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Evidence of old soil carbon in grass biosilica particles

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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 grasslands 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 is found that phytC is partially derived from soil carbon that can be several thousand years old. The accumulation of old soil organic matter derived carbon in plant biosilica suggests that Si absorption and phytolith production promote old soil organic carbon mobilization. Although the magnitude of this mechanism still needs to be properly assessed at plant and ecosystem scales, its confirmation alone argues against attempts to use phytC as a proxy of plant carbon and call for the reexamination of phytolith atmospheric CO_2 biosequestration estimates.

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 a small amount of carbon (C) occluded during silica precipitation (Alexandre et al., 2015), commonly termed as phytC or phytOC and

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assumed to be of photosynthetic origin (Carter, 2009; Piperno, 2006) (Fig. 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). Furthermore, triggered by excess quantities of carbon dioxide (CO_2) in the atmosphere (Mauna Loa Observatory; NOAA-ESRL data at http://www.esrl.noaa.gov/) and its direct association to climate change, a set of recent studies has advanced the idea that grasslands, that are 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, 2009; Parr and Sullivan, 2005; Song et al., 2013, 2014; Toma et al., 2013). If correct, portions of 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 all those 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, phytC concentration estimates of several % of d.wt. have been reported (Song et al., 2014 and references therein). However, differences in the efficiency of phytolith extraction protocols may have contributed to the inconsistencies in phytC quantification (from 0.1 to 20 % of phytolith d.wt.) (Corbineau et al., 2013 and references therein). Third, systematic offsets of phytC ^{14}C ages relative to the ^{14}C ages of the plant tissues from which phytoliths originate have been published (Santos et al., 2010, 2012a, b, Sullivan and Parr, 2013; Yin et al., 2014). These offsets can be as large as hundreds to several thousands of years (Santos et al., 2010, 2012a, b, Sullivan and Parr, 2013; Yin et al., 2014), regardless of the chemical protocol used for phytolith extractions, indicating the presence of a secondary source of C to

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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) (Fig. 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 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 grassland 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 vs. 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.

Here, we assess the old soil C contribution to phytC hypothesis on the basis of > 200 isotopic results ($\delta^{13}C$ and ^{14}C) obtained from grass tissue, atmospheric CO_2, soil (or substrate) organic matter and pure phytolith concentrates acquired from sets of above and below-ground C manipulation experiments. Phytolith concentrates were extracted via optimized protocols in four laboratories. Cutting-edge techniques assured phytolith purity, and multiple analyses of carbon isotope reference materials assured high quality and reproducibility of the isotopic results. Chemical-thermal decomposability analyses of phytC were also carried out, as an indicator for more complex attributes (e.g., phytC distribution between biochemically labile and stable fractions).

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2 Material and methods

2.1 Samples

2.1.1 Above ground C manipulation experiments

To determine whether the ^{14}C signatures of phytC are solely of photosynthetic origin, we compared the artificially altered plant C isotope content of photosynthetically assimilated depleted-$^{14}CO_2$ to plant material grown under normal atmospheric CO_2 conditions from Free Air Carbon Enrichment (FACE) experiments. 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 (Fiorenzuola d'Arda, Italy) in 2011–2012 (Badeck et al., 2012). For each experiment, a plot cultivated under ambient atmospheric CO_2 was compared to a plot cultivated under atmosphere enriched by 160–200 ppm in fossil hydrothermal CO_2 (Badeck et al., 2012; Leavitt, 1994; Ottman et al., 2001). Two samples of mixed stems and leaves were collected at the sorghum site, while four separated samples of stems and leaves were collected at the durum wheat site.

To determine the precise ^{14}C activity of the plant materials, radiocarbon measurements were conducted before the phytolith extractions started. Since the commercial CO_2 used in both FACE enrichment sites was from a fossil source, its ^{14}C signature as fraction of modern carbon (FmC or Fm^{14}C; Stuiver and Polach, 1977) was close to zero. Therefore, the ^{14}C signature of the enriched CO_2 was highly depleted compared to ambient air, and the plant tissues were tagged accordingly. Radiocarbon signatures of the plant tissue yielded FmC values of 0.640 (~ 3.6 kyr BP; ^{14}C years before present or 1950) and 0.556 (~ 4.7 kyr BP) at the sorghum and durum sites, respectively.

Plant tissue from ambient CO_2 plots was expected to yield the prescribed atmospheric $^{14}CO_2$ values of the given year that the growing season took place. At the sorghum site, the FmC value of the bulk biomass harvested at the ambient CO_2 plot

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matched with the FmC value of the CO_2 of the year of harvest (e.g. FmC ≈ 1.097, equivalent to the atmospheric $^{14}CO_2$ signature measured from clean air in 1999 – http://calib.qub.ac.uk/CALIBomb/ database and calibration software). This ^{14}C signature is higher than the present-day ambient CO_2 due to the nuclear weapon tests carried out during the 1950s and 1960s (Levin, 1997; Levin et al., 2013). The nuclear weapon tests double the ^{14}C content in the atmosphere, which created an isotopic chronometer (the ^{14}C bomb peak) during the last 60 years for all living organisms. At the durum wheat site the ^{14}C signature of the biomass harvested at the ambient CO_2 plot was slightly depleted (FmC ≈ 1.017), as expected for CO_2 above urban areas in Europe in the early 2010's. For comparison, the ^{14}C signature of atmospheric-clean CO_2 stations in Central Europe was FmC = 1.040 in 2012 (Levin, 1997; Levin et al., 2013).

At the sorghum site the enriched CO_2 had a $\delta^{13}C$ value of −40 ‰ from 1995 to 1998 and of −4.36 ‰ during 1998–1999, while the background air was −8 ‰ (Leavitt et al., 2001). At the durum wheat site, the commercial fossil CO_2 had a $\delta^{13}C$ of −6.07 ‰, which was slightly positive compared to the ambient CO_2 value of −8 ‰.

Eight soil samples (~ 5 g each) collected from the furrows of the sorghum plots at depths of 0–15, 15–30, 30–45 and 45–60 cm were obtained from the archives of the Laboratory of Tree-Ring Research, University of Arizona (USA). Two soil samples were collected from the durum wheat plots at a depth of 0–15 cm (~ 15 g each) during plant biomass harvesting.

2.1.2 Below ground C manipulation experiment

The second experiment relies on the simultaneous response of phytC to different carbon amendment treatments of grasses grown under photosynthetic natural conditions (i.e, ambient CO_2 air). *Sorghum bicolor* plants were grown outdoors in a ventilated area at the University of California, Irvine (UCI, USA), in six well-drained 40 L planters (A, B, C, D, E and F) filled with mineral substrates. Five of the planters were enriched with

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organic nutrients characterized by a broad range of ^{14}C signatures (from bomb spiked to fossil – Tables 1 and 2), while the last contained an inorganic nutrient devoid of C as a control (planter F). Although much concerning the direct root absorption of natural carbon remains unknown, beneficial responses of root and plant growth have been reported in association with the addition of either inorganic carbon (Hibberd and Quick, 2002) and/or humic acids (Nardi et al., 2002). Consequently, we chose as substrate for Planter B a natural carbonate-based sedimentary deposit mixed with organic carbon detritus of equal/even-age. For Planter E, fossil humic acids (extracted from leonardite) were chosen as the OC source.

Plants were fed as needed solely with 2 L of ultra-pure water (Planter A), or with a combination of ultra-pure water and their respective fertilizers and SiO_2 providers (Planters B–F) at a concentration of 1 % (v/v) (Table 2). Additionally, the CO_2 in the air surrounding the planters was isotopically monitored by collecting air in evacuated 6 L cylinders for the duration of the experiment with the purpose of characterizing the local atmospheric CO_2 close to planters. After 3.5 months the S. bicolor (stem and leaf) was harvested in preparation for phytolith extractions and isotopic analyses.

2.2 Laboratory procedures

2.2.1 Plant treatment and phytolith extraction

Stems and leaves samples (50–100 g each) were thoroughly rinsed with warm ultra-pure water to remove air-dust, dried at 60 °C and ground using an industrial mill (IKA® M20 Universal Mill). About 10 mg of each sample was kept for bulk tissue ^{14}C and $\delta^{13}C$ analyses.

Four phytolith extraction protocols with increasing aggressiveness relative to silica dissolution and C removal were used for the samples from the above ground C manipulation experiment (Fig. 2). The protocols are based either on acid digestion and alkali immersion (protocol 1a and 1b), or on dry ashing and acid digestion (protocol 2a and 2b) and have previously been described in detail (Corbineau et al., 2013).

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In order to assess local C contamination during chemical extractions, four laboratories were involved in the extractions. They are UCI (USA), CEREGE (France), the Soils and Sediments Analysis Lab (SSAL, the University of Wisconsin-Madison, USA), and the National Lacustrine Core Facility (LacCore, the University of Minnesota, Twin Cities, USA). Due to the limited amount of plant biomass produced by the below ground C manipulation experiment, only two protocols were tested (1a and 2b) at only three of the laboratories (UCI, CEREGE and LacCore). Overall, a total of twenty-one phytolith concentrates were produced for the above and below ground experiments.

Aliquots of pre-baked (900 °C/(3 h)$^{-1}$) silicon dioxide powder (SiO_2; mesh# −325, Sigma Aldrich, St. Louis, 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).

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 1 N HCl baths at 60 °C. The refractory (alkali-insoluble) fraction was further isolated via multiple baths in 1 M NaOH at 60 °C, followed by 1 N 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 some small aliquots of greensand (GS), allowing us to isolate the organic fraction from its bulk mixture of inorganic and organic C to determine its $\delta^{13}C$ and ^{14}C values (as shown in Table 1).

2.2.3 CO_2 flux measurements

In the frame of the below ground C manipulation experiment, the rate of CO_2 respired from *S. bicolor* foliage (after sprouting), root systems and substrate was measured us-

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ing 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 min at 0.5 L min^{-1}, 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

The purity of the phytolith concentrates was verified by Scanning Electron Microscopy with Energy-dispersive X-ray spectroscopy (SEM-EDS; Corbineau et al., 2013). Extracted phytoliths, mounted directly on pre-cleaned aluminum stubs, were analyzed with a Schottky Thermal Field Emission FEI/Philips XL-30 SEM with back-scattering electron detector. EDS semi-quantitative analyses of C and Si were obtained from 10 to 30 µm locations on selected particles. Special attention was paid to organic-like particles showing tissue-like or non-phytolith morphologies. A total of ~ 30 analyses per sample were made. Samples with all C : Si peaks area ratios < 0.1 were reported as devoid of organic particles. The equal/even accuracy and precision of the EDS analyses were evaluated by multiple measurements (mean value (M) = 1.17; standard deviation (SD) = 0.02; n = 21) of a silicon carbide (SiC) standard (#9441, Micro-Analyses Consultant Instrument LTD, St. Ives, UK).

2.3.2 Stable isotope analysis

Stems/leaves, SOM fractions, nutrients/fertilizers and phytolith samples were analyzed for their total C content and stable C isotope ratio ($\delta^{13}C$) using a continuous flow stable isotope ratio mass spectrometer (Delta-Plus CFIRMS) interfaced with a Fisons NA-1500NC (for solid materials) and a Gasbench II (for CO_2 input).

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About 10 mg of phytoliths and 25 mg of soil were weighed out into pre-baked (100 °C for 2 hours) tin capsules (5 × 9 mm capsules from Costech Analytical Technologies Inc., Valencia, CA, USA) using a pre-calibrated microbalance (Sartorius AG, Göttingen, Germany). For accurate integration and calibration of carbon peaks of phytolith samples (~ 0.1 %C), measurements were obtained by decreasing the helium carrier flow rate, and by measuring several size-matched aliquots of standards from the National Institute of Standards Technology. Aliquots of SiO_2 blanks and fossil phytoliths (MSG70) used as an internal standard at CEREGE (Alexandre et al., 2015; Crespin et al., 2008) were included for background corrections and accuracy (Santos et al., 2010), respectively. For the bulk tissue samples, aliquots of CO_2 gas were recovered after combustion, and sent to CFIRMS, which has a typical precision of 0.1 ‰. Stable isotope results are reported as δ values in ‰ relative to the Vienna Pee Dee Belemnite (vPDB).

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 remove absorbed CO_2 from 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.

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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 extraction protocols showed similar procedural blanks (~ 0.003 mg of modern C and ~ 0.002 mg of ^{14}C-free). 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 FmC and when appropriate were discussed as ages.

2.3.4 Thermal analysis

Chemical compositional insights on carbonaceous materials can be obtained via oxidation reactivity to thermal treatments. For instance, carbon single bonded structures would have a lower thermal stability than those dominated by double bonds, conjugated and aromatic structures (Harvey et al., 2012). Here, we make use of the same chemical-thermal stability concept to evaluate the C bond energy of phytC. We performed thermal analysis 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 min for surface cleansing. The stepwise temperature ramp started at 50 °C and ended at 850 °C 50 min later. Pure oxygen (65 mL min^{-1}) 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 in-

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frared 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

3.1 ^{14}C results from above- and below-ground C manipulation experiments

From the twenty-one phytolith concentrates extracted from both experiments, 52 ^{14}C targets were produced from phytC and measured by ^{14}C-AMS. The results are presented in Figs. 2 and 3. The other ^{14}C results shown are from the stems/leaves, SOM fractions, nutrients/fertilizers, and CO_2 extracted from 6 L flasks and flux chambers. The complete set of results is tabulated in the Supplement (Tables S1–S3).

In the above ground C manipulation experiments, phytC concentrations were consistent for a given extraction method but showed a clear decreasing trend with increasing protocol aggressiveness. PhytC yield averages ranged from 0.24 to 0.06 % d.wt for the less aggressive protocols 1a and 1b and from 0.05 and 0.002 % d.wt. for the more aggressive protocols 2a and 2b (Fig. 2a, and Tables S1 and 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 (Fig. 2a). However, significant 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). For durum wheat ambient phytC ^{14}C absolute offsets

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varied from 105 (UCIAMS123572) to 1925 years (UCIAMS125986), while phytC offsets from enriched plots varied from 310 (UCIAMS123570 and -123571) to 2885 years (UCIAMS125983). However, the crucial point is that the phytC ^{14}C offsets shifted linearly towards positive values if the oldest SOM fraction was older than the biomass of origin (Sorgum Ambient and Durum wheat Ambient, Fig. 2a), and towards negative values when the oldest SOM fraction was younger (Sorghum Enriched, Fig. 2a). Moreover, the agreement in phytC ^{14}C values obtained from stems and leaves indicated that the offsets were not linked to plant anatomy.

In the below ground C manipulation experiments, 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). However, significant offsets of phytC ^{14}C values relative to the stem and leaf $Fm^{14}C$ values were again evidenced (Fig. 3), varying from positive 320 years (UCIAMS130348; protocol 1a – Planter A) to negative 3610 years (UCIAMS 104366; protocol 2b – Planter B). The increase in discrepancies (towards thousands of years old) is again associated with the increase of protocol aggressiveness from 1a to 2b. However, even for phytoliths extracted simultaneously using just one protocol (such as 1a), 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 Fig. 3, Table S3). Note that 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 ($Fm^{14}C = 0$; close to ~ 43 kyr BP; $n = 3$) (Tables 1 and 2). 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 6 L cylinders during the growing season. The small discrepancies between the stem and leaf ^{14}C values (e.g. from 25 to 65 years) (Table S3) are attributed to C distribution heterogeneities 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 (Fig. 3) to verify their radiocarbon activity. As expected, once early-fixed photosynthetic

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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 are depleted towards amendment ^{14}C bulk signatures (Fig. 3), soil CO_2 plant tissue refixation via photosynthesis (and its influence on phytC) was found to be negligible. CO_2 fluxes from the planters' substrates upon sprouting varied from 0.34 to 1.72 $ppm\,s^{-1}$ ($\approx 10^{-5}\,g\,m^{-2}\,yr^{-1}$) (Table S3), indicating very little microbial activity. For comparison, global soil CO_2 fluxes vary from 60 to 1000 $g\,m^{-2}\,yr^{-1}$ (Raich and Sclesinger, 1992).

3.2 $\delta^{13}C$ results

In the above ground C manipulation experiments, $\delta^{13}C$ offsets relative to the tissue of origin did not systematically trend towards SOM $\delta^{13}C$ values, except for the Sorghum Ambient phytC undergoing the 2b protocol (as indicated in Fig. 4). As described earlier, this protocol tends to isolate the most recalcitrant phytC fraction. The difference between phytC $\delta^{13}C$ values of durum wheat and sorghum was higher (~ 15.7 ‰) than the difference between those and the $\delta^{13}C$ 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, 2010).

In the below ground C manipulation experiments, the $\delta^{13}C$ offset 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 C-soil was not a significant source of phytC (Fig. 5). Also in Fig. 5, we show the stable isotopic signatures of the CO_2 fluxes collected using closed dynamic soil CO_2 flux chambers (Czimzik et al., 2006). The results fell mostly between the air and bulk plant tissue averages, as expected for CO_2 produced from above- and below-ground biomasses, supporting our previous observations of negligible effects of soil CO_2 respired to phytC.

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However, without discriminating the molecular composition of SOM-derived C absorbed by the plant roots, further in-depth discussion of the $\delta^{13}C$ differences between phytC and plant biomass is difficult. Nevertheless, the observed differences between phytC and stems and leaves $\delta^{13}C$ values were consistent with previous calibration studies, and can be explained by preferential occlusions of plant molecular ^{13}C-depleted compounds in phytoliths (Webb and Longstaffe, 2010). Furthermore, our phytC $\delta^{13}C$ results demonstrated no presence of a massive isotopic fractionation offset due to the phytolith extraction procedures that could possibly correct the anomalous ^{14}C signatures.

3.3 Thermal stability of phytC

Thermograms obtained from phytoliths of the durum wheat leaves and weathered phytoliths from soils (MSG70) indicated a continuum of phytC CO_2 with different degrees of resistance or accessibility (Fig. 6). Although the overall production of CO_2 was lower for MSG70, the continuum temperature-dependency pattern of phytC was preserved. For example, at 250 °C both phytolith extracts produced CO_2, however the leaf phytoliths show lesser amounts of CO_2 evolved than soil phytoliths. At 500 °C half of the phytC CO_2 in both samples had been evolved, and at 800 °C all of the phytC has been completely removed. It is important to note that although phytoliths typically melt at ~ 573 °C (Deer et al., 1992), embedded metals (e.g. Al, Fe, etc) within their structures could lead to a decrease in temperature stability (Wu et al., 2014). Nevertheless, the fact that some amounts of phytC required much higher temperatures (> 573 °C) to fully oxidize, places it at the upper-end of the carbon recalcitrance continuum (Cheng et al., 2013; Harvey et al., 2012; Plante et al., 2005; Rosenheim et al., 2013). Furthermore, even if char occurred during combustion leading to some elemental carbon formation, it does not explain the Fm^{14}C phytC discrepancies obtained here (Figs. 2 and 3) or elsewhere (Santos et al., 2010, 2012a, b; Sullivan and Parr, 2013; Yin et al., 2014). Santos et al. (2012a) and Yin et al. (2014) heated phytolith aliquots from a single extract, and observed shifts in ^{14}C ages towards older values. Basically, if the C pool

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is homogeneous and from a single source, the ^{14}C results from all CO_2 temperature-fractions should be in absolute agreement, as Fernandez et al. (2014) demonstrated by subjecting carbonaceous materials to ramp pyrolysis and subsequently measured them by ^{14}C-AMS.

4 Discussion

4.1 Testing the hypothesis of a contribution of old SOM-derived C to phytC

Results from the above ground C manipulation experiments showed that the ^{14}C offsets between phytC and stems and leaves pointed toward the oldest SOM ^{14}C values. The hypothesis that there is a contribution of the oldest SOM-derived C to phytC was tested using a mixing equation between a pool consisting of the stems and leaves of origin (assigned oldest SOM-derived C contribution value of 0) and a pool consisting of the oldest extracted SOM fraction (assigned value of 1). We used for this purpose the $Fm^{14}C$ average value of the oldest SOM-C fractions measured in 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; Table S1 and S2). The mixing hypothesis is presented in Fig. 2b. The $Fm^{14}C$ values of two phytC samples from the Sorghum Ambient CO_2 experiment obtained using protocol 1a (UCIAMS123579 and -123580) and one phytC sample from the Durum wheat Enriched CO_2 experiment obtained using protocol 1b (UCIAMS130339) were higher than the stems and leaves of origin, indicating that the soil pool still has remnants of ^{14}C-labeled OC from the 1950s thermonuclear tests. In this case the associated results were assigned the SOM-derived C value of 0, instead of 1 in Fig. 2b. Conversely, some of the phytC $Fm^{14}C$ values from the Durum wheat Enriched CO_2 experiment, obtained using protocols 1a, 2a and 2b (UCIAMS123566, -123567, -125985, -130334, -130335), were lower (^{14}C age older) than the $Fm^{14}C$ value of the oldest SOM fraction or 1 in Fig. 2b. This pattern suggests that the so-called oldest SOM fraction, which is a mixture of old and young SOM

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(Schrumpf et al., 2013) may still be "younger" than present-day in terms of its ^{14}C signatures, if the C pool is still bearing some bomb-produced ^{14}C OM or much older if aromatic complexes are dominant. For the sorghum experiment this trend was particularly obvious, as the ambient CO_2 and the upper soil layers were clearly imprinted with bomb ^{14}C (Levin, 1997; Levin et al., 2013). Therefore, Fig. 2b clearly showed that the phytC $Fm^{14}C$ values unambiguously trend toward the $Fm^{14}C$ value (or ^{14}C age) of the oldest SOM fraction.

Although the contribution of old SOM-derived C to total phytC may have been initially small (< 10 %), its relative proportion increased significantly with phytolith extraction aggressiveness. This confirmed that a fraction of the old SOM-derived C occluded in phytoliths was more resistant to oxidation than the occluded C derived from recent photosynthesis. This behavior mirrors that in a recent study showing an increase in ^{14}C age offsets of phytoliths with increasing combustion temperature (Yin et al., 2014), and also the thermal decomposability pattern illustrated in the phytC thermograms in Fig. 4. Once the most labile-accessible C had been removed, a resistant/older fraction became dominant.

For the below ground C manipulation experiment, the contribution of amendment-derived C to phytC was estimated as above, using a mixing equation. We assigned values of 0 and 1 to the $Fm^{14}C$ associated with stems and leaves of origin and amendments, respectively (Fig. 3). Similar to the above ground C manipulation dataset, the phytC $Fm^{14}C$ values of the sorghum plants decreased by several hundreds of years from phytolith extraction protocol 1a to 2b, even in planter C despite its low amount of available carbon relative to all others (Tables 1 and 2). The depleted phytC $Fm^{14}C$ values showed again that the oldest phytC fraction was the least accessible to oxidation in the silica structure.

Recent 3-D X-Ray microscopy and NanoSIMS measurements of a phytolith sample from the Durum wheat enriched CO_2 experiment (TD-F-L/1a-CEREGE, Table S1; Alexandre et al., 2015) suggested two locations for phytC: in micrometric internal cavities and within the silica network. Rapid opening of internal cavities during the disso-

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lution process resulted in losses of phytC found in these locations, which is expected when phytoliths are subject to rapid oxidation. Conversely, phytC in the silica network is homogeneously distributed at the micrometric scale, and is less accessible to oxidation. These two pools of phytC may account for the most and least labile pools, as evidenced in both C manipulation experiments.

The two experiments thus support the hypothesis that phytoliths occlude fractions of SOM-derived C that can be several thousand years old. This finding implies that SOM-derived C is absorbed by the roots, transferred to the stems and leaves and finally occluded into phytoliths. In the bulk stems and leaves the old SOM-derived C amount is far too small to be ^{14}C detected, as it is masked by the photosynthetic atmospheric carbon tissue. In addition, its preferential distribution (if any) is still unknown. On the other hand, in phytoliths the old SOM-derived C becomes more concentrated when the most labile phytC starts to be oxidized. However, our experiments did not allow further quantification of the concentration of this old SOM-derived C in phytC as: 1) it was impossible to assess whether or not protocol 1a did start dissolving silica and oxidizing phytC and, 2) the ^{14}C ages of the oldest SOM fraction are averaged bulk values that do not yield any precise assessment of the fine-scale ^{14}C age of the molecules from the entire SOM-derived C. Since ^{14}C alone cannot discriminate young soil-C age signatures from a photosynthetic signal, we cannot truly rule out the possibility of a much larger SOM-derived C contribution to phytC. Consequently, our estimate of 10 % soil-C absorption by roots and relocation within phytoliths should be viewed as a very conservative minimum.

4.2 Rebuttals to possible arguments against the SOM derived-C contribution to phytC hypothesis

Our experiments and dataset allow the rejection of several hypotheses for the "anomalously" old ^{14}C ages for phytC. First, bias due to exogenous C contamination during the phytolith extractions performed simultaneously by several laboratories and artifacts of errors in background corrections are highly unlikely. In these cases the ^{14}C offsets

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would trend in a single direction, rather than being both positive and negative (Figs. 2b and 3). In addition, aliquots of SiO_2 blank and fossil phytoliths (MSG70) reference material yielded ^{14}C values in close agreement with the expected results, giving no indication of the presence of unusual contaminants. Second, natural- or spectrometer-produced anomalous $\delta^{13}C$ shifts of phytC were not observed here (Figs. 4 and 5) nor elsewhere (Santos et al., 2010, 2012b; Sullivan and Parr, 2013). Third, contributions of soil respired CO_2 to mature plant tissue (and phytC) were also negligible. Fourth, phytC ^{14}C results were not biased by organic matter residues, as the efficiency of the phytolith extraction protocols was fully checked by SEM-EDS analyses (e.g. acceptance threshold of C : Si $\leq$ 0.1 of 30 frames or more) (Corbineau et al., 2013), a method superior to microscopic evaluation alone (Figs. S1 and S2 in the Supplement; Kameník et al., 2013; Santos et al., 2012a). Moreover, our extracts were consistently reproducible regarding phytC yields and thermal decomposability properties across all labs involved (Tables S1–S3). Since it has been established that plants do not photosynthesize all carbon found within their tissues (details in Sect. 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

The evidence of phytC occluding SOM-derived C coupled with higher (previous studies – e.g. Wilding, 1967; Kelly et al., 1991; McClaran and Umlauf, 2000; Santos et al., 2010, 2012a; Sullivan and Parr, 2013) and lower (present investigation) phytC concentrations allow us to suggest that the anomalous thousands of years old age results are due to two factors. 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 (Fig. 7), which can be detected via C : Si peaks with SEM-EDS

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(Corbineau et al., 2013), may be undetected under natural light microscopy. For instance, Santos et al. (2010, 2012a) reported phytC ^{14}C age offsets of 2.3 to 8.5 kyr BP in association with large OM remnants on phytolith concentrates extracted from living grasses using conventional digestion protocols, such as Kelly et al. (1991). 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 kyr (Santos et al., 2012b; Sullivan and Parr, 2013). 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). This cumulative effect would also explain 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 (termed "protocol 2" in Fig. 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 (Yin et al., 2014 and this work). Since the range of old SOM-derived C content left by a given protocol can be large (Fig. 2), and can vary in association to the abundances of C fractions within the substrates and their respective ^{14}C signatures (Fig. 3), 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 depths (Sivandran and Bras, 2012) than the ones sampled here, where the proportion of intrinsic-older organic compounds is likely to rise (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 contribu-

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tion to phytC. Further work is still needed to assess the full impact of the SOM (e.g., the different fractions of labile vs. recalcitrant carbon; Han et al., 2007) to the phytC pool. Regarding phytolith $\delta^{13}C$ records, the presence of SOM-derived C in phytC may bias the $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ signatures of phytC as a dating tool or as a proxy of plant or atmospheric CO_2 signatures should be reappraised.

4.4 Implications for long-term atmospheric CO_2 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, these 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), only 10 % of phytoliths produced annually are in fact preserved

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for extended periods, the remaining 90 % being rapidly dissolved due to weathering (Oleschko et al., 2004). Recently, NanoSIMS investigations of the phytC distribution in the silica structure suggests that substantial portions of phytC are indeed prone to early dissolution, depending on its location within the phytolith structure (Alexandre et al., 2015).

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 measured Si fluxes (Alexandre et al., 2011), allow to recalculate a global grassland phytC-sink. It decreases from ca 3.7×10^6 t C yr^{-1} (Song et al., 2014 and references therein) to 4.1×10^4 t C yr^{-1}. This amount is insignificant when compared to the 2.6×10^9 t C yr^{-1} estimate for the land C sink (I.P.C.C. Staff, 2007), or to the 0.4×10^9 t C yr^{-1} global mean long term soil C accumulation rate (Schlesinger, 1990). This suggests that previous conclusions on the importance of developing silica accumulator crops for increasing atmospheric C sequestration should be reconsidered.

4.5 Implications for our understanding of soil C pools mobilization

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

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older SOM fraction weakly accessible to mineralization was reached by the roots, uptaken and transported to the stem and leaves tissues.

The fact that roots can also acquire soil C in a molecular form has been previously inferred from its detection in roots, stems and shoots of polycyclic aromatic hydrocarbons (PAH) (Gao et al., 2010; Yu et al., 2013), and soil amino acids (AA) (Paungfoo-Lonhienne et al., 2008; Warren, 2012; Whiteside et al., 2012, 2009). Although reported PAH concentrations were three orders of magnitude below phytC concentrations (e.g. 10^{-9} vs. 10^{-6} g g^{-1}, assuming 0.1 % d.wt. for both phytolith concentration in plants and phytC content in phytoliths), AAs make up several tenths of % of the plant nitrogen requirements (Lipson and Näsholm, 2001). Arbuscular mycorrhizal fungi, which colonize 70 % of plant families (Talbot and Treseder, 2010; Treseder and Turner, 2007) are probably at the base of the transfer of molecular C from the rhizosphere to the roots, although intact protein has also been shown to enter root cells without the help of mycorrhizae, most likely via endocytosis (Paungfoo-Lonhienne et al., 2008). At lower scales, AA transporters were shown to confer the ability of plants to absorb molecular C from the soil solution (Lipson and Näsholm, 2001; Tegeder, 2012). Root acquisition of humic substances (active and passive) and its positive effect on plant nutrient uptake has been also reported (Trevisan et al., 2010). The incorporation of below-ground physical, chemical and biological processes in the rhizosphere (e.g. microbial priming effect or nitrogen (N) and C cycles interactions) have also been proposed (Heimann and Reichstein, 2008 and references therein). The results of the present study go a step further by demonstrating that part of the soil molecular C absorbed by roots is several thousand years old. Recent studies also show that old, supposedly poorly accessible SOM (Kleber, 2010; Petsch et al., 2001; Schmidt et al., 2011), can be decomposed by organisms or catalytic enzymes (Dungait et al., 2012; Marín-Spiotta et al., 2014). We suggest that roots access this old C during Si uptake, a process that can be genetically controlled (Ma et al., 2006), and transport it in association with Si until the formation of biosilica in the epidermal cells of stems and leaves. Common sources of dissolved Si for plants are clay minerals and amorphous silicates (allophane, imogolite). Due to

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their small size, high surface functional groups, area, and porosity, these minerals stabilize SOM either by adsorption onto their surface or by aggregation (Basile-Doelsch et al., 2007; Jones and Singh, 2014; Kögel-Knabner et al., 2010). Plant exudates such as oxalic acid have been recently shown to promote disruption of soil mineral-organic associations and mobilization of soil C (Keiluweit et al., 2015). Dissolution of Si-bearing forms during active uptake of Si may also promote old SOM mobilization, ready to be chelated with Si, absorbed by the roots and translocated to the stems and leaves.

5 Conclusions

Although photosynthesis is the main source of C in plant tissue, we have demonstrated here that grass biosilica (phytoliths) occlude SOM-derived C that can be several thousand years old, debunking the common assumption of phytC photosynthetic carbon exclusivity. This finding suggests causes for previous anomalously older phytC ^{14}C ages found in the literature. Moreover, the fact that phytC is not uniquely constituted of photosynthetic C further limits the usefulness of phytC as a proxy of plant C and as a significant sink of atmospheric CO_2. Revised estimates of atmospheric CO_2 biosequestration by phytoliths led to values that are insignificant compared to the total land C or soil C sinks. Last but not least, by demonstrating that old SOM-derived C is accessible to roots and builds-up in plant biosilica, this study constitutes a basis to further investigate the mechanism and amplitude of old SOM recycling by roots for a better understanding of the C cycle at the soil/plant interface.

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Author contributions. G. M. Santos conceived the study. G. M. Santos, A. Alexandre, P. E. Reyerson, and R. Corbineau designed the experiments and conceived the strategies for phytolith

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extraction and purity analyses. G. M. Santos, P. E. Reyerson, A. Alexandre, A. Harutyunyan, R. Corbineau and H. A. Martinez De La Torre performed the experiments and contributed to analysis tools. F. Badeck and L. Cattivelli provided bulk tissue and soil samples from T. Durum FACE. G. M. Santos, A. Alexandre, and P. E. Reyerson interpreted the data and wrote the paper. All authors discussed the results and implications, and commented on the manuscript.

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

Name	Major contents	% C (mass)[a]	FmC	±1σ	^{14}C age	±1σ	$\delta^{13}C$ (‰)	±1σ
Miracle Gro® (MG)	*Sphagnum* Moss, Perlite, Compost, NH_4NO_3, $(NH_4)_3PO_4$, $Ca_3(PO_4)_2$, K_2SO_4	49.5 (n = 2)	1.0849 1.0123	0.0028 0.0028	−650[b] −95[b]	25 25	−26.1 −25	0.1 0.1
Greensand (GS)		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_3PO_4, HNO_3, K_2SO_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_2MoO_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_2PO_4, $MgSO_4$, $Ca(NO_3)_2$, KNO_3	–	–	–	–	–	–	–
Silica Blast (SB)[d]	Na_2SiO_3, K_2SiO_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 based on stable isotopic measurements. The remaining C pool is from marine carbonates. Nevertheless, the FmC ^{14}C values of the organic C and bulk fractions are similar, and are shown here as an average value. The $\delta^{13}C$ 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.

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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.

	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

In bold: main amendment.
[a] IG has a very low %C. Therefore, its C contribution to planters B and C after dilution into solution (e.g. ~ 0.02 g 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.

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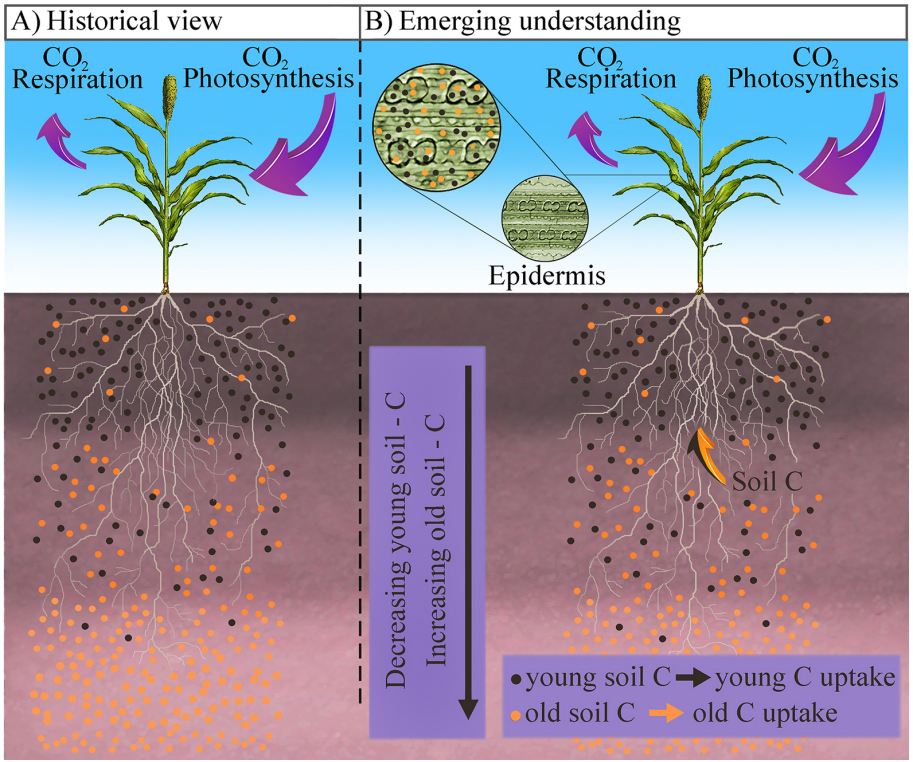

Figure 1. Sketch of **(a)** the conventional hypothesis of plant C occlusion during silica precipitation based solely on atmospheric CO_2 as a source, and **(b)** the emerging hypothesis of a dual origin (atmospheric CO_2 and SOM) for plant C (phytC). Young and old soil C in leaf epidermis and phytoliths (illustrated by the bilobate type) are represented by black and orange dots, respectively, in the microscope image.

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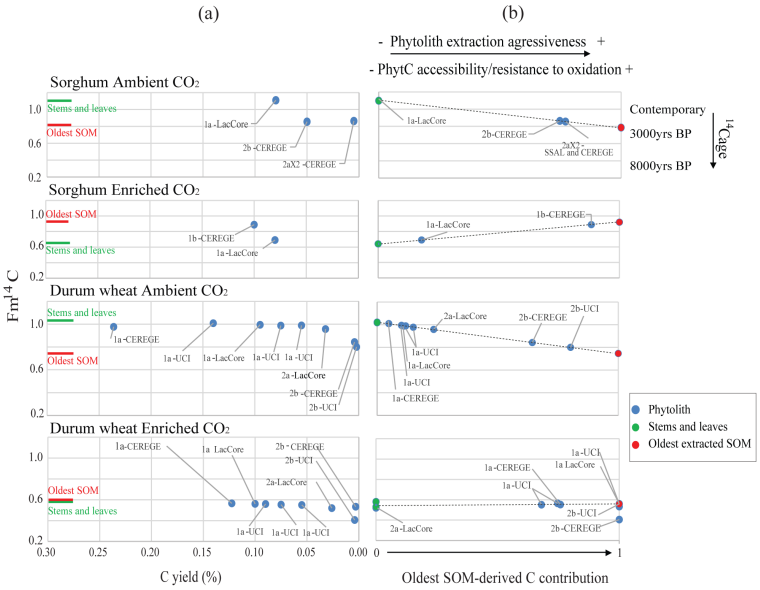

Phytolith extraction protocol

	Dominant steps	Characteristic steps	Silica dissolution
1a	Acid/Alkaline	KOH solution @pH 11	+
1b	Acid/alkaline	KOH solution @pH 14	++
2a	Acid/Combustion	6H 500°C combustion	-
2b	Acid/Combustion	12H 500°C combustion	-

Figure 2. Above ground C manipulation procedure. **(a)** Averaged $Fm^{14}C$ values vs. average C yields obtained for phytC. Averaged $Fm^{14}C$ values obtained for stems and leaves (SL) of origin and the oldest extracted SOM fraction are indicated. **(b)** PhytC expressed as a mixture between photosynthetically-derived C and the oldest extracted SOM fraction-derived C (oldest SOM-derived C contribution = $(Fm^{14}C_{SOM} - Fm^{14}C_{SL})/(Fm^{14}C_{phytC} - Fm^{14}C_{SL})$). Phytolith samples are labeled according to the extraction protocol (1a, 1b, 2a, 2b described in caption) used and the laboratory of extraction.

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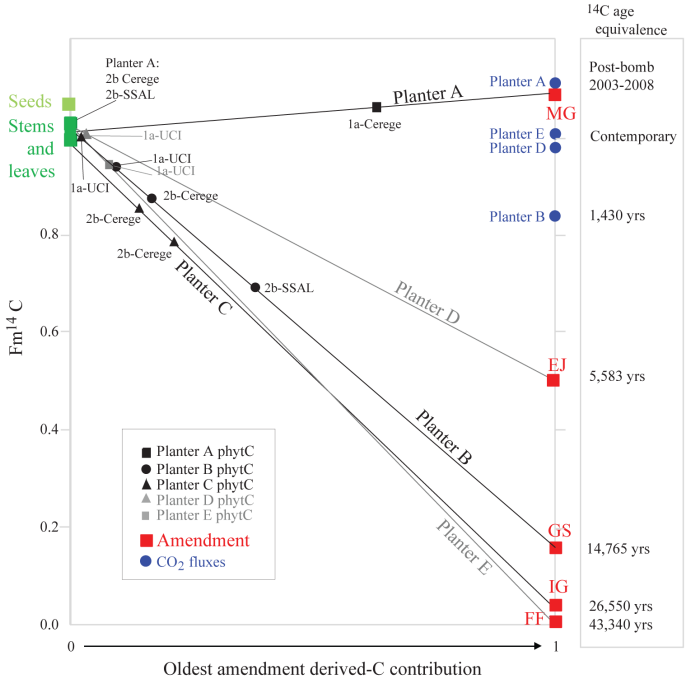

Figure 3. Below ground C manipulation procedure: PhytC expressed as a mixing between SL-derived C and the oldest extracted amendment-derived C (Am) (oldest amendment-derived C contribution = $(Fm^{14}C_{Am} - Fm^{14}C_{SL})/(Fm^{14}C_{phytC} - Fm^{14}C_{SL})$). $Fm^{14}C$ values of CO_2 fluxes are given for indication. 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.

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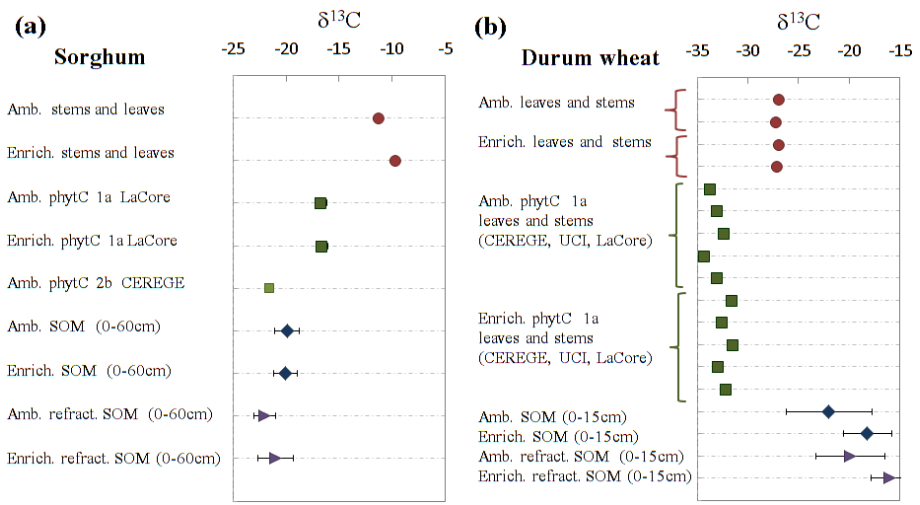

Figure 4. Above-ground C manipulation experiment. $\delta^{13}C$ values of stems and leaves, phytC, and soil SOM fractions obtained for a) sorghum and b) durum wheat experiments. To facilitate comparisons between groups, samples from ambient and enriched CO_2 plots are plotted next to each other. Values 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.

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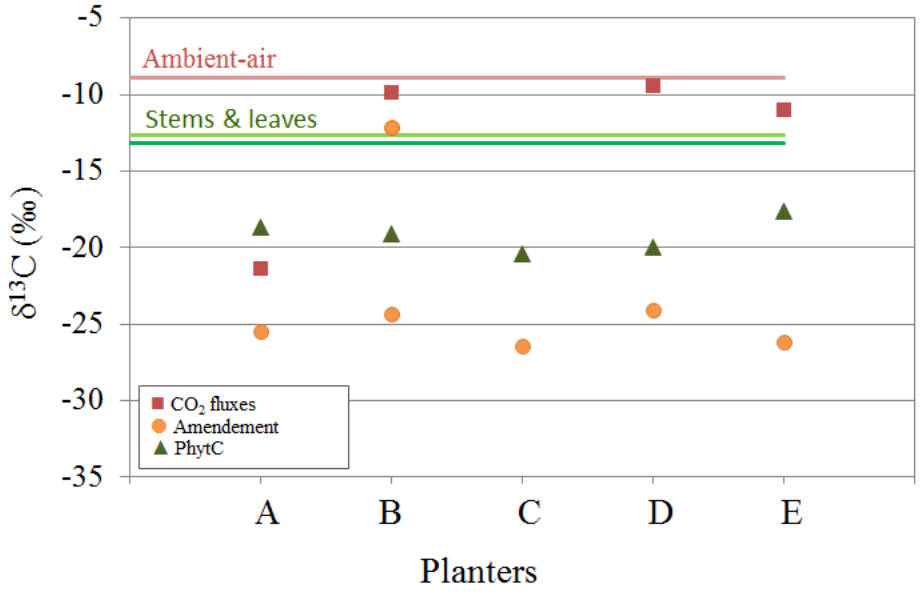

Figure 5. Below-ground C manipulation experiment. $\delta^{13}C$ values of the respired CO_2, stems and leaves, amendments and phytC for the five planters enriched in organic carbon nutrients (A–E). Values are reported as per mil (‰) related to PDB, and individual symbols represent single results as 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.

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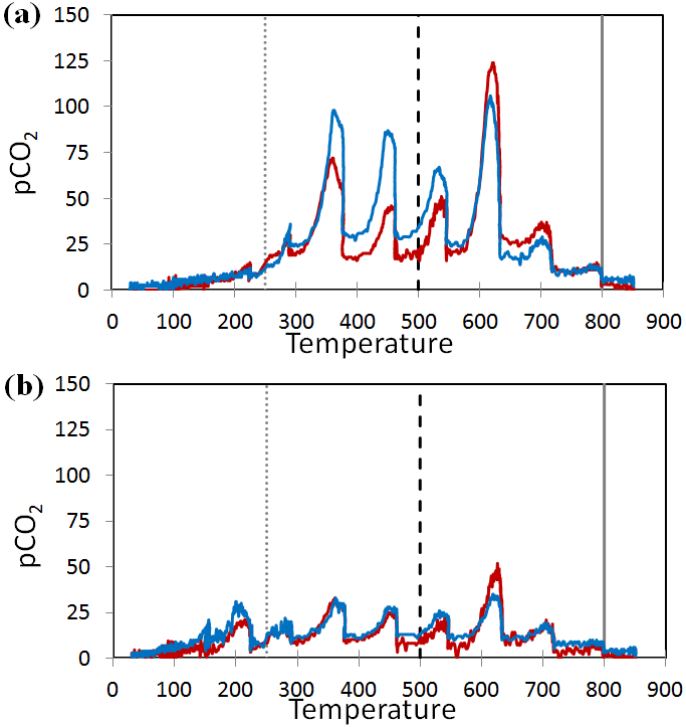

Figure 6. Thermograms (n = 2; blue and red lines) of phytoliths obtained from **(a)** durum wheat leaves, phytoliths extracted following protocol 1a (Table S2), and **(b)** soil phytoliths MSG70 extracted using a conventional protocol adapted to soil and sediment materials. Peaks are artifacts of the 100 °C temperature-step increments. Vertical lines indicate main temperature thresholds, as explained in text.

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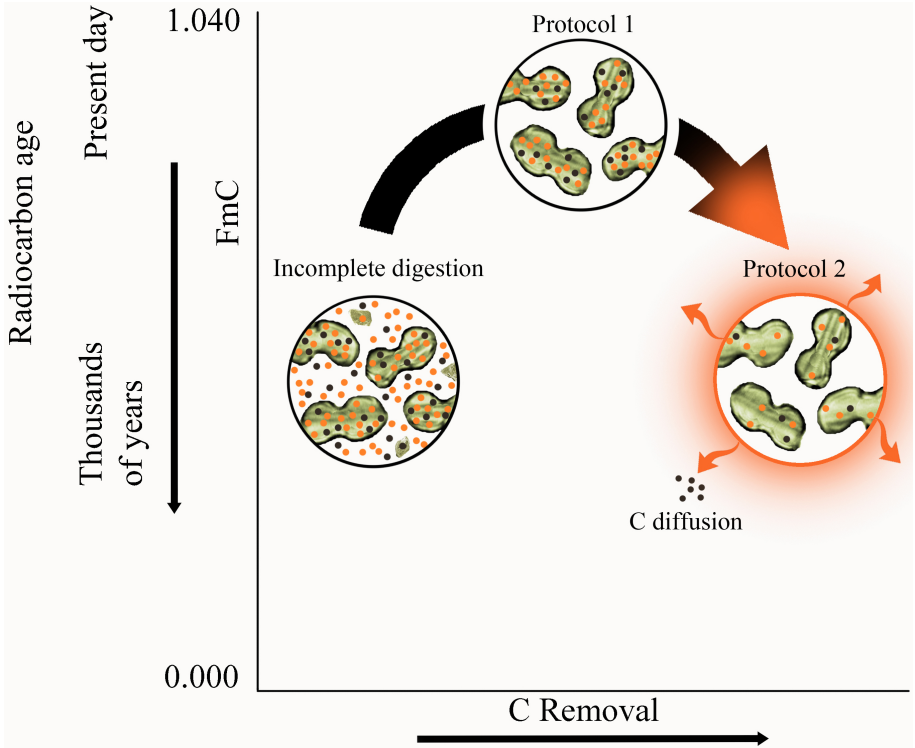

Figure 7. Conceptualization of the impact of phytolith extraction aggressiveness and C removal on ^{14}C age of phytoliths. Incomplete digestion leads to an accumulation of old SOM residues on phytolith extract surfaces. Protocol 1 removes all surface OM and preserves the dual source phytC signature. 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 Fig. 1b).

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