhytOC) isolated from contemporary plant materials can produce ages that are incompatible, being often several kyr older, with both their known recent origin and the ^{14}C age of the bulk plant material. In their article, Santos et al. (2012) propose that the anomalously old ^{14}C carbon dates of PhytOC from harvested plant materials are based on plants taking up “old” dissolved soil carbon to the plant by roots during nutrient uptake. They then propose that this old soil-derived carbon is subsequently partitioned from the general plant biomass into either the silica phytoliths they produce, or as recalcitrant organic matter elsewhere in the plant. We suggest that the full data available for PhytOC ^{14}C dating does not support this hypothesis. Santos et al. (2012) also address the important issue of contamination of PhytOC by general plant biomass material that can occur with procedures that incompletely extract phytoliths. Whilst we agree that such contamination needs to be avoided when examining the nature of PhytOC, we also point out that the converse problem, i.e. removal of PhytOC by over-vigorous extraction procedures, can also have important adverse consequences.

1 Discussion

Santos et al. (2012) examine the reliability of ^{14}C dating of PhytOC from modern plant materials as well as the utility of techniques for the isolation of phytoliths and the determination of PhytOC. The authors show that ^{14}C dating of the carbon occluded in silica phytoliths isolated from contemporary plant materials often produce ages that are several kyr older than both their known recent origin and the ^{14}C age of the bulk plant material. These authors also note that ^{14}C dates using phytoliths isolated from soil often do not correlate well with the dates provided by other dating methods. Santos et al. (2012) examine this phenomenon by using recently harvested plant specimens,

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taking considerable effort to exclude contamination during phytolith preparation, and by reviewing other published data.

Santos et al. (2012) hypothesise that the anomalously old ^{14}C carbon dates of PhytOC from harvested plant materials are based on plants taking up “old” dissolved soil carbon to the plant by roots during nutrient uptake. This old soil-derived carbon is subsequently strongly partitioned from the general plant biomass into either the silica phytoliths they produce (as PhytOC), or as recalcitrant organic matter elsewhere in the plant (n.b. as the authors demonstrate in their paper, if inefficient phytolith extraction techniques are used to isolate phytoliths from plant materials, such recalcitrant organic matter may remain after the phytolith extraction procedure and be erroneously included in the PhytOC fraction). In effect, the authors are proposing the existence of a powerful, but not defined, carbon fractionation mechanism occurring within plants.

In their examination of the data available Santos et al. (2012) selectively use the results of our unpublished progress report to a funding agency (Sullivan et al., 2008). (Note: the authorship of this unpublished progress report was incorrectly attributed in Santos et al. (2012): it is corrected here.) Our study radiocarbon dated PhytOC isolated from mature leaves harvested when alive from a mature stand of bamboo, and from eleven sub-layers of the thick leaf litter layer beneath this bamboo grove. This litter layer was comprised of:

1. a thin sub-layer of recently fallen leaves (as indicated by both their location on the very top of the litter layer and, although in an air-dry condition, their green coloration indicating an intermediate stage of chlorophyll degradation),
2. an underlying sub-layer of intact straw-colored bamboo leaves down to 9 cm depth, and;
3. an underlying 7 cm thick sub-layer composed primarily of bamboo leaves in various stages of decomposition (see Fig. 1).

All of these samples underwent identical sample preparation, phytolith extraction (i.e. a microwave digestion procedure modified from Parr et al., 2001) and subsequent

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analytical procedures to derive radiocarbon ages at the Australian Nuclear Science and Technology Organization AMS facility. Litter fall traps at this bamboo site indicate a leaf fall rate of approximately 8 cm per annum indicating that all the bamboo leaves in these litter layers are only a few years old since leaf fall.

5 Santos et al. (2012) cite only two of the twelve data points for recent bamboo leaf materials available in our report to support their hypothesis that the anomalous ^{14}C dating PhytOC results arise from plants strongly partitioning the old carbon they take up from the soil into either the silica phytoliths or other recalcitrant organic matter plant fractions included in PhytOC. Examination of the complete data set from our study
10 (Fig. 1) shows such strong partitioning of old soil-derived C could only apply to the two samples Santos et al. (2012) chose to cite, i.e. the mature harvested leaves and the recently fallen leaves. The other ten ^{14}C dates of PhytOC extracted from the other recent leaf materials for this site, provided in Sullivan et al. (2008) and shown in Fig. 1, are far more in accord with their recent formation having either “modern” dates or close to
15 “modern” dates. Surprisingly, given their awareness of this report, Santos et al. (2012) assert that “to their knowledge there is only one “modern” phytolith ^{14}C age in the literature that of Piperno and Blecker (1996)” and ignore these other “modern” phytolith ^{14}C ages.

It does not seem plausible that the mature stand of bamboo plants at our study site
20 had – at the time of sampling – only recently begun to take up ancient soil carbon from the soil and, subsequently, strongly preferentially partitioned this ancient carbon into either their phytoliths or as recalcitrant materials, when such strong ancient carbon partitioning is not observed in the other ten leaf litter layers which represent the immediate past 2 to 3 yr of leaf fall at this same site. If the uptake and preferential partitioning
25 of ancient soil-derived carbon is occurring as uniformly as Santos et al. (2012) suggest, then why do none of the ten other recent bamboo leaf materials from this very same bamboo site exhibit the same strongly anomalous ^{14}C dating of PhytOC?

The reason for the often strongly anomalous ^{14}C dating of PhytOC from recent plant materials we believe still remains unclear. Whilst we agree with Santos et al. (2012)

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conclusion that the anomalous ^{14}C results for the two samples of ours they cited can not be attributed to isotopic fractionation of the ^{14}C in the PhytOC by any known fractionation mechanism, equally the mechanism responsible for the strong phytochemical carbon partitioning that Santos et al. (2012) propose is similarly not known. Furthermore the operation of such a possible mechanism is not uniformly supported by the full dataset available.

Santos et al. (2012) provide a discussion on the need to isolate PhytOC from plants (or soil) without having extraneous carbon attached and provided strong evidence to indicate that the phytolith extraction technique they used (i.e. Kelly et al., 1991) was prone to this problem by use of SEM-EDAX. As Santos et al. (2012) stress, this is an important issue as the variations in PhytOC yield between different plant types and even cultivars of the same crop, are increasingly being looked to as an approach with potential for enhancing carbon biosequestration in agriculture and forestry (e.g. Parr and Sullivan, 2005, 2011; Parr, et al., 2009, 2010; Jansson et al., 2010; Zuo and Yuan, 2011). However, to suggest solely on the basis of morphology in SEM images published elsewhere that phytolith extraction techniques they did not examine (such as the microwave digestion technique) “seem” unable to extract extraneous carbon, is speculative. Speculation is fine when indicated as such, but elsewhere Santos et al. (2012) move from speculation on this issue to the following definitive statement “We show that current extraction protocols are inefficient since they do not entirely remove recalcitrant forms of C from plant tissue.” We would argue Santos et al. (2012) actually show, and this is a valuable contribution, that the phytolith extraction technique they used is prone to this problem, rather than showing that all current phytolith extraction protocols cannot entirely remove non-phytolith carbon from plant tissue.

Although not addressed by Santos et al. (2012) we regard the converse problem to that above, namely employing phytolith extraction techniques that alter or deplete PhytOC, to also be an important problem for purposes such as ^{14}C dating of PhytOC, the accurate determination of the PhytOC yields of plants, and examinations of the nature of PhytOC. For example, Watling et al. (2011) used Raman, infrared and X-ray

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photoelectron spectroscopy to examine in detail the effect of three different phytolith extraction procedures on recently harvested bamboo leaves from the same site in Sullivan et al. (2008) and showed that the phytolith extraction procedure employed could alter the chemical nature of the PhytOC considerably. Santos et al. (2012) regard the considerable loss of carbon (i.e. over 50 %) from their Kandara phytolith concentrate after exposure to rinses of 8 % HClO₄ and HNO₃, to be extraneous organic matter that was cleaned from the surface of phytoliths (Table 1). However, without confirmation of the removal of organic coatings or extraneous organic matter by detailed examination of phytoliths after such “cleaning” techniques have been used – by, e.g. SEM and low temperature ashing (Sullivan and Koppi, 1987) – such a loss of carbon could just as equally represent a loss of PhytOC itself rather than of extraneous organic matter. Interestingly, the “cleaner” this Kandara phytolith sample became after further purification procedures, the older the PhytOC ages of this phytolith sample (Table 1). For example, extra wet oxidation steps performed on the Kandara phytolith concentrate resulted in the measured ¹⁴C age of the PhytOC of this material to increase markedly from 430 yr BP to between 2000–2760 yr BP depending on the type of acid used for the additional oxidation step. Thus even when PhytOC was most likely partially removed by these further purification procedures, the ¹⁴C ages of the Kandara PhytOC diminished further.

2 Summary

We believe that Santos et al. (2012) proposed hypothesis that the anomalously old ¹⁴C carbon dates of PhytOC from harvested plant materials are the result of plants taking up “old” dissolved soil carbon and subsequently strongly partitioning this carbon into either the silica phytoliths they produce or as recalcitrant organic matter elsewhere in the plant is not supported by the full dataset available. The data available indicates that there is another carbon fractionation/partitioning mechanism that operates only sometimes when using PhytOC extracted from fresh plant materials (especially) to provide ¹⁴C dates. The nature of this carbon fractionation/partitioning mechanism is

not apparent from the data available. Further whilst Santos et al. (2012) show that contamination of PhytOC by general plant biomass material can occur with the procedure they employed to extract phytoliths, we argue that the converse problem, i.e. removal of PhytOC by over-vigorous extraction procedures, can also have important adverse consequences when examining the nature of PhytOC.

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Table 1. Measured ^{14}C age of extracted Kandara soil phytolith concentrate after exposure to additional purification steps (data from Santos et al., 2012).

Extraction/purification procedure	Average measured ^{14}C age, yr BP (mean, $\pm \sigma$)	Carbon content %C (mean)
1. Baking (exposure to 200 °C for 3 h, or overnight at 160 °C)	430, 20	0.90
2. Rinsing with HClO_4 and HNO_3	2000, 230	0.41
3. Baking (exposure to 500 °C overnight)	2120, 300	0.17
4. Rinse with HCl and then baking overnight at 160 °C	2760, (no σ as only one sample tested)	0.55

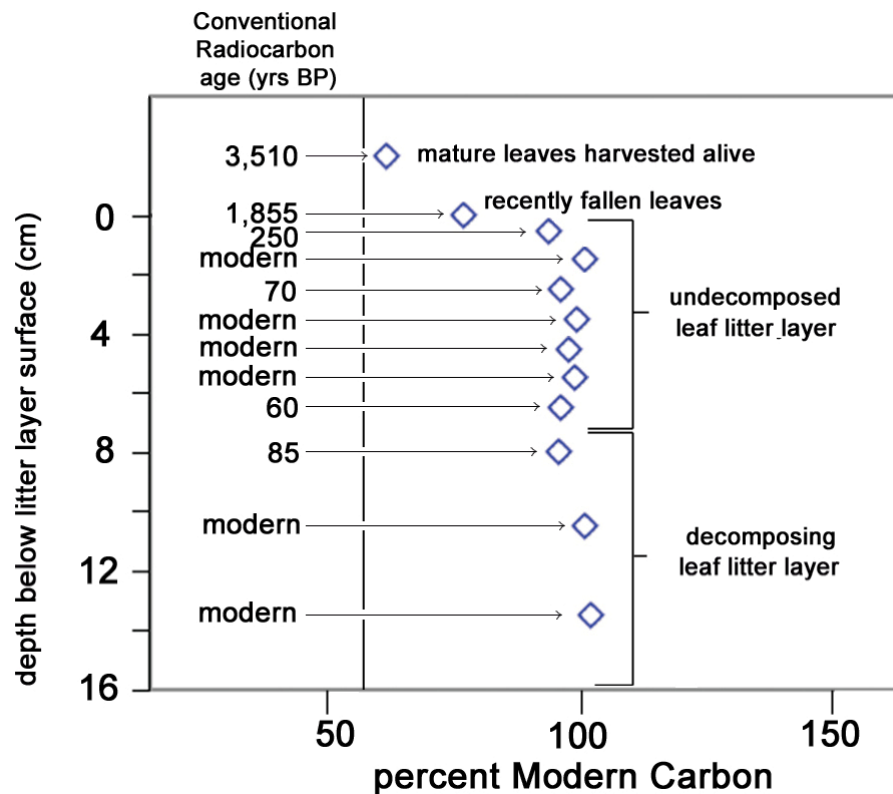


Fig. 1. Radiocarbon ages measured by the Australian Nuclear Science and Technology Organization AMS facility of PhytOC isolated from leaves of a bamboo (*Bambusa vulgaris* cv. *Vittata*) and the underlying litter layer (data from Sullivan et al., 2008).

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