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EARTH SYSTEM SCIENCE DEPARTMENT

B321 CROUL HALL
UNIVERSITY OF CALIFORNIA
IRVINE, CALIFORNIA 92697-3100
949-824-3674
gdossant@uci.edu
www.ess.uci.edu/ams

January 26, 2016

Dr. Roland Bol
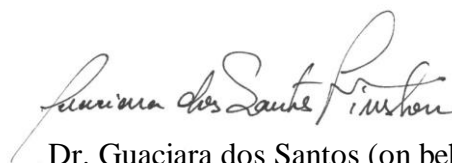Associate Editor
Biogeosciences

Dear Dr. Roland Bol,

Thank you for providing us with the opportunity to address the reviewer's comments and willingness to consider a revised version of our manuscript titled "Evidence of old soil carbon in grass biosilica particles" by P. E. Reyerson et al.

We thank the 3 anonymous reviewers for their time and work on our manuscript. In response to reviewer comments#2 and 3, we have re-organized the manuscript with more clarity in the results and discussion sections. We also clarified portions of the methodology as well as expanded the reasoning behind them. Consequently, we feel that the title did not optimally reflect our study content (explanation below in response to reviewer #2 comment), and we suggest the following new title:

"Unambiguous evidence of old soil carbon in grass biosilica particles"

Following your recommendations, all changes were done as concise as possible, and therefore 6 extra citations were added in the revised manuscript version. We believe our manuscript is much improved, and hope that it will meet with your satisfaction as well as that of the reviewers. Below, we detail the changes we have made to each of the reviewers comments (text in black). We are grateful for the chance to publish our work in BG.

Sincerely,

Dr. Guaciara dos Santos (on behalf of co-authors),
Research Project Scientist
Keck Carbon Cycle AMS Facility,
University of California, Irvine

Reviewer #2 comments

Introduction: Many vascular plants produce siliceous bodies known as phytoliths, and these can contain carbon occluded into their structure – termed phytC. The 13C of phytC and their 14C activities have been used for either paleoenvironmental reconstructions or dating, and recent work has repeatedly argued that phytC may represent a sizable atmospheric CO2 sink. But as Reyerson et al. note, there are several conceptual inconsistencies and knowledge gaps regarding our understanding of phytC, including a) poor knowledge of any relationship between 13C of phytC and climate, and b) systematic offsets between phytC 14C ages and the plant tissue 14C ages. These problems may be compounded by the poor inter-laboratory repeatability of phytC quantification. These issues led workers to posit a non-photosynthetic origin for some fraction of phytC, i.e. from some pool of 'older' soil carbon. This is the central issue that this manuscript seeks to address.

Reyerson et al. use a variety of experimental approaches, including above- and belowground C isotope manipulations. They also incorporate aspects of an inter-laboratory comparison/protocol test, CO2 flux measurements and a phytC thermal stability experiment. As a result, and because the experimental protocols are not fully described, it is often hard to identify exactly what has been done. Therefore, although the conclusions seem sensible, it is often hard to assess the extent to which they are supported by the data. At the level of individual sentences, this is a well-written manuscript. But because of these issues, at level of paragraphs to whole sections, I find the structure to be confusing and difficult to follow.

In general, the manuscript presents useful data and sensible conclusions that will be of interest to many workers and fall within the scope of Biogeosciences. I recommend the authors spend some time re-evaluating the structure of the manuscript – particularly the results - and considering carefully the degree to which the extra analyses they performed actually contribute to the results and conclusions. These extraneous details might even include the FACE experimental setup. I think the manuscript would be much stronger if it was reduced to the key messages – which I understand to be that phytC 13C and 14C can be explained as a mixing between soil carbon and photosynthetic (ambient) carbon, and that true phytC concentrations are lower than reported elsewhere.

We agree with the reviewer that we have a rather large and complex dataset to resolve the anomalous (lower than expected) phytC ^{14}C.

As the photosynthetic CO_2 to phytC had a strong intuitive appeal, a scientific inquiry of it was not properly done (or worst, considered an unnecessary expenditure). In Wilding (1967), the first to use ^{14}C of ancient phytoliths, the difference between expected and phytC ^{14}C age are in the order of ~10ka yrs. Although Wilding (1967) acknowledged that his phytolith ^{14}C result was unexpected, his article has been continuously cited as proof-of-concept that phytC ^{14}C are reliable (more details in Santos et al. 2010 and 2016). Consequently, phytC has been used as a dating material in ^{14}C geochronology for decades, and more recently as a potential atmospheric CO_2 sink.

A systematic evaluation of procedural chemical blank assessments on phytolith extractions reveals a non-photosynthetic origin of phytC (Santos et a. 2010), where phytC ^{14}C offsets as large as 8ka yrs BP between phytolith extracts and plant tissue have been found. While blank analyses ruled out any possibility of exogenous impurities, a less intuitive mechanism involving soil C adsorption by roots through amino-acids was put forward (Santos et al. 2012a). Consequently, the "key message" that the reviewer refers to, has been already demonstrated and reported elsewhere. Even though the previous evidence was robust (Santos et al. 2010) and reasoning for it was discussed in Santos et al. (2012a), similar phytC ^{14}C discrepancies than those

reported earlier and conceptual inconsistencies on ^{14}C interpretation of phytC in this field continued (e.g. Sullivan and Parr 2013, and most recently Piperno 2015). Santos et al. (2012b, 2016) asserted the conceptual inconsistencies on those studies and reinterpreted the datasets shown. Unfortunately, poor laboratory practices (the omission of blank assessment and detailed information on ^{14}C analysis) called for a comprehensive study on the subject. The point is that those works (Sullivan and Parr 2013, Piperno 2015) just add more confusing to the controversy surrounding the use of phytoliths for direct ^{14}C dating, or as a viable C sequestration mechanism. Thus, if we wish to unequivocally establish that a fraction of phytC is indeed from soils, we must consider and rule out all other factors that can possibly bias the isotopic signatures of phytC.

In the revised version, we are making this message stronger to help the readers understand the phytC ^{14}C previous inaccuracies. We also suggest making a small modification to the title to better reflect this point. Moreover, we modified the end of the introduction, several portions of the results and discussions (now together), and conclusions to better reflect the issues discussed above (highlighted in red in the main text for your viewing).

In order to provide recent examples of problems on phytolith analyses, we are also citing in the text two recent articles. The first shows extra anomalous phytC ^{14}C results from post-bomb living vegetation in Neotropical regions (Piperno 2015), and the second addresses the issues of phytolith ^{14}C misinterpretations (Santos et al. 2016), as mentioned above.

Major comments: The four different extraction protocols for phytoliths from modern plant material are referred to many times throughout the manuscript, but besides a brief indication in a figure legend it is not mentioned what they are or why some are more aggressive than others. At some points in the results/discussion, more confusion is introduced: e.g. what does it mean when phytoliths are 'extracted simultaneously using just one protocol'?

Besides citing Corbineau et al. (2013) where the protocols we use were described in detail, we are now summarizing the main differences of the four extraction protocols we employed in the text (section 2.2.1 - Plant treatment and phytolith extraction). Moreover, following both reviewers' suggestions, we split the above- and below-ground C manipulation experiments results into two subsections (e.g. 3.1 and 3.2, respectively), now under section 3 (Results and Discussions).

The statement, 'extracted simultaneously using just one protocol' refers to a subset of 6 targets from the below-ground C manipulation experiment produced from the 6 individual planters/substrates (A to F). The phytoliths from those were extracted simultaneously using just one protocol (e.g. 1a). For clarity, the statement was completely modified (section 3.2):

Relatedly, the different extraction protocols clearly have a important effect on the quantification of phytC (cf Pg. 15380-1) and on phytC 13C (cf. Fig 4). Therefore it would be nice if there was some recommendations for future work.

Recommendations on phytolith cleaning and purity screening methods (such as SEM-EDS) have been already offered in Corbineau et al. (2013), as we demonstrated that even very small amounts of surface C is enough to bias isotopic values (evidenced in Figure 2 of Santos et al. 2012a). Nevertheless, since phytC contains a broad continuum of carbon with complex mixture of chemical compounds of different turnover times (as evidenced by the thermograms of phytoliths as well – Figure 6), insufficient to excessive oxidations can move ^{14}C dates from thousands, to hundreds, to back to thousands years old (as represented in Figure 7). Since soil-C compounds and ages are highly variable in soils (Telles et al. 2003, Torn et al. 2009), and soil/amendment properties can modify the molecular makeup of phytC (this work), any

heterogeneous pool of C will be partitioned differently upon different degrees of OM oxidation (or protocols). Therefore, there is no way to correct for the soil C fraction within phytC, so that phytC can be used as a reliable dating material. We are now making those messages stronger in our revised version. A summarized paragraph with the above message was added to section 4.1 (The SOM-derived C to phytC hypothesis set of evidence), followed by the citations Telles et al. 2003 and Torn et al. 2009 (added to the revised manuscript).

Also, are there inter-laboratory differences when using the same protocols?

We have not identified inter-laboratory differences when using the same protocols from the subsamples of the same biomass, and/or when evaluating procedural blank materials (added to every batch analyzed). This fact has been already reported in the third paragraph of section 2.3.3. (Radiocarbon Analysis), but now we added a succinct description of accuracy/precision based on our duplicate/triplicates and secondary standard at the beginning of section 3.1 (as per reviewer# 3 recommendation).

The results are not presented in a clear and logical manner. To my mind, it would make more sense to separate first into the above- and below-ground experiments, and then have subheadings for the different analyses (phytC, ^{13}C, ^{14}C: : :). At the moment, section 3.1 (for example) includes (i) phytC concentrations (ii) 14C results from all substrates (including some not mentioned before: : :) (iii) extraction protocol differences and (iv) CO_2 fluxes. Overall, this makes for confusing reading. I can not follow how 21 phytolith concentrates was arrived at (Pg 15376, ln. 8). If I understand correctly, there are six biomass samples from the above-ground experiment and 6 from the below-ground experiment. Multiplied by four labs and four protocols – this should be more than 21! And later (Pg 15380, ln 8) – why do they become 52 14C measurements?

In agreement with this reviewer remarks, we separated the above- and below-ground C experiments, and consequently the discussions of the respective set of results are now also separated. However, we felt that adding too many subheadings is unnecessary, as it will break the present text flow. Now each set of results are self-contained in their respective subsection (e.g. 3.1 and 3.2). This change made the results and discussions section a bit longer, but easier to follow.

Since the above- and below-ground C experiments are now separated, the number of phytolith extracts produced, and the number of duplicates and triplicates targets produced from those extracts (reported in the supplement tables) are better accounted for, and described.

The rationale for including the CO2 chamber flux measurements (section 2.2.3) and the thermal analysis (2.3.4) in this manuscript is unclear. When these are returned to in the results (briefly in section 3.2, and section 3.3 respectively), or the discussion, they seem to add little, and instead just distract from the main message.

The present experiments and multiple analyses were designed to definitively explain the anomalously low ^{14}C values of phytC extracts, as mentioned earlier. We realize that our message regarding the need of the CO_2 chamber flux measurements (section 2.2.3) and the thermal analysis (2.3.4) in our manuscript was not as clear as we planned.

While it might seem strange, speculative discussions regarding over excessive oxidative protocols altering the natural ^{14}C signatures of phytC, or pesticide contaminations, directly fixation of atmospheric CO_2 from both fossil fuel emissions and fires as well as soil dissolved inorganic carbon (DIC) to whole-plant carbon have been continuously invoked by researchers

(Sullivan and Parr 2013, Parr and Sullivan 2014, Piperno 2015) to explain phytC ^{14}C discrepancies. In all those cases, no further isotopic measurements to support offsets have been provided, illustrating the inconsistencies in this field. Unfortunately, those articles have been cited as proof that phytC ^{14}C works (Hodson 2015, for example). Thus, if we wish to unequivocally establish that a fraction of phytC is indeed from soils, we must consider and rule out all other factors that can possibly bias the isotopic signatures of phytC (as mentioned earlier). The CO_2 soil fluxes concentrations and associated isotopic values help us to demonstrate that there is no possibility to invoke CO_2 soil/amendment respiration, DIC or otherwise, to reconcile phytC ^{14}C ages (as well as any other "outside" factor).

Regarding the thermograms, we show that phytC has the same physicochemical properties observed from complex carbon matrices, such as the carbon found in soils and sediments. We feel that this evidence is important, because the soil C to phytC hypothesis is by far less intuitive, and just recently (last 10 years or so) researchers were able to show that old, supposedly poorly accessible SOM can be decomposed by organisms or catalytic enzymes (as we addressed in section 4.5).

In our revised version, at the end of the introduction (section 1) we explain better the rationality behind the thermograms. We further address our thermogram profiles at the beginning of section 3.3. (Thermal stability of phytC), as well as add the citations of Plante et al. (2011, 2013), which show similar spectra from soils and sediments. We better explained what one should expect, from a mixed carbon pool such as phytC (the effects on %C losses and ^{14}C age changes due to partitioning of labile and recalcitrant carbon) once samples are subjected to thermal treatments. With those concepts at hand, we can set the stage to explain the phytC ^{14}C depletions by heat treatments observed in Yin et al. (2014) and (Santos et al. 2012a), as well as in this study (last paragraph of section 3.3, and second paragraph in section 4.3 - Implications for the use of phytC as a proxy of plant C).

Minor comments The figures could be larger, with more detailed captions.

Figures 2 was reformatted and enlarged. Furthermore, the captions under figures 1, 2 and 4 were modified to add clarity to the points addressed.

Pg 15379, line 9: what is 14C-free? - Although the ^{14}C-free term is widely used in the radiocarbon community, we are now adding a footnote to clarify it in section 2.3.3 (Radiocarbon Analysis).

Pg 15384, line 3: measuring, not measured.

It has been fixed.

Fig 2b – is phytC 'accessibility' really what is meant here? Should it not be the opposite?

No. We used the term accessibility to reflect labile-accessible or prone to oxidation. So, in figure 2b, when the labile-accessible carbon is not removed ^{14}C results are as close as possible to the values of the tissue of origin (e.g. phytC ^{14}C offsets are hundreds, rather than thousands years old). Once the labile-accessible carbon is removed, or better the C fraction resistant to oxidation is isolated, the ^{14}C ages as well as the %C in phytoliths tend to decline. So, the term is been used correctly.

The soil extraction fractions, bulk SOM and refractory (alkali-insoluble), were described at section 2.2.2, and the individual results from both fractions are shown in the Supplement (Tables S1 and S2). In this case, we have more than one measurement to represent the soil carbon pools. However, to test the mixing equation between the atmospheric CO_2 pool and SOM-derived C contributions, we make use the $Fm^{14}C$ average value of the oldest SOM-C fraction measured from each experiment (i.e., the $Fm^{14}C$ average value of the SOM 45-60 cm fraction for *S. bicolor* plots, and the refractory 0-15 cm fraction for *T. durum* plots). This was addressed previously in the discussion section, and in the revised manuscript it can be now found in section 3.1 (Isotopic results from above-ground C manipulation experiments).

Reviewer #3 comments

We agree that it may appear confusing to have two terms to define the carbon embedded in phytoliths, e.g. phytC and phytOC. The term phytOC was born based on the assumption that carbon embedded in phytoliths is from a CO_2 photosynthetic origin. Moreover, the term also

implies that all the carbon embedded in phytoliths must be organic-based, a fact not yet established.

At the present study, just one amendment (in Planter B) contained inorganic-carbon from a natural deposit (greensand) as well as competing amounts of organic carbon of similar ^{14}C age than its inorganic C fraction. PhytC stable isotope results from Planter B suggested that the inorganic-carbon contribution (if any) was undetectable (Figure 5, in this study). As yet, and based on one single amendment, we do not have other ways to infer if "inorganic carbon-containing compounds" cannot be encapsulated by phytoliths. Note that the term phytC has been also used in Santos et al. 2010, 2012a, 2012b, 2016; Corbineau et al. 2013, Gallagher et al. 2015, Alexandre et al. 2015, and recently Alexandre et al. 2015b (accepted in BGD).

- p15370, l 1-15: it would interesting to read somewhere in the ms a bit more about the processes behind the mobilization of old SOM and the implication of this mobilization.

This is indeed an interesting question that we cannot properly address in the present work, as the pathways of soil C (byproduct or biologically-driven) were not the main focus here. Some of our previous analysis (NanoSIMS coupled with 3D-Xray microscopy analysis of the silica structure - Alexandre et al. 2015) suggested that the phytC occlusions occur at a very early stage of the inner cell silicification. Soil C may be concentrated in the vacuole as an unmetabolizable waste product, without any link with Si, and eventually occluded in the silica structure during silica precipitation and vacuole destruction. On the other hand, relationships between OM and other types of biosilicas (BSi), such as diatoms, or deep-sea sponges (spicules) have been already established. Ma et al. (2006) suggested that the uptake of dissolved Si in higher plants may be genetically controlled. So, the assumption of a connection between soil C and BSi (for phytoliths) may be feasible. In order to provide further BSi mechanistic information, involving the encapsulation of carbon in phytoliths, further analyses are necessary.

- P15372, lines 23-24: what do you mean by "optimized"?

We replaced "optimized" by "modified" protocols, and cite Corbineau et al. (2013), where those protocols are fully described.

- P15376, lines 14-23: this part is not clear. Could you please precise/clarify which extraction procedure is used for which SOM fraction.

Besides of the description on SOM chemical extractions at section 2.2.2 (Soil extraction fractions), we have added at the end of the first paragraph of the section 3.1 (Isotopic results from above-ground C manipulation experiments) a statement to clarify that SOM was chemically separated in two carbon fractions for isotopic analyses, e.g. labile-accessible and recalcitrant (alkali-insoluble).

- P15380, line 8: you have to clarify which sample is analyzed. This is not clear which experimentation is carried out for which sample: 21 phytolith concentrates and 52 14C targets ??
- P15380, lines 13-14: what do you mean? Could you please clarify?

In agreement with both reviewers, we separated the above- and below-ground C experiments (see reply to reviwer#2).

✓ The 4 protocols are now described in the text.
✓ The above- and below-ground C experiments were split (see reply to reviwer#2).
✓ phytC yield is now defined in section 3.2 and in the caption of figure 2.

The link is established by the phytC thermograms. Since soil/amendment properties can modify the molecular makeup of phytC (this study), once roots absorb heterogeneous pool of C from soils, upon different degrees of OM oxidation, and heat treatments (see the description of protocols in section 2.2.1) the phytC will be partitioned differently (as we demonstrated in Figure 6). This last sentence was added to section 3.2 (Isotopic results from below-ground C manipulation experiments) to clarify the reviewer's remark.

The results and discussion sections have been completely reformatted.

We agree that Figure 2 is complex and that it can be a bit difficult to understand. With that said, we considered multiple variations of the figure before arriving at the present format. We feel this format is the most concise and parsimonious arrangement of the data to visually demonstrate the reduction of %C in phytoliths and its association with the isolation of the oldest SOM C fraction. In addition, Figure 4 follows the same format and concept of Figure 2 panel b (e.g. how phytC ^{14}C moves away from the photosynthetic ^{14}C signature of bulk plant tissue and towards the soil/amendment ^{14}C values, as the amount of soil C within phytoliths is isolated).

Recently, researchers were able to show that old, supposedly poorly accessible SOM can be decomposed by organisms or catalytic enzymes. Consequently, those old pools of carbon are not completely stable, but rather turning over in a much slower rate than the labile/fast carbon pool (Torn et al. 2009). Furthermore, roots can also acquire soil C in a molecular form (through amino acids, among other molecules). Although the phenomenon might be small it has not been

assessed at the ecosystem scale. Those discussions are in section 4.5 (Implications for our understanding of soil C pools mobilization).

- P15385, lines 17-20: the mixing equation should be further explained.

The mixing equation was explained at the caption of Figure 2. Now it is presented and further explained in the text (section 3.1. Isotopic results from above-ground C manipulation experiments).

- P15386, lines 1-5: not clear what is new compared to Alexandre et al. 2015?

Indeed, this point was already discussed in Alexandre et al., 2015. However we found it worth being recalled, as it explains the heterogeneity of phytC pool accessibility. The paragraph was slightly changed to make it clear.

- P15386, line 8-11: Could you present a synthesis of the accuracy and reproducibility of extraction of OC and 14C measurement on your extracts? This would support your study and evidences.

We have added a succinct discussion on phytC 14C accuracy and reproducibility at the beginning of section 3.1 (Isotopic results from above-ground C manipulation experiments).

- P15387, 6-7: "Third, : : :" I don't see how this part is supported by your data?

The reviewer refers to, "Third, contributions of soil respired CO_2 to mature plant tissue (and phytC) were also negligible." The statement is supported by our CO_2 chamber flux measurements (section 2.2.3), as discussed above. To make the message clear, we now refer to the section 2.2.2, where the statement appears.

- P15389: I don't really get the reason why some authors measure 1.5-3% of PhytOC while Santos et al. measured <0.1%. Did you isolate environmental factors (type of vegetation, climate, geology, soil type) that can influence the concentration of OC occluded in phytoliths? Could you please clarify?

Indeed, there are many estimates of phytC quantities (as %) from phytoliths. We suspect that those high estimates of 1.5-3% of phytOC are products of insufficient cleaning during the extraction process. It should also be noted that the methods used by Santos et al. (2010); Santos et al. (2012); Corbineau et al. (2013), and this study (images and associated spectra in Supplement) are the only ones which use sample blanks in order to determine if the cleaning process itself can lead to contamination. Further, all of these papers (except Santos et al. (2010) used scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDS) as a way to verify that phytolith surfaces were devoid of organic residues. Checking for residues/contaminants by optical microscopy is simply not adequate. The amount of phytOC within phytoliths is extremely small – a few hundred parts per million at best. Thus, even very small amounts of surface C is enough to bias the isotopic result. We already discussed those issues in sections 4.2, but for further clarification they are also briefly recalled in section 4.3 and 4.4.

Unfortunately several articles continue to promote the idea that phytoliths are quite stable in soils, and that they can persist for several thousands of years (Song et al 2014 and references therein). This is true only for a very small fraction of all phytoliths produced. Thus, soil phytoliths are not a stable sink for Si and C, but rather they are dynamic, the result of inputs (production) and outputs (dissolution).

The reviewer is right, but we just do not have many studies which directly measure phytolith stability factors to provide a better assessment to phytolith stability in soils. Therefore, section 4.4 (Implications for long-term atmospheric CO_2 biosequestration) was rewritten to take into account this valuable remark.

The results and discussions sections were reformatted, as suggested by reviewers.

The sentence was removed and the revised section dealing with soil C pools mobilization made clearer and shorter.

In agreement with this comment, this section was shortened and speculating parts were removed.

References added to the revised manuscript:

Piperno, D.R. Phytolith radiocarbon dating in archaeological and paleoecological research: a case study of phytoliths from modern Neotropical plants and a review of the previous dating evidence. J. Archaeol. Sci. 2015, (doi: 10.1016/j.jas.2015.06.002), *in press*

Plante, A.F., Fernández, J.M., Haddix, M.L., Steinweg, J.M., Conant, R.T. Biological, chemical and thermal indices of soil organic matter stability in four grassland soils. Soil Biology & Biochemistry 43(5), 1051–8, 2011.

Plante, A.F., Beaupré, S.R., Roberts, M.L. and Baisden, T. Distribution of Radiocarbon Ages in Soil Organic Matter by Thermal Fractionation. Radiocarbon, 55(2–3), 1077-1083, 2013.

Santos, G.M., Alexandre, A., Prior, C.A. From radiocarbon analysis to interpretation: A comment on "Phytolith Radiocarbon Dating in Archaeological and Paleoecological Research: A Case Study of Phytoliths from Modern Neotropical Plants and a Review of the Previous Dating Evidence", Journal of Archaeological Science (2016), doi: 10.1016/j.jas.2015.06.002." by Dolores R. Piperno, Journal of Archaeological Science 66:36-43, 2016 (doi: 10.1016/j.jas.2015.11.012), *in press*

Telles, E.C.C., Camargo, P.B., Martinelli, L.A., Trumbore, S.E., Costa, E.S., Santos, J., Higuchi, N., Oliveira Jr., R.C.: 2003. Influence of soil texture on carbon dynamics and storage potential in tropical forest soils of Amazonia. Global Biogeochem. Cycles 17 (2), 1040, 2003 (http://dx.doi.org/10.1029/2002GB001953)

Torn, M.S., Swanston, C.W., Castanha, C., Trumbore, S.E.: Storage and turnover of organic matter in soil. In: Senesi, N., Xing, B., Huang, P.M. (Eds.), Biophysicochemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems. International. Union of Pure and Applied Chemistry (IUPAC), New York, NY, pp. 219-272, 2009.

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Hodson, M.J. The development of phytoliths in plants and its influence on their chemistry and isotopic composition. Implications for palaeoecology and archaeology. J. Archaeol. Sci. doi:10.1016/j.jas.2015.09.002, 2016, *in press*

Parr, J. F., and Sullivan, L. A. Comparison of two methods for the isolation of phytolith occluded carbon from plant material. Plant and soil, 374(1-2), 45-53, 2014.

All other references cited in this reply are listed in our original manuscript.

1   Unambiguous evidence of old soil carbon in grass biosilica particles

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3   Paul E. Reyerson[1,2, †], Anne Alexandre[3], Araks Harutyunyan[1], Remi Corbineau[3], Hector A.

4   Martinez De La Torre[1], Franz Badeck[4], Luigi Cattivelli[4], Guaciara M. Santos[1,*]

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6   [1]Earth System Science, University of California, Irvine, USA.

7   [2]Department of Botany, University of Wisconsin-Madison, USA.

8   [3]Aix Marseille Université, CNRS, IRD, CEREGE UM34, 13545 Aix-en-Provence Cedex 4,

9   France

10   [4]Consiglio per la Ricerca in Agricoltura e l'analisi dell'economis agraria - Genomics Research

11   Centre, Fiorenzuola d'Arda, Italy.

12   [†]Current address: Department of Geography and Earth Science, University of Wisconsin-La

13   Crosse, USA.

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18   *Corresponding author. Guaciara M. Santos, Department of Earth System Science, 1222 Croul

19   Hall, University of California, Irvine, Irvine, CA, 92697-3100, USA, phone: (+1-949-824-3674),

20   fax: (+1-949-824-3256), E-mail address: gdossant@uci.edu

Abstract

Plant biosilica particles (phytoliths) contain small amounts of carbon called phytC. Based on the assumptions that phytC is of photosynthetic origin and a closed system, claims were recently made that phytoliths from several agriculturally important monocotyledonous species play a significant role in atmospheric CO_2 sequestration. However, anomalous phytC radiocarbon (^{14}C) dates suggested contributions from a non-photosynthetic source to phytC. Here we address this non-photosynthetic source hypothesis using comparative isotopic measurements (^{14}C and $\delta^{13}C$) of phytC, plant tissues, atmospheric CO_2, and soil organic matter. State-of-the-art methods assured phytolith purity, while sequential stepwise-combustion revealed complex chemical-thermal decomposability properties of phytC. Although photosynthesis is the main source of carbon in plant tissue, it was found that phytC is partially derived from soil carbon that can be several thousand years old. The fact that phytC is not uniquely constituted of photosynthetic C limits the usefulness of phytC either as a dating tool or as a significant sink of atmospheric CO_2. It additionally calls for further experiments to investigate how SOM-derived C is accessible to roots and accumulates in plant biosilica, for a better understanding of the mechanistic processes underlying the silicon biomineralization process in higher plants.

1. Introduction

Silicon (Si) is the most abundant element in the Earth's crust and is widely recycled by higher plants. Si is acquired by roots from soils and precipitated in or between the cells as micrometric hydrous amorphous biosilica particles called phytoliths. Phytolith abundances range from <1% of dry weight (d.wt) in many plants to several % d.wt in grasses that are Si-accumulators (Geis, 1973, Runge, 1999, Webb and Longstaffe, 2000; Raven, 2003). Phytoliths contain small amounts of carbon (C) occluded during silica precipitation (Alexandre et al., 2015), commonly termed as phytC (or phytOC) and assumed to be of photosynthetic origin (Carter 2009, Piperno 2006) (Figure 1a). Thus, phytC isotopic signatures ($\delta^{13}C$ and ^{14}C) obtained from buried soils and sedimentary archives have been interpreted in terms of paleoenvironmental changes (Kelly et al., 1991, Carter, 2009; McInerney et al., 2011), or used as a dating tool (McClaran and Umlauf, 2000; Piperno and Stothert, 2003; Parr and Sullivan, 2005, Piperno, 2006).

Motivated by anthropogenic emissions of carbon dioxide (CO_2) (Mauna Loa Observatory; NOAA-ESRL data at http://www.esrl.noaa.gov/) and their direct association with climate change, a set of recent studies has advanced the idea that many monocotyledonous crop species (bamboo, sugarcane, maize, rice, etc.) as well as grasslands in general (among the largest ecosystems in the world - Suttie et al., 2005) may play a significant role in C sequestration through a newly evidenced mechanism: CO_2 biosequestration in grass biosilica particles (Parr and Sullivan, 2011, Parr et al., 2010, Parr et al., 2009, Parr and Sullivan, 2005, Song et al., 2013, Song et al., 2014, Toma et al., 2013). If correct, encapsulated atmospheric CO_2 can be slowly and steadily accumulated in soils, with turnover times on the order of several hundreds to thousands of years (Parr and Sullivan, 2005). Selective use of silica accumulator crops could further enhance this sequestration mechanism (Song et al., 2013).

However, the validity of these interpretations has recently been challenged. First, attempts to properly calibrate the geochemical signals borne by phytC were inconclusive (Wilding, 1967, Kelly et al., 1991, McClaran and Umlauf, 2000, Smith and White, 2004, Webb and Longstaffe, 2010). Second, differences in the efficiency of phytolith extraction protocols may have contributed to inconsistencies and overestimations in phytC quantification (from 0.1 to 20% of phytolith d.wt.) (Corbineau et al., 2013 and references therein, Song et al. 2014 and references therein). Third, systematic offsets of phytC ^{14}C ages relative to the ^{14}C ages of the

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plant tissues from which phytoliths originate have been published (Santos et al. 2010, Santos et al. 2012a,b, Sullivan and Parr 2013, Yin et al. 2014, Piperno 2015, Santos et al. 2016). These offsets can be as large as hundreds to several thousands of years, regardless of the chemical protocol used for phytolith extractions, indicating the presence of a secondary contributor of C to phytC. Together, these observations led to the hypothesis that a whole or a fraction of phytC may come from old soil C (Santos et al., 2012a) (Figure 1b). Previous analyses of macromolecules embedded in phytoliths suggested a variety of organic molecules (Bauer et al., 2011 and references therein), but there is no direct evidence that they are solely synthesized by the plant. Moreover, a recent Nano Secondary Ion Mass Spectrometry (NanoSIMS) investigation of phytC distribution in the silica structure suggests that a significant part of phytC can be lost at the very first stage of phytolith dissolution (Alexandre et al., 2015), thus dissociating the concept of phytC protection from phytolith stability.

Therefore, if the soil C to phytC hypothesis is definitively confirmed, it casts doubt on the efficiency of paleoenvironmental reconstructions based on phytC as a proxy of plant C, and raises questions regarding the present estimates of crop and grasslands phytolith efficiency in sequestering atmospheric CO_2, as well as its assessment of long-term stabilization in soils based on fossil phytolith ^{14}C dating (decades versus hundreds, or thousands of years, as suggested by Parr and Sullivan, 2005). Additionally, confirmation of a dual origin (soil organic matter (SOM) and photosynthetic) of phytC would open new questions regarding plant-soil interactions and SOM recycling, relevant for our understanding of the role of terrestrial ecosystems in the C cycle.

To unequivocally establish that a fraction of phytC is indeed from soils, a robust dataset is produced here by considering and ruling out all other factors that can possibly bias the isotopic signatures of phytC. We reassess the old soil C contribution to phytC hypothesis (Santos et al. 2012a) on the basis of >200 isotopic results (δ^{13}C and/or ^{14}C) of phytoliths and associated materials (grass tissues, SOM fractions, amendments and hydroponic solutions, CO_2 respired from substrates or extracted from air). Pure phytolith concentrates were acquired from sets of above and below-ground C manipulation experiments. Phytolith concentrates were extracted using several protocols with different degrees of aggressiveness (Corbineau et al. 2013) in four different laboratories. Cutting-edge techniques assured phytolith purity, and multiple analyses of carbon isotope reference materials assured high quality and reproducibility of the isotopic

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results. Furthermore, to establish a link between phytC heterogeneity in the sense of molecular complexity and resistance to oxidation (labile vs. recalcitrant), we subjected duplicates of pure phytolith extracts to thermal treatments. The multi-methodology approach used in this study allows us to completely address: a) the anomalous ^{14}C results associated with phytC in the literature, b) the implications of a soil C contribution to phytC for ^{14}C geochronology dates, and c) the shortcomings of using phytC as an atmospheric CO_2 sink.

2. Material and Methods

2.1. Samples

Our experimental design is based on a two-step process. First, in order to evidence whether the ^{14}C signatures of phytC are solely of photosynthetic origin, we select samples from known-year specimens, and compare plant material grown under normal atmospheric CO_2 conditions to the artificially altered plant C isotope content of photosynthetically assimilated depleted-$^{14}CO_2$ from Free Air Carbon Enrichment (FACE) experiments (section 2.1.1). Second, we seek to establish a causal connection between soil C and phytC by selecting samples from plant material grown under normal atmospheric CO_2 conditions, but altered substrate carbon pools (section 2.1.2). In both cases phytC and an array of samples associated with it were selected.

2.1.1. Above ground C manipulation experiments

The FACE experiments exposed the plants to elevated atmospheric CO_2 concentrations by continuously releasing CO_2 through jets from tubes installed in the surroundings and within the enclosures of the cultivation plots. Target mixing ratios of atmospheric and geologic CO_2 were maintained on plots until leaves were senescent and/or ready for harvesting.

Two grass species (*Sorghum bicolor* and *Triticum durum*) were grown in two FACE experiments, respectively: at the Maricopa Agricultural Center (University of Arizona, USA) in 1998-1999 (Ottman et al., 2001), and at the Genomics Research Centre of CREA (Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria) in Fiorenzuola d'Arda, Italy, in 2011-2012 (Badeck et al., 2012 - http://centrodigenomica.entecra.it/research/durumFACE). For each experiment, a plot cultivated under ambient atmospheric CO_2 was compared to a plot cultivated

131 under atmosphere enriched by 160-200ppm in fossil hydrothermal CO_2, and therefore free of ^{14}C

132 (Leavitt, 1994, Ottman et al., 2001, Badeck et al., 2012). In terms of stable isotopic labelling, at

133 the sorghum site the enriched CO_2 had a $\delta^{13}C$ value of -40‰ from 1995 to 1998. This stronger

134 isotopic label was obtained from a mixture of natural CO_2 from the Springerville, Arizona, USA

135 geologic wells with 15% petroleum-derived CO_2. During 1998-1999 only fossil hydrothermal

136 CO_2 was used ($\delta^{13}C$ = -4.36 ‰), while the background air $\delta^{13}C$ was -8‰ (Leavitt et al., 2001).

137 At the durum wheat site, the commercial fossil CO_2 from the Rapolano Terme, Poggio S. Cecilia

138 (Tuscany) well had a $\delta^{13}C$ of -6.07‰, which was slightly positive compared to the ambient CO_2

139 value of -8‰.

140 Two samples of mixed stems and leaves (~100 g) were obtained from the sorghum site,

141 while four separated samples (300-400 g each) of stems and leaves were collected at the durum

142 wheat site. Eight soil samples (~5 g each) collected from the furrows of the sorghum plots at

143 depths of 0-15, 15-30, 30-45 and 45-60 cm were also obtained from the archives of the

144 Laboratory of Tree-Ring Research, University of Arizona, USA. While two soil samples were

145 collected from the ongoing durum wheat experimental plots at a depth of 0-15 cm (~15 g each)

146 during plant biomass harvesting.

147 To determine the precise ^{14}C activity of the plant materials, radiocarbon measurements

148 were conducted before the phytolith extractions started. Since the commercial CO_2 used in both

149 FACE enrichment sites was from a fossil source, its ^{14}C signature as fraction of modern carbon

150 (FmC or $Fm^{14}C$; Stuiver and Polach, 1977) was close to zero. Therefore, the ^{14}C signature of the

151 enriched CO_2 was highly depleted compared to ambient air, and the plant tissues were tagged

152 accordingly. Radiocarbon signatures of the plant tissue yielded $Fm^{14}C$ values of 0.640 (~3.6 kyrs

153 BP; ^{14}C years before present or 1950; UCIAMS53273 and 53274; Table S1 in Supplement) and

154 0.556 (~4.7 kyrs BP; UCIAMS109000 and 109001; Table S2 in Supplement) at the sorghum and

155 durum sites, respectively. Alternatively, plant tissue from ambient CO_2 plots was expected to

156 yield the prescribed atmospheric $^{14}CO_2$ values of the given year that the growing season took

157 place. At the sorghum site, the $Fm^{14}C$ value of the bulk biomass harvested at the ambient CO_2

158 plot matched with the $Fm^{14}C$ value of the CO_2 of the year of harvest (e.g. $Fm^{14}C \approx 1.097$,

159 equivalent to the atmospheric $^{14}CO_2$ signature measured from clean air in 1999 -

160 http://calib.qub.ac.uk/CALIBomb/ database and calibration software). This ^{14}C signature is

161 higher than the present-day ambient CO_2 due to nuclear weapon tests carried out during the

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162 1950s and 1960s (Levin, 1997, Levin et al., 2013). The nuclear weapon tests doubled the ^{14}C

163 content in the atmosphere, which created an isotopic chronometer (the ^{14}C bomb peak) during

164 the last 60 years for all living organisms. At the durum wheat site, however, the ^{14}C signature of

165 the biomass harvested at the ambient CO_2 plot was slightly depleted (Fm^{14}C $\approx$ 1.017), as

166 expected for CO_2 above urban areas in Europe in the early 2010s. For comparison, the ^{14}C

167 signature of atmospheric-clean CO_2 stations in Central Europe was Fm^{14}C = 1.040 in 2012

168 (Levin, 1997, Levin et al., 2013).

169

170 2.1.2. Below ground C manipulation experiment

171 The second experiment relies on the simultaneous response of phytC to different carbon

172 amendment treatments of grasses grown under photosynthetic natural conditions (i.e., ambient

173 CO_2 air). *Sorghum bicolor* plants were grown outdoors in a ventilated area at the University of

174 California, Irvine (UCI, USA), in six well-drained 40 L planters (A, B, C, D, E and F) filled with

175 mineral substrates. Five of the planters were enriched with organic nutrients characterized by a

176 broad range of ^{14}C signatures (from bomb spiked to fossil - Tables 1 and 2), while the last

177 contained an inorganic nutrient devoid of C as a control (planter F). Although much concerning

178 the direct root absorption of natural carbon remains unknown, beneficial responses of root and

179 plant growth have been reported in association with the addition of either inorganic carbon

180 (Hibberd and Quick, 2002) and/or humic acids (Nardi et al., 2002). Consequently, we chose as

181 substrate for Planter B a natural carbonate-based sedimentary deposit mixed with organic carbon

182 detritus of equal/even-age. For Planter E, fossil humic acids (extracted from leonardite) were

183 chosen as the OC source.

184 Plants were fed as needed solely with 2 L of ultra-pure water (Planter A), or with a

185 combination of ultra-pure water and their respective fertilizers and SiO_2 providers (Planters B-F)

186 at a concentration of 1% (v/v) (Table 2). Additionally, the CO_2 in the air surrounding the planters

187 was isotopically monitored by collecting air in evacuated 6 L cylinders for the duration of the

188 experiment with the purpose of characterizing the local atmospheric CO_2 close to planters, and to

189 serve as a reference for the ^{14}C signatures expected from plant tissue organs. Also, we

190 isotopically measured commercial (sorghum) seeds to check if their ^{14}C signatures were recent.

191 Finally, CO_2 fluxes respired from the planter substrates were also sampled to evaluate their

192   putative contribution to the phytC ^{14}C signature. After 3.5 months the *Sorghum bicolor* plants

193   (stem and leaf) were harvested in preparation for phytolith extractions and isotopic analyses.

194

2.2. Laboratory Procedures

2.2.1. Plant treatment and phytolith extraction

197   Stems and leaves samples (50-100 g each) were thoroughly rinsed with warm ultrapure

198   water to remove air-dust, dried at 60 °C and ground using an industrial mill (IKA$^{®}$ M20

199   Universal Mill). About 10 mg of each sample was kept for bulk tissue ^{14}C and $δ^{13}$C analyses.

200   Four phytolith extraction protocols with increasing aggressiveness (via organic

201   compound oxidation and silica dissolution) were used to treat the samples from the above ground

202   C manipulation experiment (Fig. 2). The protocols have been previously described in detail by

203   Corbineau et al. (2013). They are based either on acid digestion and alkali or on multi-step dry

204   ashing and acid digestion. They are summarized below and in Figure 2.

i.   *Protocols 1a and 1b* - Plant samples were subject to strong wet-digestion steps in
      order to oxidize the organic matter (e.g., 1N HCl/2 hours, hot H_2SO_4/24 hours plus
      30% H_2O_2 for 2-3 days, and > 65% HNO_3 plus 1 g $KClO_3$ for 24 hours). This was
      followed by 30 min of immersion in KOH solution at pH11 (protocol 1a) or pH13
      (protocol 1b). The KOH immersions allowed final removal of any alkali-soluble
      forms of organic compounds remaining on phytolith surfaces.

ii.   *Protocols 2a and 2b* - Plant samples were subjected to dry-ashing. Stepwise increases
      in temperature were used from 300°C to 500°C and the samples were then kept at
      500°C for 6 hours (protocol 2a) or 12 hours (protocol 2b). Samples were then
      digested in a >65% HNO_3 and 70% $HClO_4$ mixture (2:1).

215

216   In order to assess local ^{14}C contamination during chemical extractions, four laboratories

217   were involved in the extractions. They are UCI (USA), CEREGE (France), the Soils and

218   Sediments Analysis Lab (SSAL, the University of Wisconsin-Madison, USA), and the National

219   Lacustrine Core Facility (LacCore, the University of Minnesota, Twin Cities, USA). Aliquots of

220   pre-baked (900°C/3 hours) silicon dioxide powder (SiO_2; mesh# -325, Sigma Aldrich, St. Louis,

221   MO, USA) were chemically pre-treated in parallel with the plant samples, and later analyzed as

phytolith extract to provide independent blank data for each laboratory following the procedures described in Santos et al. (2010).

Due to the limited amount of plant biomass produced by the below ground C manipulation experiment (session 2.1.2), only two protocols were tested (1a and 2b) at only three of the laboratories (UCI, CEREGE and LacCore), followed by blank sample materials as required.

2.2.2. Soil extraction fractions

Soils from the above ground C manipulation experiment were physically cleaned of roots and stones. The bulk SOM fraction was isolated after carbonate removal in 1N HCl baths at 60 °C. The refractory (alkali-insoluble) fraction was further isolated via multiple baths in 1M NaOH at 60 °C, followed by 1N HCl rinses (Santos and Ormsby, 2013). Upon chemical treatment, samples were adjusted to pH neutral and dried in a vacuum oven (Savant RT 100A refrigerated vapor vacuum pump system).

Amendments from the below ground C experiment were not subject to any chemical pretreatment, except for the tests performed to small aliquots of greensand (GS, Table 1), allowing us to isolate the organic fraction from its bulk mixture.

2.2.3. CO_2 flux measurements

In the frame of the below ground C manipulation experiment, the rate of CO_2 respired from *Sorghum bicolor* foliage (after sprouting), root systems and substrate was measured using closed dynamic soil CO_2 flux chambers (Czimzik et al., 2006). Chamber headspace gasses were circulated through an infrared gas analyzer (840, 1400, LI-COR, Lincoln, NE, USA,) for 6 minutes at 0.5 L per minute, and the CO_2 concentration was recorded every second. Once headspace CO_2 concentrations reached twice that of ambient-air, the CO_2 was collected in a molecular sieve trap for isotopic analysis, followed by ambient-air samples to serve as references.

2.3. Analytical procedures
2.3.1. Phytolith concentrate purity analysis

252 Small particulate organic contamination of phytolith concentrates may considerably

253 bias isotopic and quantitative analyses of phytC. The purity of the phytolith concentrates was

254 thus verified by Scanning Electron Microscopy with Energy-dispersive X-ray spectroscopy

255 (SEM-EDS) (Corbineau et al., 2013). Extracted phytoliths, mounted directly on pre-cleaned

256 aluminum stubs, were analyzed with a Schottky Thermal Field Emission FEI/Philips XL-30

257 SEM with back-scattering electron detector. EDS semi-quantitative analyses of C and Si were

258 obtained from 10 to 30 μm locations on selected particles. Special attention was paid to organic-

259 like particles showing tissue-like or non-phytolith morphologies. A total of ~30 analyses per

260 sample were made. Samples with all C:Si peaks area ratios <0.1 were reported as devoid of

261 organic particles. The equal/even accuracy and precision of the EDS analyses were evaluated by

262 multiple measurements [Mean value *(M)=1.17;* Standard Deviation *(SD)=0.02; n=21*] of a

263 silicon carbide (SiC) standard (#9441, Micro-Analyses Consultant Instrument LTD, St. Ives,

264 UK).

265

266 **2.3.2. Stable Isotope Analysis**

267 Stems/leaves, SOM fractions, nutrients/fertilizers and phytolith samples were analyzed

268 for their total C content and stable C isotope ratio (δ^{13}C) using a continuous flow stable isotope

269 ratio mass spectrometer (Delta-Plus CFIRMS) interfaced with a Fisons NA-1500NC (for solid

270 materials) and a Gasbench II (for CO_2 input).

271 About 10 mg of phytoliths and 25 mg of soil were weighed out into pre-baked (100 °C

272 per 2 hours) tin capsules (5 x 9 mm capsules from Costech Analytical Technologies Inc.,

273 Valencia, CA, USA) using a pre-calibrated microbalance (Sartorius AG, Göttingen, Germany).

274 For accurate integration and calibration of carbon peaks of phytolith samples (~0.1% C),

275 measurements were obtained by decreasing the helium carrier flow rate, and by measuring

276 several size-matched aliquots of standards from the National Institute of Standards Technology.

277 Aliquots of SiO_2 blanks and fossil phytoliths (MSG70) used as an internal standard at CEREGE

278 (Alexandre et al., 2015, Crespin et al., 2008) were included for background corrections and

279 accuracy (Santos et al., 2010), respectively. For the bulk tissue samples, aliquots of CO_2 gas

280 were recovered after combustion, and sent to CFIRMS, which has a typical precision of 0.1‰.

281 Stable isotope results are reported as δ values in ‰ relative to the Vienna Pee Dee Belemnite

282 (vPDB).

283

2.3.3. Radiocarbon Analysis

Stems/leaves, SOM fractions, nutrients/fertilizers, CO_2 and phytolith samples were processed for ^{14}C accelerator mass spectrometry (AMS) analyses. About 2 mg of plant tissue, 20-100 mg of SOM and 15-300 mg of phytoliths were loaded for tube-sealed combustion (Santos et al., 2004). To avoid CO_2 adsorption on phytolith surfaces, the loaded samples were kept and transferred warm (at 160 °C) to the evacuated line for sealing (Santos et al., 2010). Liquid solutions were freeze-dried directly into tubes prior to combustion. Atmospheric CO_2 was extracted from 6 L collection flasks of whole air, by attaching the flasks to an evacuated line. A similar procedure was used to recover the CO_2 collected in molecular sieve traps (from flux chambers). Once the CO_2 was cryogenically separated from other gasses, it was then transferred to a Pyrex tube at a flame-off port and sealed (Santos et al., 2010). Samples of CO_2 from tube-sealed combustions, flanks and traps were cryogenically isolated, and reduced to graphite (Santos et al., 2007, Xu et al., 2007), or transferred to Gasbench II CFIRMS for isotopic analysis.

The ^{14}C measurements were performed at the Keck-CCAMS Facility (UCI). Precision and accuracy in measurements on >0.7 mg of near-modern carbon samples are typically 0.2–0.3% (Beverly et al., 2010), and 1% on samples in the 0.01 mgC range (Santos et al., 2007). The instrument provides the isotopic ratio $^{13}C/^{12}C$, allowing for fractionation effects (either spectrometer induced or arising from biochemical processes) to be corrected for all targets measured.

Blanks from SiO_2 aliquots were also measured to provide background corrections. All labs and phytolith extraction protocols showed similar procedural blanks (~0.003 mg of modern C and ~0.002 mg of ^{14}C free[a]. Those values were subtracted from the ^{14}C data, including the results obtained from the MSG70 reference material, for accuracy. Details on such background subtractions can be found elsewhere (Santos et al., 2010). Radiocarbon results were expressed as $Fm^{14}C$ and when appropriate were discussed as ages.

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[a] The term ^{14}C free is used in association with materials from which the original ^{14}C radioisotope content has been reduced to zero or close to zero. However, those materials obviously continue to maintain their stable amounts of ^{12}C and ^{13}C. Consequently, the addition of ^{14}C free (or organic carbon from subfossil ^{14}C signatures) to pools of C containing present-day atmospheric CO_2 signals will lower the overall ^{14}C signature of the pool. For each 1% fossil C present, an offset of 80 years is expected (Santos et al. 2016).

2.3.4. Thermal Analysis

We performed thermal analysis of phytoliths on a modified Thermal-Optical Carbon Aerosol Analyzer (RT 3080, Sunset Laboratory Inc.) (Bae et al., 2004). Phytolith concentrates of 7-10 mg were loaded onto a customized spoon (Jelight Company, Inc. USA), placed into the instrument and kept at 50 °C for ~10 minutes for surface cleansing. The stepwise temperature ramp started at 50 °C and ended at 850 °C 50 minutes later. Pure oxygen (65 mL/min) was used to avoid refractory carbon (char) formation. The CO_2 evolved was injected into a manganese dioxide oven at 870 °C, and later quantified by a non-dispersive infrared detector. Typical multi-point calibration curves, when analyzing known quantities of C ranging from 2-120 μg, yielded correlation coefficients greater than 0.998.

Two phytolith samples were analyzed. Durum wheat leaf phytoliths extracted using protocol 1a, and the CEREGE internal standard, MSG70, made of highly weathered fossil phytoliths (Alexandre et al., 2015, Crespin et al., 2008).

3. Results and Discussions

3.1. Isotopic results from above-ground C manipulation experiments

A total of 21 individual phytolith concentrates were produced for the above-ground experiments by all laboratories involved in this project. Those samples are tabulated in the Supplement (Tables S1 and S2), followed by details on the sample processing (protocols, laboratory and measurement identifiers). Note that when sufficient plant material was available (which was the case for the durum wheat samples) some labs could replicate the extraction (i.e. processing the same pool of biomass following the same protocol).

From those 21 phytolith concentrates, 51 ^{14}C results were produced to determine the phytC ^{14}C signatures (number of targets includes duplicates and/or replicates, as specified in Tables S1 and S2). Two phytC ^{14}C targets from MSG70, a fossil phytolith internal standard at CEREGE, were also produced to evaluate measurement reproducibility. Overall, the precision and accuracy of the phytC ^{14}C data were better than 0.3‰, based on duplicates and triplicates of graphite samples > 0.5 mgC. For the smaller sized samples, 1‰ or better were recorded in most cases, even after background corrections based on measurements of multiple SiO_2 aliquots were propagated into individual uncertainties (Tables S1 and S2). We have not identified significant

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differences in inter-laboratory analyses when using the same protocol on subsamples of the same biomass sample, and/or when evaluating procedural blank materials (added to every batch analyzed – details in section 2.3.3). To help with determining the phytC carbon sources, other ^{14}C results shown in tables S1 and S2 are from the stems/leaves and SOM fractions (e.g. the carbon pools associated with the labile-accessible and recalcitrant (alkali-insoluble)).

PhytC concentrations were consistent for a given extraction method but showed a clear decreasing trend with increasing protocol aggressiveness. The phytC yields (phytC % relative to the d.wt of phytoliths) averages ranged from 0.24 to 0.06% for the less aggressive protocols 1a and 1b and from 0.05 and 0.002% for the more aggressive protocols 2a and 2b (Figure 2a, and Tables S1, S2).

Phytoliths extracted from either sorghum or durum wheat using protocol 1a produced phytC ^{14}C signatures closest to the values of the stems and leaves of origin regardless of air CO_2 concentration (ambient *vs* enriched CO_2) and grass species (Figure 2b). However, phytC ^{14}C offsets were still evident when compared to the expected values given the year of harvest or artificial tagging (Table S1). For sorghum, absolute offsets varied from 85 (UCIAMS123579 and -123580) to 610 years (UCIAMS123577 and -123578) when using protocol 1a. The maximum offset increased when using protocols 1b (2633 years; UCIAMS95338), 2a (1920 years; UCIAMS130339), and 2b (1990 ^{14}C years; UCIAMS95335 to -95337). Durum wheat ambient phytC ^{14}C absolute offsets varied from 105 (UCIAMS123572) to 1925 years (UCIAMS125986), while phytC offsets from enriched plots varied from 310 (UCIAMS123570 and -123571) to 2885 years (UCIAMS125983).

The hypothesis that there is a contribution of SOM-derived C to phytC was tested estimating phytC as a mixture of i) C derived from plant photosynthesis and ii) C derived from the oldest SOM fraction measured. The mixing equation (eq.1) is:

Oldest SOM-derived C contribution = $(Fm^{14}C_{SOM} - Fm^{14}C_{SL})/(Fm^{14}C_{phytC} - Fm^{14}C_{SL})$ (eq. 1)

where the ^{14}C signatures of the oldest SOM, stems and leaves (SL) and phytC are expressed as $Fm^{14}C_{SOM,}$ $Fm^{14}C_{SL}$ and $Fm^{14}C_{phytC}$. $Fm^{14}C_{phytC}$ was expressed relative to $Fm^{14}C_{SOM}$ (assigned a contribution value of 1) and $Fm^{14}C_{SL}$ (assigned a contribution value of 0). The average $Fm^{14}C$ values of the oldest SOM-C fractions measured in each experiment (i.e., the $Fm^{14}C$ average

13

371   value of the SOM 45-60 cm fraction for *S. bicolor* plots – Table S1 in Supplement, and the

372   refractory 0-15 cm fraction for *T. durum* plots – Table S2 in Supplement) were used for

373     $Fm^{14}C_{SOM.}$

374   The mixing curves associated with the SOM-derived C to phytC hypothesis are presented

375   in figure 2b. The $Fm^{14}C$ values of two phytC samples from the Sorghum Ambient CO_2

376   experiment obtained using protocol 1a (UCIAMS123579 and -123580) and one phytC sample

377   from the Durum wheat Enriched CO_2 experiment obtained using protocol 1b (UCIAMS130339)

378   were higher than $Fm^{14}C$ values of the stems and leaves of origin, indicating that the soil pool still

379   has remnants of ^{14}C-labeled OC from the 1950s thermonuclear tests (Levin, 1997, Levin et al.,

380   2013). In this case the SOM-derived C was assigned a contribution value of 0, and the stems and

381   leaves a contribution value of 1 in figure 2b. Conversely, some of the phytC $Fm^{14}C$ values from

382   the Durum wheat Enriched CO_2 experiment, obtained using protocols 1a, 2a and 2b

383   (UCIAMS123566, 123567, 125985, 130334 and 130335), were lower (^{14}C age older) than the

384     $Fm^{14}C$ value of the oldest SOM fraction or 1 in figure 2b. This pattern suggests that the so-called

385   oldest SOM fraction, which is a mixture of old and young SOM (Schrumpf et al., 2013) may still

386   be "younger" than present-day in terms of its ^{14}C signatures, if the C pool is still bearing some

387   bomb-produced ^{14}C OM or much older if aromatic complexes are dominant (Teller et al. 2003,

388   Torn et al. 2009). For the sorghum experiment this trend was particularly obvious, as the ambient

389   CO_2 and the upper soil layers were clearly imprinted with bomb ^{14}C (Levin 1997). Therefore,

390   figure 2b clearly showed that the phytC $Fm^{14}C$ values unambiguously trend toward the $Fm^{14}C$

391   value (or ^{14}C age) of the oldest SOM fraction. Overall, the crucial point to be noticed is that the

392   phytC ^{14}C offsets shifted linearly towards positive values if the oldest SOM fraction was older

393   than the biomass of origin (Sorghum Ambient and Durum wheat Ambient, Figure 2a), and

394   towards negative values when the oldest SOM fraction was younger (Sorghum Enriched, Figure

395   2a). Thus, phytC ^{14}C differences were clearly linked to the SOM ^{14}C ages. Moreover, the

396   agreement in phytC ^{14}C values obtained from stems and leaves indicated that the offsets were not

397   linked to plant anatomy.

398   Regarding $\delta^{13}C$ values, the phytC offsets relative to the tissue of origin did not

399   systematically trend towards SOM $\delta^{13}C$ values, except for the Sorghum Ambient phytC

400   undergoing the 2b protocol (-21.6±0.1‰ (*n=2*) as indicated in Figure 3; UCIAMS95335 and

401   95336). As described earlier, this protocol tends to isolate the most recalcitrant phytC fraction.

The difference between phytC δ^{13}C values of durum wheat and sorghum was higher (~15.7‰) than the difference between δ^{13}C values of the stems and leaves of origin (e.g. ~5.6 vs ~7.2‰ for wheat and sorghum, respectively), as previously reported for grasses with C_3 and C_4 photosynthetic pathways (Webb and Longstaffe, 2000, Webb and Longstaffe, 2010). Without further discrimination of the molecular composition of SOM-derived C absorbed by the plant roots, in-depth discussion of the δ^{13}C differences between phytC and plant biomass is difficult. Nevertheless, the observed differences between phytC and stems and leaves δ^{13}C values were consistent with previous calibration studies, and were explained by preferential occlusions of plant molecular ^{13}C-depleted compounds in phytoliths (Webb and Longstaffe, 2010).

3.2. Isotopic results from below-ground C manipulation experiments

A total of 12 individual phytolith concentrates and phytC ^{14}C targets were produced for the below-ground experiments, with duplicates or triplicates from the same biomass samples (from Planters A, B and C), but subjected to different degrees of oxidation (e.g. protocols 2a and 2b). Other ^{14}C results shown are from the stems/leaves, nutrients/fertilizers, and CO_2 extracted from 6 L flasks and flux chambers (Figure 4). The complete set of isotopic results and sample processing details are tabulated in the Supplement (Tables S3).

Phytoliths produced phytC yields ranging from 0.08 to 0.1% d.wt. when using the less aggressive protocol 1a and from 0.01 to 0.04% d.wt when using the more aggressive protocol 2b (Table S3).

Significant offsets of the phytC ^{14}C values relative to the stem and leaf Fm^{14}C values were again found in association with the C sources in the soils (e.g. substrates/amendments). The highest phytC ^{14}C offset of 3610 years (UCIAMS104366) was obtained from the phytC ^{14}C from Planter B when using protocol 2b, showing again that the increased age discrepancies were due to protocol aggressiveness (e.g. from 1a to 2b). The effect is also observed in the phytoliths associated with Planter C, which received very low amounts of below-ground organic carbon relative to all other treatments (Tables 1 and 2). Specifically, the Planter C phytC ^{14}C offsets increased from 160 (UCIAMS130346; protocol 1a) to 1150 (UCIAMS104362; protocol 2a), and finally to 1760 years (UCIAMS104900; protocol 2b).

Even when we processed biomass samples from all Planters following the same protocol (such as the less aggressive 1a protocol), ^{14}C age discrepancies between phytC and the plant of

origin were highly evident, and correlated to the ^{14}C signatures of amendments (UCIAMS130344 to 130348). PhytC ^{14}C offsets were greater for amendments containing sufficient amounts of C of extreme ^{14}C-signatures (e.g. positive 320 years to Planter A, and negative 680 years to Planter E in Table S3). Note that the Planter A substrate was composed of rich bulk-complex OC imprinted with ^{14}C-bomb values (or $Fm^{14}C$ signatures higher than present-day values), while the Planter E substrate received a solution of fossil OC ($Fm^{14}C \approx 0$; close to ~43 kyr BP; n=3) (Tables 1 and 2). As in the above C manipulation experiment, in figure 4 we assigned values of 0 and 1 to the $Fm^{14}C$ associated with stems and leaves of origin and amendments, respectively (Table S3), and used the same mixing equation (eq.1).

The bulk stems and leaves produced $Fm^{14}C$ signatures that were very similar to the local ambient air $^{14}CO_2$ values collected in the 6L cylinders during the growing season, excluding any possibility that the phytC ^{14}C depletions are a product of urban fossil atmospheric CO_2 fixation. The small discrepancies between the stem and leaf ^{14}C values (e.g. from 25 to 65 years) (Table S3) are attributed to heterogeneities in C distribution within plant cells during C fixation (Pausch and Kuzyakov, 2011, Wichern et al., 2011). The commercial seeds of sorghum were also measured by ^{14}C-AMS (Figure 4) to verify their recent radiocarbon activity (UCIAMS83120 and 83121; Table S3). As expected, once early-fixed photosynthetic CO_2 became dominant, remobilized ^{14}C from seeds made little contribution to mature biomass tissue.

Although $Fm^{14}C$ values of substrate CO_2 fluxes were depleted towards amendment ^{14}C bulk signatures (UCIAMS83842 to 83845, Table S3), soil CO_2 plant tissue refixation via photosynthesis (and its influence on phytC) was found to be negligible, and cannot be invoked to explain the anomalous phytC ^{14}C results. CO_2 fluxes from the planters' substrates upon sprouting varied from 0.34 to 1.72 ppm/sec ($\approx 10^{-5}$ $g/m^2/yr$) (Table S3), indicating very little microbial activity. For comparison, global soil CO_2 fluxes vary from 60 to 1000 $g/m^2/yr$ (Raich and Sclesinger, 1992).

$\delta^{13}C$ offsets between phytC and stems and leaves were ~ 6.5‰ on average, including the phytC from Planter B (which contain a mixed C pool of OM detritus of plant origin and carbonate deposits - Table 1), showing that the inorganic fraction of the soil C was not a significant source of phytC (Figure 5). Also in Figure 5, we show the stable isotopic signatures of the CO_2 fluxes (UCIAMS83842 to 83845; Table S3) collected using closed dynamic soil CO_2 flux chambers (Czimzik et al., 2006). The results fell mostly between the air and bulk plant

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464   tissue averages, as expected for CO_2 produced from above- and below-ground biomasses,

465   supporting our previous observations of negligible effects of soil CO_2 respired to phytC.

466   This dataset clearly shows that amendment-derived C, adsorbed through root plants,

467   altered the phytC ^{14}C signatures.

468

469   **3.3. Thermal stability of phytC**

470   Chemical compositional insights on carbonaceous materials can be obtained via oxidation

471   reactivity to thermal treatments; such treatments have been frequently used on organic

472   compounds from soils and sediments (Plante et al. 2011, 2013, Rosenheim et al., 2013). For

473   instance, single bonded carbon structures usually show a lower thermal stability than those

474   dominated by double bonds, such as conjugated and aromatic structures (Harvey et al., 2012).

475   Here, we make use of the same chemical-thermal stability concept to evaluate the heterogeneity

476   of phytC in reacting to heat treatments.

477   Thermograms obtained from phytoliths of the durum wheat leaves and fossil phytoliths

478   (MSG70) indicated a continuum of phytC CO_2 with different degrees of resistance or

479   accessibility (Figure 6). Although the overall production of CO_2 was lower for MSG70, the

480   continuum temperature-dependency pattern of phytC was preserved. For example, at 250 °C

481   both phytolith extracts produced CO_2, however the leaf phytoliths show lesser amounts of CO_2

482   evolved than soil phytoliths. At 500 °C half of the phytC CO_2 in both samples had been evolved,

483   and at 800°C all of the phytC has been completely removed.

484   Phytoliths typically melt at ~573 °C (Deer et al., 1992), but embedded metals (e.g. Al, Fe,

485   etc) within their structures could lead to a decrease in temperature stability (Wu et al., 2014).

486   Nevertheless, phytC that required much higher temperatures (e.g. >> 573° C) to fully oxidize,

487   places it at the upper-end of the carbon recalcitrance continuum (Cheng et al., 2013, Harvey et

488   al., 2012, Plante et al., 2005, 2011, 2013). Furthermore, even if char occurred during combustion

489   leading to some elemental carbon formation, it does not explain the phytC ^{14}C discrepancies

490   obtained here (Figures 2 and 4) or elsewhere (Santos et al., 2010, Santos et al., 2012a, Santos et

491   al., 2012b, Sullivan and Parr, 2013, Yin et al., 2014).

492   Santos et al. (2012a) and Yin et al. (2014) intentionally heated phytolith aliquots from a

493   single extract, and observed shifts in ^{14}C ages towards older values. This effect is similar to that

494   observed in total carbon or SOM distributions in soils and sediments when subject to thermal

495 decomposability (Plante et al. 2011, 2013). Thus, phytolith extractions that employ heat

496 treatments would better isolate the oldest soil C fraction within phytoliths, as previously found

497 (in sections 3.1. and 3.2). Basically, if the C pool in phytoliths is supposedly homogeneous and

498 from a single source (100% atmospheric CO_2), the ^{14}C results from all CO_2 temperature-fractions

499 should be in absolute agreement, as Fernandez et al. (2015) demonstrated by subjecting

500 carbonaceous materials to ramp pyrolysis and subsequently measuring them by ^{14}C-AMS.

501

4.1. The SOM-derived C to phytC hypothesis set of evidence

503 Results from both above- and below-ground experiments showed that the ^{14}C offsets

504 between phytC and stems and leaves pointed toward the oldest SOM ^{14}C values (Figures 2 and

505 4). This confirmed that a fraction of the old SOM-derived C occluded in phytoliths was more

506 resistant (or less accessible) to oxidation than the occluded C derived from recent photosynthesis

507 or from recent SOM. Once the most labile (or more accessible) C had been removed, the older

508 and more resistant carbon fraction became dominant. This behavior mirrors that in a recent study

509 showing an increase in ^{14}C age offsets of phytoliths with increasing combustion temperature

510 (Yin et al., 2014), and also the thermal decomposability pattern illustrated in the phytC

511 thermograms (Figure 6).

512 Our findings also imply that a portion of SOM-derived C is absorbed by the roots,

513 transferred to the stems and leave and finally occluded into phytoliths. In the bulk plant organs,

514 the old SOM-derived C amount is far too small to be ^{14}C detected in tissue clippings, as it is

515 masked by the large amounts of photosynthetic atmospheric carbon tissue (bulk stems and leaves

516 averaged ~41% carbon; Tables S1-S3). On the other hand, in phytoliths, the old SOM-derived C

517 becomes overrepresented when the most labile-accessible phytC starts to be oxidized. It should

518 be noted that the ^{14}C ages of the oldest SOM fraction are averaged bulk values that do not yield

519 any precise assessment of the fine-scale ^{14}C age of the C that may have been absorbed. These

520 drawbacks prevent precise quantification of the old SOM (probably diluted by the young SOM)-

521 derived C contribution to phytC. The impossibility of quantifying precisely the amounts of soil C

522 and associated ^{14}C signatures in phytC precludes application of any correction that would allow

523 phytC to be used as a reliable dating material. As in any other heterogeneous carbon pool, the

524 phytC continuum can be similarly partitioned differently by distinctive chemical extractions. For

525 instance, in Piperno (2015) the entire dataset of post-bomb Neotropical plant phytolith extracts

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526   were neither accurate nor precise. While ^{14}C offsets reached discrepancies as high as 4.4 kyrs
527   between expected calendar ages and phytC, two pairs of phytolith extracts obtained by distinct
528   chemical treatments (sulfuric vs. nitric) yielded a 50 percent reproducibility rate (Table 1, Santos
529   et al. 2016).

530   Recent 3D X-Ray microscopy and NanoSIMS measurements of a phytolith sample from

531   the Durum wheat enriched CO_2 experiment (TD-F-L/1a-CEREGE, Table S1) (Alexandre et al.,

532   2015) suggested two locations for phytC: in micrometric internal cavities and within the silica

533   network. Rapid opening of internal cavities during the dissolution process resulted in losses of

534   phytC found in these locations, which is expected when phytoliths are subject to rapid oxidation.

535   Conversely, phytC in the silica network is homogeneously distributed at the micrometric scale,

536   and is less accessible to oxidation. These two pools of phytC may account for the heterogeneity

537   of phytC accessibility to oxidation.

538

539   **4.2. Rebuttals to possible arguments against the SOM derived-C contribution to phytC**

540   **hypothesis**

541   Our experiments and dataset allow the rejection of several hypotheses for the

542   "anomalously" old ^{14}C ages for phytC. First, bias due to exogenous C contamination during the

543   phytolith extractions performed simultaneously by several laboratories and artifacts of errors in

544   background corrections are highly unlikely. In these cases the ^{14}C offsets would trend in a single

545   direction, rather than being both positive and negative (Figures 2b and 4). In addition, aliquots of

546   SiO_2 blank and fossil phytoliths (MSG70) reference material yielded ^{14}C values in close

547   agreement with the expected results, giving no indication of the presence of unusual

548   contaminants. Second, natural- or spectrometer-produced anomalous δ^{13}C shifts of phytC were

549   not observed here (Figures 3 and 5) nor elsewhere (Santos et al., 2010, Santos et al., 2012b,

550   Sullivan and Parr, 2013). Third, contributions of soil respired CO_2 to mature plant tissue (and

551   phytC) were also negligible (section 2.2.3). Fourth, phytC ^{14}C results were not biased by organic

552   matter residues, as the efficiency of the phytolith extraction protocols was fully checked by

553   SEM-EDS analyses (e.g. acceptance threshold of C:Si $\leq$ 0.1 of 30 frames or more) (Corbineau et

554   al., 2013), a method superior to microscopic evaluation alone (Figures S1 and S2) (Kameník et

555   al., 2013, Santos et al., 2012a). Moreover, our extracts were consistently reproducible regarding

556   phytC yields across all labs involved (Tables S1-S3) and thermal decomposability properties

(figure 6). Since it has been established that plants do not photosynthesize all carbon found within their tissues (details in section 4.5), the uptake of SOM-derived C via the root system and its allocation to phytC is the only plausible explanation for the phytC ^{14}C offsets.

4.3. Implications for the use of phytC as a proxy of plant C

Since phytoliths (and to some extent plant tissues) contain a broad continuum of C with a complex mixture of chemical compounds of different turnover times as evidenced here (Figures 2, 4, and 6), we believe that insufficient to excessive oxidations can result in wild moves in phytC ^{14}C dates from thousands, to hundreds, to back to thousands years old (Figure 7).

While pure surface phytoliths produced from a less aggressive protocol (e.g. 1a) may minimize ^{14}C offsets to some degree, two factors remain that may explain the anomalous thousands of years old age of phytC indicated in the literature (Wilding, 1967, Kelly et al., 1991, McClaran and Umlauf, 2000, Santos et al., 2010, Santos et al., 2012a, Sullivan and Parr, 2013, and recently, Piperno 2015 and Santos et al. 2016). The first factor is the incomplete removal from phytolith concentrates of refractory SOM residues, either extraneous in the case of litter and soil samples or from the plant tissue itself. The accumulation effect of small quantities of residual recalcitrant (and somewhat older) SOM derived-C from concentrates due to incomplete digestion (Figure 7), which can be detected via C:Si peaks with SEM-EDS (Corbineau et al. 2013), may be undetected under natural light microscopy. For instance, Santos et al. (2010) reported phytC ^{14}C age offsets of 2.3 to 8.5 kyrs BP on phytolith concentrates extracted from living grasses using conventional digestion protocols, such as Kelly et al. (1991). Later, OM remnants in association with those anomalous ^{14}C results were detected by SEM-EDS on phytolith concentrates (Figure 2 in Santos et al. 2012a), thus demonstrating that even very small amounts of surface C were enough to bias the phytC ^{14}C results. Attempts to reproduce the atmospheric $^{14}CO_2$ bomb-peak in phytC from bamboo litter and mature leaves subjected to microwave digestions, also yielded offsets of several hundreds to 3.5 kyrs (Santos et al., 2012b, Sullivan and Parr, 2013). Similarly, a set of post-bomb Neotropical plant phytolith extracts produced by two protocols yielded phytC ^{14}C ages that were highly inaccurate, e.g. phytC ^{14}C offsets range from several decades to 4.4 kyrs (Santos et al. 2016). In those cases, preferential bias due to post-depositional occlusion of SOM was unlikely. All phytolith extracts analyzed were obtained from living or close to living vegetation, undergoing different extraction

procedures coupled with optical microscope analyses (for purity evaluations). Cumulative effects of OM remnants on phytoliths would also explain the higher phytC yields (Kelly et al., 1991, Li et al., 2014, Parr and Sullivan, 2005, Santos et al., 2010, Song et al., 2014). The second factor is the increasing relative proportion of old SOM-derived C in phytC when phytolith extraction aggressiveness is high enough to remove the phytC fraction most sensitive to oxidation (e.g. the labile-accessible C fraction termed 'protocol 2' in Figure 7). Once carbon partitioning takes place via either further chemical extractions or increased combustion temperatures, phytC concentrations tend to drop followed by increased ^{14}C offsets to thousands of years old (Santos et al. 2012a, Yin et al., 2014 and the present work).

Since the range of old SOM-derived C content in phytC left by a given protocol can be large (Figure 2), and can vary in association to the abundances of C fractions within the substrates and their respective ^{14}C signatures (Figure 4), any attempt to apply a systematic correction to obtain a phytC Fm^{14}C signature derived solely from photosynthesis is likely to fail. We can also assume that when grasses are forced to reach greater rooting depths (Sivandran and Bras, 2012) than the ones sampled here, where the proportion of intrinsic-older organic compounds is likely to rise (Teller et al. 2003, Torn et al. 2009, Kleber, 2010, Petsch et al., 2001), old SOM-derived C in phytC and its Fm^{14}C depletions would also increase. Furthermore, by themselves the ^{14}C signatures of phytC pools with competing ^{14}C ages (recent SOM-derived C vs present-day atmospheric ^{14}CO$_2$) are insufficient to distinguish them. Therefore, the old soil-C to phytC contributions found here in the ^{14}C signatures of phytoliths extracted from living grasses are likely to be only a very small fraction of the total SOM contribution to phytC, as discussed earlier.

Further work is still needed to assess the full impact of SOM (e.g., the different fractions of labile vs. recalcitrant carbon; Han et al., 2007) to the phytC pool. At natural conditions the presence of SOM-derived C in phytC may bias the δ^{13}C signature to a lesser extent if the SOM and the plants of origin have similar photosynthetic pathways (C$_3$ or C$_4$). The bias may however be significant if they are not. The δ^{13}C signature of SOM can be hard to assess, especially in the case of phytoliths extracted from sedimentary archives. Thus, we suggest that the use of ^{14}C and δ^{13}C signatures of phytC as a dating tool or as a proxy of plant or atmospheric CO$_2$ signatures should be reappraised in the light of the present findings.

618

4.4. Implications for long-term atmospheric CO₂ biosequestration

      The evidence for a SOM-derived C contribution to phytC decreases the putative effectiveness of grasslands and crops to sequester atmospheric CO_2 for two reasons. Besides negatively affecting phytolith C storage capacity, our findings most importantly invalidate phytC accumulation rates estimated from direct ^{14}C dating of soil phytoliths (Parr and Sullivan, 2005). In addition, other issues may also come into play. For instance, the phytolith biosequestration hypothesis is based essentially on the following premises. First, high phytC concentrations are required. Values of 1.5-3% d.wt. have been quantified (e.g. Li *et al.*, 2013, Parr and Sullivan, 2011, Parr *et al.*, 2010). These values are more than 10 times higher than the concentrations recently measured by others (<0.1% d.wt. [Santos et al., 2010]). Differences in the efficiency of phytolith extraction protocols (Kameník et al., 2013), combined with the lack of proper control (blanks) and reproducibility of results (Corbineau et al., 2013) may have contributed to these high phytC concentrations. Second, a soil phytolith stability factor of 70 to 90% based on a few ^{14}C measurements of soil phytoliths (e.g. Parr and Sullivan, 2005) has been estimated and widely used (Li et al., 2014) regardless of soil type. These high percentage estimates differ from those of biogenic Si fluxes, based on Si pool measurements in tropical soil-plant systems. For instance, according to Alexandre et al. (2011) investigating two soil/plant systems in intertropical areas, only 10% of phytoliths produced annually are in fact preserved for extended periods, the remaining 90% being rapidly dissolved due to weathering (Oleschko et al., 2004). These proportions would reasonably depend on environmental conditions such as activity of elements (Si, Al, Fe, H+) in soil solution, morphology of phytoliths (and thus vegetation type), elemental concentration of phytoliths (and thus soil type).

      Only as an exercise, we used the highest phytC yield measured in the frame of the present study (0.3% of phytoliths) coupled with the 10% phytolith stability factor estimated from Alexandre et al. (2011), to recalculate a global grassland phytC-sink. We obtain a value of r 4.1 $\times 10^4$ tC yr^{-1}, which is roughly one hundred times lower than the 3.7×10^6 tC yr^{-1} value reported elsewhere (Song et al., 2014 and references therein). This amount is insignificant when compared to the 2.6×10^9 tC yr^{-1} estimate for the land C sink (I.P.C.C. Staff, 2007), or to the 0.4×10^9 tC yr^{-1} global mean long term soil C accumulation rate (Schlesinger, 1990). This

648 suggests that previous conclusions on the importance of developing silica accumulator crops for
649 increasing atmospheric C sequestration should be reconsidered.

650

651 **4.5. Implications for our understanding of soil C pools mobilization.**

652 Our findings have important implications for our understanding of the mobilization of
653 soil C pools. Several studies have shown that terrestrial plant roots can uptake soil dissolved
654 inorganic carbon (DIC). DIC can be transported directly by the transpiration stream or fixed in
655 mycorrhizal and root tissues and subsequently translocated in the form of amino acid (Gioseffi et
656 al., 2012, Rasmussen et al., 2010, Talbot and Treseder, 2010). DIC can represent 1 to 3% of
657 total leaf-fixed CO_2 (Ford et al., 2007, Ubierna et al., 2009). However, as DIC is expected to be
658 in equilibrium with soil CO_2 respired from autotrophic and heterotrophic sources, its ^{14}C
659 signature should reflect an average of SOM ^{14}C signatures, close to contemporary. Assuming soil
660 DIC as the soil end-member in Figure 2, the phytC samples from ambient CO_2 experiments
661 would plot along mixing lines with lower slopes than the actual ones. The ^{14}C age of several
662 thousand years systematically measured for the most resistant phytC, rather suggests that an
663 older SOM fraction supplies the SOM-derived C absorbed by the roots, up-taken and transported
664 to the stem and leaves tissues.

665 The fact that roots can also acquire soil C in a molecular form has been previously
666 inferred from the detection in roots, stems and shoots of polycyclic aromatic hydrocarbons
667 (PAH) (Gao et al., 2010, Yu et al., 2013), and soil amino acids (AA) (Paungfoo-Lonhienne et al.,
668 2008, Warren, 2012, Whiteside et al., 2012, Whiteside et al., 2009). Although reported PAH
669 concentrations were three orders of magnitude below phytC concentrations (e.g. 10^{-9} g/g vs. 10^{-6}
670 g/g, assuming 0.1% d.wt. for both phytolith concentration in plants and phytC content in
671 phytoliths), AAs make up several tenths of % of the plant nitrogen requirements (Lipson and
672 Näsholm, 2001). Arbuscular mycorrhizal fungi, which colonize 70% of plant families (Talbot
673 and Treseder, 2010, Treseder and Turner, 2007) are probably at the base of the transfer of
674 molecular C from the rhizosphere to the roots, although intact protein has also been shown to
675 enter root cells without the help of mycorrhizae, most likely via endocytosis (Paungfoo-
676 Lonhienne et al., 2008). At lower scales, AA transporters were shown to confer the ability of
677 plants to absorb molecular C from the soil solution (Lipson and Näsholm, 2001, Tegeder, 2012).
678 Root acquisition of humic substances (active and passive) and its positive effect on plant nutrient

679 uptake has been also reported (Trevisan et al., 2010). The incorporation of below-ground

680 physical, chemical and biological processes in the rhizosphere (e.g. microbial priming effect or

681 nitrogen (N) and C cycles interactions) have also been proposed (Heimann and Reichstein, 2008

682 and references therein). The results of the present study go a step further by demonstrating that

683 part of the soil molecular C absorbed by roots is several thousand years old. Recent studies also

684 show that old, supposedly poorly accessible SOM (Kleber, 2010, Petsch et al., 2001, Schmidt et

685 al., 2011), can be decomposed by organisms or catalytic enzymes (Dungait et al., 2012, Marín-

686 Spiotta et al., 2014). Common sources of dissolved Si for plants are clay minerals and

687 amorphous silicates (allophane, imogolite). Due to their small size, high surface functional

688 groups, area, and porosity, these minerals stabilize SOM either by adsorption onto their surface

689 or by aggregation (Basile-Doelsch et al., 2007, Jones and Singh, 2014, Kögel-Knabner et al.,

690 2010). Further studies are needed to investigate whether dissolution of Si-bearing forms during

691 active uptake of Si (Ma et al., 2006) may also promote old SOM mobilization, ready to be

692 chelated with Si, absorbed by the roots and translocated to the stems and leaves.

693

694 **5. Conclusion**

695 Although photosynthesis is the main source of C in plant tissue, we have demonstrated

696 here that grass biosilica (phytoliths) occlude SOM-derived C that can be several thousand years

697 old, debunking the common assumption of phytC photosynthetic carbon exclusivity. This finding

698 suggests causes for previous anomalously older phytC ^{14}C ages found in the literature. Moreover,

699 the fact that phytC is not uniquely constituted of photosynthetic C limits the usefulness of phytC

700 either as a dating tool or as a significant sink of atmospheric CO_2. Revised estimates of

701 atmospheric CO_2 biosequestration by phytoliths led to values that are insignificant compared to

702 the total land C or soil C sinks. All in all, by demonstrating that old SOM-derived C is accessible

703 to roots and builds-up in plant biosilica, this study constitutes a basis to further investigate the

704 mechanism and amplitude of old SOM recycling by roots for a better understanding of the C

705 cycle at the soil/plant interface.

706

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Table 1: Below-ground experiment. Details of substrate amendments, their carbon content, radiocarbon values (as $Fm^{14}C$ and ^{14}C age) and C isotopic signatures.

Name	Major Contents	%C (mass)[a]	$Fm^{14}C$	±1σ	^{14}C age	±1σ	$δ^{13}C$ (‰)	±1σ
Miracle Gro® (MG)	*Sphagnum Moss, Perlite, Compost, NH$_4$NO$_3$, (NH$_4$)$_3$PO$_4$, Ca$_3$(PO$_4$)$_2$, K$_2$SO$_4$*	49.5 (n=2)	1.0849	0.0028	-650[b]	25	-26.1	0.1
			1.0123	0.0028	-95[b]	25	-25	0.1
Greensand (GS)	*Glauconite with organic and inorganic detritus, MnO$_2$, SiO$_2$*	0.10[c]	0.1591 (n=2)	0.0016	14765	78	-24.3(OC; n=4) -12.6(bulk)	0.1
Ionic Grow (IG)	*Ca(NO$_3$)$_2$, KNO$_3$, H$_3$PO$_4$, HNO$_3$, K$_2$SO$_4$*	0.8	0.0374 (n=2)	0.0101	26550	2192	-26.4	0.1
Earth juice (EJ)	*Kelp meal, MgSO$_4$ borax, CoSO$_4$, FeSO$_4$, MnSO$_4$, Na$_2$MoO$_4$, ZnSO$_4$*	15.44 (n=2)	0.4991 (n=3)	0.0013	5583	24	-24.1 (n=2)	0.2
Fossil Fuel (FF)	*Humic acids (from leonardite or lignite coal)*	33.04 (n=2)	0.0055	0.0003	43340	1700	-26.2 (n=2)	0.2
Inorganic in-house fertilizer (IF)[d]	*NaH$_2$PO$_4$, MgSO$_4$, Ca(NO$_3$)$_2$, KNO$_3$*	------	------	------	------	------	------	------
Silica Blast (SB)[d]	*Na$_2$SiO$_3$, K$_2$SiO$_3$*	------	------	------	------	------	------	------

[a]Total percent carbon was determined by manometric measurements of CO_2 after combustion of solids. Those values are estimates only, as it does not take in account volatile organic C losses during the drying procedure of the amendments as solutions; [b]negative ^{14}C ages are associated with material that fixed C during the post-nuclear testing period (e.g. Post-AD 1950 to present); [c]GS %C is based on its total C amount by d.wt., with 0.06% of it constituted of organic matter detritus with the remaining C pool from marine carbonates. %C estimates of independent fractions were based on stable isotopic measurements of bulk and HCl treated (OC fraction) subsamples (section 2.2.2). Nevertheless, the ^{14}C values of the organic C and bulk fractions are similar, and are shown here as an average value. The $δ^{13}C$ values of both fractions are shown as reference; [d]attempts to produce CO_2 from solids (upon freeze-dry) confirmed the absence of C in those amendments, and therefore those are not shown.

Table 2: Below-ground experiment. Planters' major features: substrates and amendments, living plant appearance, biomass by d.wt. and phytolith yields. All nutrients and fertilizers were administered in aqueous solutions, except for MG. In bold: main amendment.

| | **Planters** | | | | | |
	A	**B**	**C**	**D**	**E**	**F**
Substrate	**MG**	**GS**	Baked Sand	Baked Sand	Baked Sand	Baked Sand
Amendments	In MG	**In GS**, IG[a]	**IG**[a]	**EJ**, IF[b]	**FF**, IF[b]	**IF**[b]
Silica Provider	In MG	In GS	SB	SB	SB	SB
Appearance	Dark green	Dark green	Dark green	Green	Yellowish green	Green
Biomass (g)	98.57	79.09	89.24	86.67	54.78	53.37
Phytolith yield[c]	0.12	0.78	0.83	0.83	1.77	1.35

[a]IG has a very low %C. Therefore, its C contribution to planters B and C after dilution into solution (e.g. ~ 0.02 grams of C per feeding) was found to be very small, a conclusion supported by isotopic analyses (Table S3); [b]IF (which does not contain measurable amounts of C) was added to those planters to supply micronutrients to support plant growth; [c]as % of dry leaf and stem biomass combined.
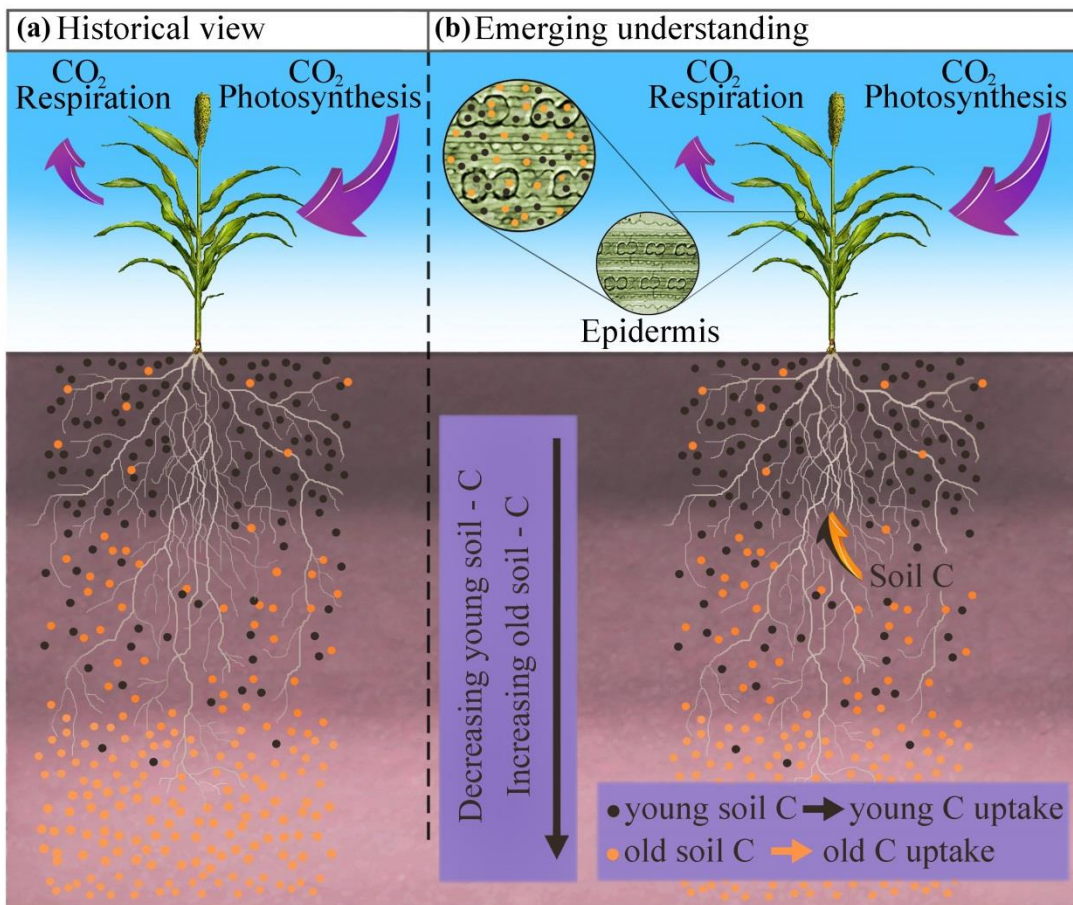
Fig. 1: Sketch of a) the conventional hypothesis of plant C occlusion during silica precipitation based solely on atmospheric CO_2 as a source, and b) the emerging hypothesis of a dual origin (atmospheric CO_2 and SOM) for plant C (and phytC). Young and old soil C distributed in leaf epidermis (green tissue) and phytoliths (illustrated by the bilobate type shape outlined in black) are represented by black and orange dots, respectively, in the microscope diagram.
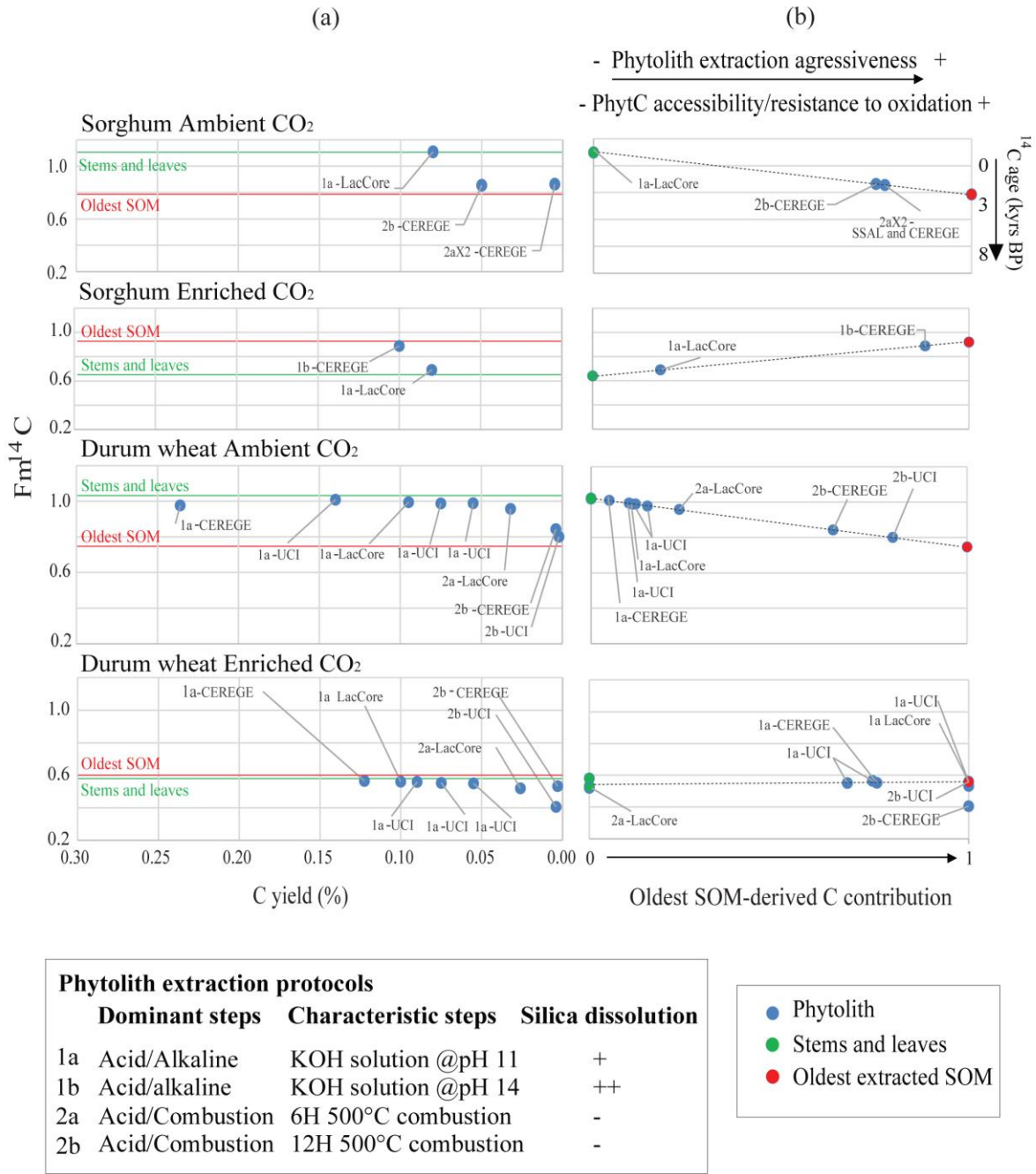
Fig. 2: Above ground C manipulation procedure. a) Averaged Fm^{14}C values versus averaged phytC yields (or concentration in % of phytoliths). Constant solid lines correspond to the averaged Fm^{14}C values obtained for stems and leaves (SL) of origin and the oldest extracted SOM fraction. b) Oldest SOM-derived C contribution to phytC calculated using the mixing equation (eq. 1) presented in the text expressing the ^{14}C signature of phytC as the result of mixing between the C derived from plant photosynthesis and the C derived from the oldest

Fig. 3. Above-ground C manipulation experiment. δ^{13}C values of stems and leaves, phytC, and soil SOM fractions obtained for A) sorghum and B) durum wheat experiments. To facilitate comparisons between groups, samples from ambient and enriched CO_2 plots are plotted next to each other. Values are reported as per mil (‰) related to PDB. Results of the bulk and refractory SOM fractions were averaged; consequently results and uncertainties indicate multiple data points. Individual results are shown in Tables S1 and S2.
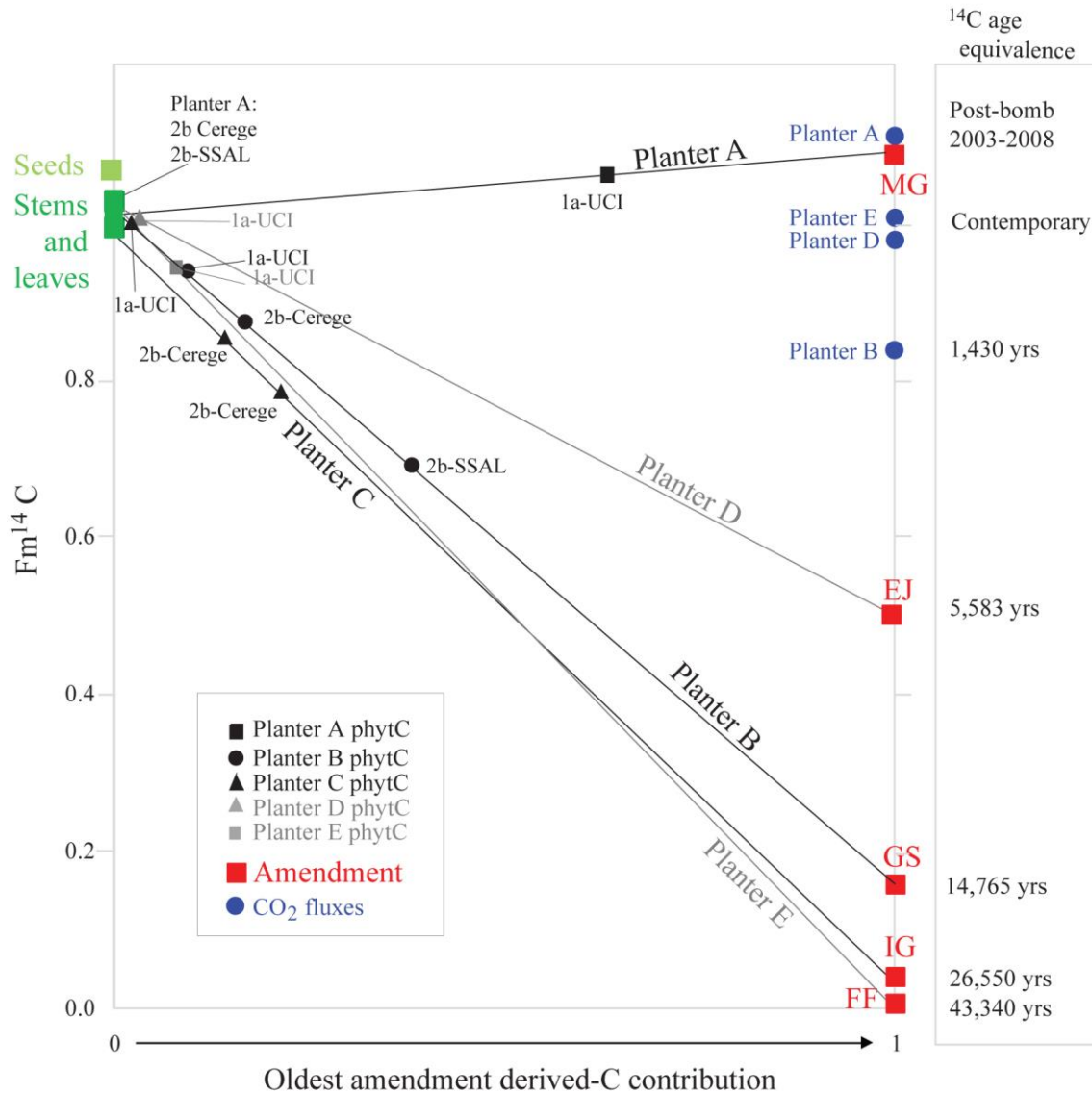
Fig. 4. Below ground C manipulation procedure: Oldest amendment-derived C contribution to phytC calculated using the mixing equation (eq. 1) presented in the text expressing the ^{14}C signature of phytC as the result of mixing between C derived from plant photosynthesis (seeds, stems and leaves represented by the green squares) and C derived from the oldest amendment (MG, EJ, GS, IG, FF defined in table 1and represented by the red squares). Phytolith samples are labeled according to the phytolith extraction protocol used (1a and 2b) and the laboratory of extraction (UCI, CEREGE and SSAL). Selected age benchmarks from substrate amendments and soil CO_2 fluxes are shown for reference on the right axis.
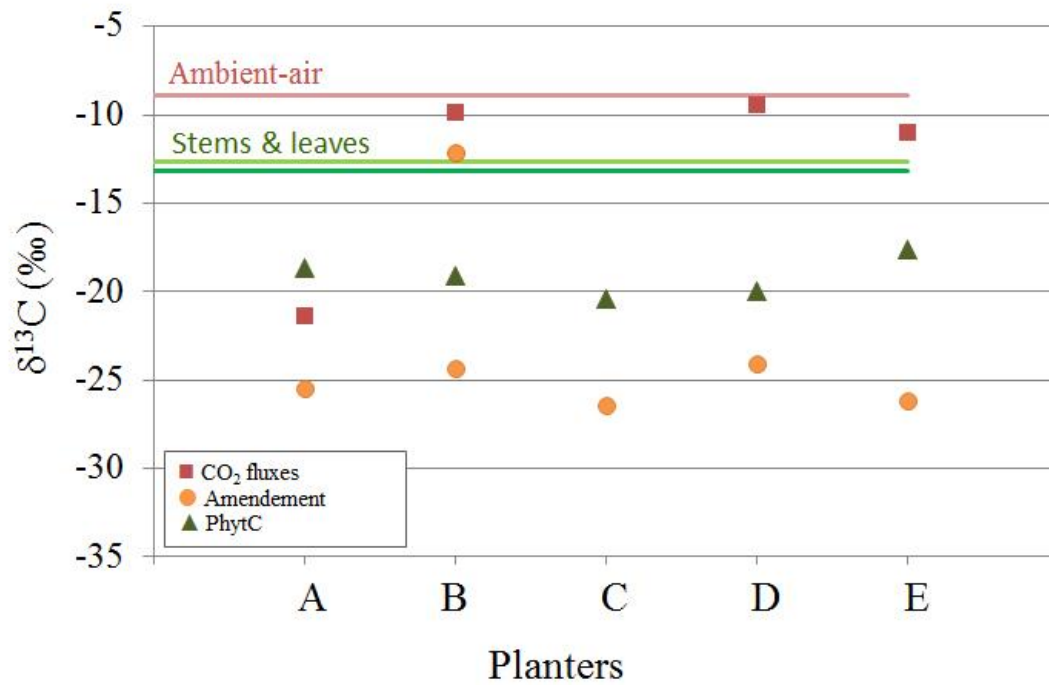
Fig. 5. Below-ground C manipulation experiment. $\delta^{13}C$ values of the respired CO_2, stems and leaves, amendments and phytC for the five planters enriched in organic carbon nutrients (A-E). Values are reported as per mil (‰) related to PDB, and individual symbols represent single results as reported in Table S3. For planter B we report two values, its OC fraction (-24.3‰) and its bulk fraction (-12.1‰ – a mixture of OC and inorganic carbon) (Table 1). Constant solid lines correspond to the average $\delta^{13}C$ values of ambient-air CO_2 and bulk plant tissues.
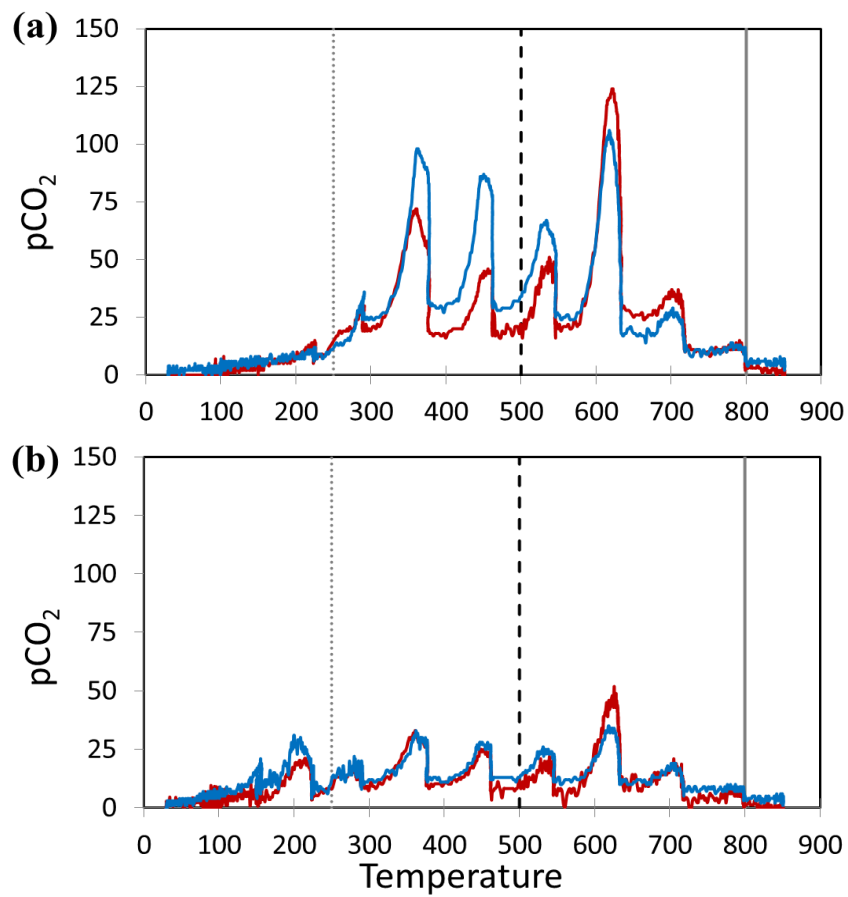
Fig. 6: Thermograms (*n=2*; blue and red lines) of phytoliths obtained from a) durum wheat leaves, phytoliths extracted following protocol 1a (Table S2), and b) soil phytoliths MSG70 extracted using a conventional protocol adapted to soil and sediment materials. Peaks are artifacts of the 100°C temperature-step increments. Vertical lines indicate main temperature thresholds, as explained in text.
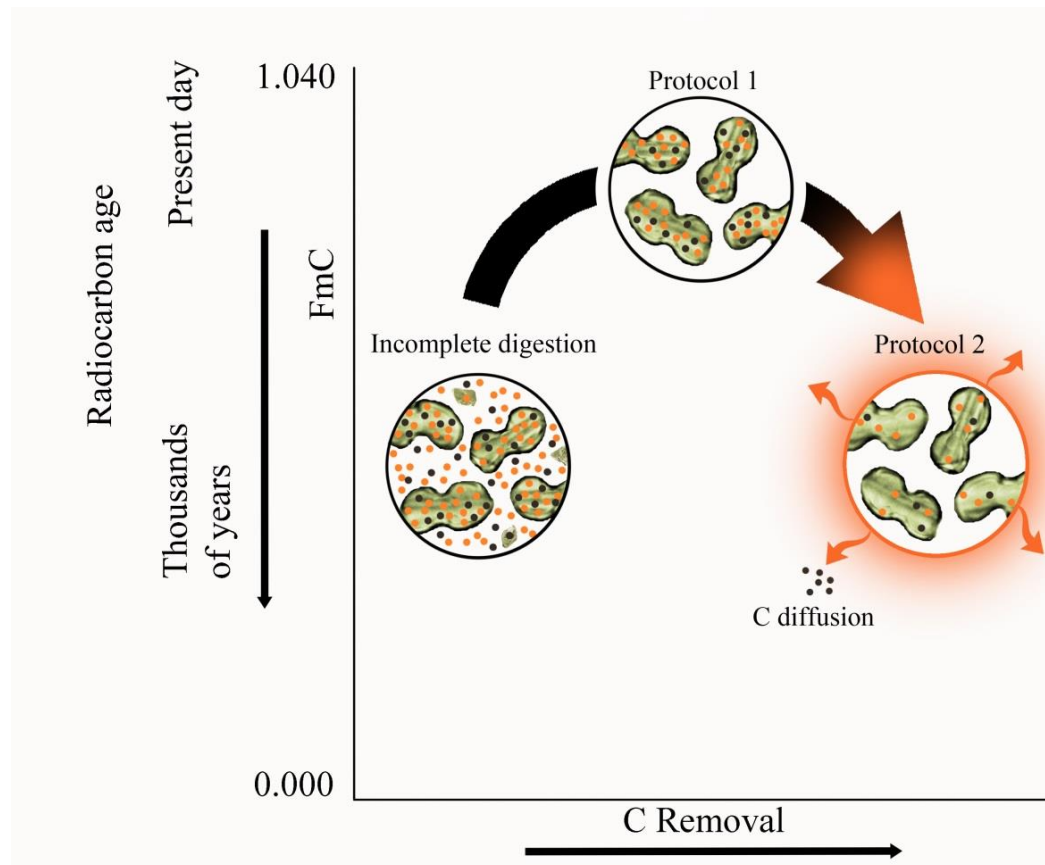
Fig. 7: Conceptualization of the impact of phytolith extraction aggressiveness and C removal on ^{14}C age of phytoliths. Incomplete digestion leads to an accumulation of old SOM residues on phytolith extract surfaces. Protocol 1 removes all surface OM and better preserves the dual source phytC signature. Protocol 2 removes all surface OM and labile (intrinsically young) phytC from inside the silica network. For illustration purposes, young and old C are represented by black and orange dots, respectively (cf Figure 1b).