Unambiguous 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 several agriculturally important monocotyledonous species play a significant role in atmospheric CO2 sequestration. However, anomalous phytC radiocarbon (14C) dates suggested contributions from a non-photosynthetic source to phytC. Here we address this non-photosynthetic source hypothesis using comparative isotopic measurements (14C and 13C) of phytC, plant tissues, atmospheric CO2, and soil organic matter. State-of-the-art methods assured phytolith purity, while sequential stepwise-combustion revealed complex chemical-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 CO2. 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) (Fig. 1a). Thus, phytC isotopic signatures (δ13C and 14C) 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 (CO2) (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: CO2 biosequestration in grass
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 (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.

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 phytC) (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 plant tissues from which phytoliths originate have been published (Santos et al., 2010, 2012a, b, 2016; Sullivan and Parr, 2013; Yin et al., 2014; Piperno, 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) (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 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 data set 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 ($\delta^{13}C$ 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 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 multimethodology 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 (Sect. 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 (Sect. 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.entecri.it/research/durumFACE). 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$_2$, and therefore free of $^{14}$C (Leavitt, 1994; Ottman et al., 2001; Badeck et al., 2012). In terms of stable isotopic labeling, at the sorghum site the enriched CO$_2$ had a $\delta^{13}$C value of $-40\%_{o}$ from 1995 to 1998. This stronger isotopic label was obtained from a mixture of natural CO$_2$ from the Springerville, AZ, USA geologic wells with 15% petroleum-derived CO$_2$. During 1998–1999 only fossil hydrothermal CO$_2$ was used ($\delta^{13}$C = $-4.36\%_{o}$), while the background air $\delta^{13}$C was $-8\%_{o}$ (Leavitt et al., 2001). At the durum wheat site, the commercial fossil CO$_2$ from the Rapolano Terme, Poggio S. Cecilia (Tuscany) well had a $\delta^{13}$C of $-6.07\%_{o}$, which was slightly positive compared to the ambient CO$_2$ value of $-8\%_{o}$.

Two samples of mixed stems and leaves ($\sim 100$ g) were obtained from the sorghum site, while four separated samples (300–400 g each) of stems and leaves were collected at the durum wheat site. Eight soil samples ($\sim 5$ g each) collected from the furrows of the sorghum plots at depths of 0–15, 15–30, 30–45, and 45–60 cm were also obtained from the archives of the Laboratory of Tree-Ring Research, University of Arizona, USA. While two soil samples were collected from the ongoing durum wheat experimental plots at a depth of 0–15 cm ($\sim 15$ g each) during plant biomass harvesting.

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 Fm$^{14}$C values of 0.640 ($\sim 3.6$ kyr BP; $^{14}$C years before present or 1950; UCIAMS53273 and 53274; Table S1 in Supplement) and 0.556 ($\sim 4.7$ kyr BP; UCIAMS109000 and 109001; Table S2 in Supplement) at the sorghum and durum sites, respectively. Alternatively, 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 Fm$^{14}$C value of the bulk biomass harvested at the ambient CO$_2$ plot matched with the Fm$^{14}$C value of the CO$_2$ of the year of harvest (e.g., Fm$^{14}$C $= 1.097$, 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 nuclear weapon tests carried out during the 1950s and 1960s (Levin, 1997; Levin et al., 2013). The nuclear weapon tests doubled 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, however, the $^{14}$C signature of the biomass harvested at the ambient CO$_2$ plot was slightly depleted (Fm$^{14}$C $= 1.017$), as expected for CO$_2$ above urban areas in Europe in the early 2010s. For comparison, the $^{14}$C signature of atmospheric-clean CO$_2$ stations in Central Europe was Fm$^{14}$C $= 1.040$ in 2012 (Levin, 1997; Levin et al., 2013).

**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 40L planters (A, B, C, D, E and F) filled with mineral substrates. Five of the planters were enriched with 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, and to serve as a reference for the $^{14}$C signatures expected from plant tissue organics. Also, we isotopically measured commercial (sorghum) seeds to check if their $^{14}$C signatures were recent. Finally, CO$_2$ fluxes respired from the planter substrates were also sampled to evaluate their putative contribution to the phytC $^{14}$C signature. After 3.5 months the *Sorghum bicolor* plants (stem and leaf) were 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 ultrapure 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 (via organic compound oxidation and silica dissolution) were used to treat the samples from the above-ground C manipulation experiment (Fig. 2). The protocols have been previously described in detail by Corbineau et al. (2013). They are based either on acid digestion and alkali or on multi-step dry ashing and acid digestion. They are summarized below and in Fig. 2.

**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 h$^{-1}$, hot H$_2$SO$_4$ 24 h$^{-1}$ plus 30% H$_2$O$_2$ for 2–3 days, and > 65% HNO$_3$ plus 1 g KClO$_3$ for 24 h). This was followed by 30 min of immersion in KOH solution at pH 11 (protocol 1a) or pH 13 (protocol 1b). The KOH immersions allowed final removal of any alkali-soluble forms of organic compounds remaining on phytolith surfaces.

**Protocols 2a and 2b.** Plant samples were subjected to dry-ashing. Stepwise increases in temperature were used from 300 to 500 °C and the samples were then kept at 500°C for 6 h (protocol 2a) or 12 h (protocol 2b). Samples were then digested in a > 65% HNO$_3$ and 70% HClO$_4$ mixture (2:1).

In order to assess local $^{14}$C contamination during chemical extractions, four laboratories were involved in the extractions. They are UCI (USA), CEREGE (CNRS, Aix-Marseille University, 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). Aliquots of pre-baked (900 °C 3 h$^{-1}$) silicon dioxide powder (SiO$_2$, mesh# ~ 325, Sigma Aldrich, St. Louis, MO, USA) were chemically pretreated 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 (Sect. 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 min 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

Small particulate organic contamination of phytolith concentrates may considerably bias isotopic and quantitative analyses of phytC. The purity of the phytolith concentrates was thus 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...
Figure 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 extracted SOM fraction. Phytolith samples are labeled according to the extraction protocol (1a, 1b, 2a, 2b described in caption and in the text) used and the laboratory of extraction (UCI, CEREGE, LacCore and SSAL).

### Phytolith extraction protocol

<table>
<thead>
<tr>
<th>Dominant steps</th>
<th>Characteristic steps</th>
<th>Silica dissolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a Acid/Alkaline</td>
<td>KOH solution @pH 11</td>
<td>+</td>
</tr>
<tr>
<td>1b Acid/alkaline</td>
<td>KOH solution @pH 14</td>
<td>++</td>
</tr>
<tr>
<td>2a Acid/Combustion</td>
<td>6H 500°C combustion</td>
<td>-</td>
</tr>
<tr>
<td>2b Acid/Combustion</td>
<td>12H 500°C combustion</td>
<td>-</td>
</tr>
</tbody>
</table>

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) inter-
faced with a Fisons NA-1500NC (for solid materials) and a Gasbench II (for CO$_2$ input).

About 10 mg of phytoliths and 25 mg of soil were weighed out into pre-baked (100 °C per 2 h) 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 %e. 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 tis-
sue, 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 gases, 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, flasks 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 mg C 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 Fm$^{14}$C and when appropriate were discussed as ages.

### 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 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 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 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 mg C. 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 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 Sect. 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 0.24–0.06 % for the less aggressive protocols 1a and 1b and from 0.05 and 0.002 % for the more aggressive protocols 2a and 2b (Fig. 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 (Fig. 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 is:

$$\text{Oldest SOM-derived C contribution} = \frac{(\text{Fm}^{14}\text{C}_{\text{phytC}} - \text{Fm}^{14}\text{C}_{\text{SL}})}{(\text{Fm}^{14}\text{C}_{\text{SOM}} - \text{Fm}^{14}\text{C}_{\text{SL}})}$$

where the $^{14}$C signatures of the oldest SOM, stems and leaves (SL) and phytC are expressed as $\text{Fm}^{14}\text{C}_{\text{SOM}}$, $\text{Fm}^{14}\text{C}_{\text{SL}}$, and $\text{Fm}^{14}\text{C}_{\text{phytC}}$. $\text{Fm}^{14}\text{C}_{\text{phytC}}$ was expressed relative to $\text{Fm}^{14}\text{C}_{\text{SOM}}$ (assigned a contribution value of 1) and $\text{Fm}^{14}\text{C}_{\text{SL}}$ (assigned a contribution value of 0). The average $\text{Fm}^{14}\text{C}$ value of the oldest SOM-C fractions measured in each experiment (i.e., the $\text{Fm}^{14}\text{C}$ average value of the SOM 45–60 cm fraction for S. bicolor plots – Table S1 in Supplement, and the refractory 0–15 cm fraction for T. durum plots – Table S2) were used for $\text{Fm}^{14}\text{C}_{\text{SOM}}$.

The mixing curves associated with the SOM-derived C to phytC hypothesis are presented in Fig. 2b. The $\text{Fm}^{14}\text{C}$ 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 $\text{Fm}^{14}\text{C}$ values of the stems and leaves of origin, indicating that the soil pool still has remnants of $^{14}$C-labeled OC from the 1950s thermonuclear tests (Levin, 1997; Levin et al., 2013). In this case the SOM-derived C was assigned a contribution value of 0, and the stems and leaves a contribution value of 1 in Fig. 2b. Conversely, some of the phytC $^{14}$C values from the Durum Wheat Enriched CO$_2$ experiment, obtained using protocols 1a, 2a and 2b (UCIAMS123566, 123567, 125985, 130334 and 130335), were lower ($^{14}$C age older) than the $\text{Fm}^{14}\text{C}$ 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 (Schrumpf et al., 2013) may still be “younger” than present-day in terms of its $^{14}$C signatures, if the soil pool is still bearing some bomb-produced $^{14}$C OC, or much older if aromatic complexes are dominant (Telles et al., 2003; Torn et al., 2009). 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). Therefore, Fig. 2b clearly showed that the phytC $\text{Fm}^{14}\text{C}$ values unambiguously trend toward the $\text{Fm}^{14}\text{C}$ value (or $^{14}$C age) of the oldest SOM fraction. Overall, the crucial point to be noticed is that the phytC $^{14}$C offsets shifted linearly towards negative values if the oldest SOM fraction was older than the biomass of origin (Sorghum Ambient and Durum Wheat Ambient, Fig. 2a), and towards positive values when the oldest SOM fraction was younger (Sorghum Enriched, Fig. 2a). Thus, phytC $^{14}$C differences were clearly linked to the oldest SOM $^{14}$C ages. Moreover, the agreement in phytC $^{14}$C values obtained from stems and leaves indicated that the offsets were not linked to plant anatomy.

Regarding $^{13}$C values, the phytC offsets relative to the tissue of origin did not systematically trend towards SOM $^{13}$C values, except for the Sorghum Ambient phytC undergoing the 2b protocol ($-21.6 \pm 0.1$‰ ($n = 2$) as indicated in Fig. 3; UCIAMS95335 and 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 ($\sim 15.7$‰) than the difference between δ$^{13}$C values of the stems and leaves of origin (e.g., $\sim 5.6$ vs. $\sim 7.2$‰ for wheat and sorghum, respectively), as previously reported for grasses with C$_3$ and C$_4$ photosynthetic pathways (Webb and Longstaffe, 2000, 2010). 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, two of those from the same biomass samples (from Planters A and C), but subjected to different degrees of oxidation (e.g., protocol 2b). Other $^{14}$C results shown are from the stems/leaves, nutrients/fertilizers, and CO$_2$ extracted from 6 L flasks and flux chambers (Fig. 4). The complete set of isotopic results and sample processing details are tabulated in the Supplement (Tables S3).

Phytoliths produced phytC yields ranged from 0.08 to 0.1 $\%$ d wt when using the less aggressive protocol 1a and from 0.01–0.04 $\%$ 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 $\text{Fm}^{14}\text{C}$ values were again found in association with the carbon 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 2b), and 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 $^{14}C$ = 0; close to $\sim$ 43 kyr BP; $n$ = 3) (Tables 1 and 2). As in the above C manipulation experiment, in Fig. 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 6 L cylinders during the growing season, excluding any possibility that the phytC $^{14}C$ depletions are a product of urban fossil atmospheric $^{14}CO_2$ 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 (Fig. 4) to verify their recent radiocarbon activity (UCIAMS83120 and 83121; Table S3). As expected, once early-fixed photosynthetic $^{14}CO_2$ 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 re-fixation 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 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).

$\delta^{13}C$ offsets between phytC and stems and leaves were $\sim$ 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 (Fig. 5). Also in Fig. 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 (Czemzik et al., 2006). The results fell mostly between the air
Figure 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 1 and 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.

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.

This data set clearly shows that amendment-derived C, adsorbed through root plants, altered the phytC $^{14}$C signatures.

### 3.3 Thermal stability of phytC

Chemical compositional insights on carbonaceous materials can be obtained via oxidation reactivity to thermal treatments; such treatments have been frequently used on organic compounds from soils and sediments (Plante et al. 2011, 2013; Rosenheim et al., 2013). For instance, single-bonded carbon structures usually show a lower thermal stability than those dominated by double bonds, such as conjugated and aromatic structures (Harvey et al., 2012). Here, we make use of the same chemical-thermal stability concept to evaluate the heterogeneity of phytC in reacting to heat treatments.

Thermograms obtained from phytoliths of the durum wheat leaves and fossil phytoliths (MSG70) indicated a continuum of phytC CO$_2$ with different degrees of resistance.
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.

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

Phytoliths typically melt at $\sim$573°C (Deer et al., 1992), but embedded metals (e.g., Al, Fe) within their structures could lead to a decrease in temperature stability (Wu et al., 2014). Nevertheless, phytC that required much higher temperatures to fully oxidize (e.g., beyond of the phytolith melting point of 573°C) would be placed at the upper end of the carbon recalcitrance continuum. (Cheng et al., 2013; Harvey et al., 2012; Plante et al., 2005, 2011, 2013). Furthermore, even if char occurred during combustion leading to some elemental carbon formation, it does not explain the Fm$^{14}$C phyC discrepancies obtained here (Figs. 2 and 4) 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) also heated phytolith aliquots from a single extract, and observed shifts in $^{14}$C ages towards older values. This effect is similar to that observed in total carbon or SOM distributions in soils and sediments when subject to thermal decomposability (Plante et al., 2011, 2013). Thus, phytolith extractions that employ heat treatments would better isolate the oldest soil C fraction within phytoliths, as previously found (in Sects. 3.1. and 3.2). Basically, if the C pool in phytoliths is supposedly homogeneously and from a single source (atmospheric CO$_2$), the $^{14}$C results from all CO$_2$ temperature-fractions should be in absolute agreement, as Fernandez et al. (2015) demonstrated by subjecting carbonaceous materials to ramp pyrolysis and subsequently measuring them by $^{14}$C-AMS.

3.4 The SOM-derived C to phytC hypothesis set of evidence

Results from both above- and below-ground experiments showed that the $^{14}$C offsets between phytC and stems and leaves pointed toward the oldest SOM $^{14}$C values (Figs. 2 and 4). This confirmed that a fraction of the old SOM-derived C occluded in phytoliths was more resistant (or less accessible) to oxidation than the occluded C derived from recent photosynthesis or from recent SOM. Once the most labile (or more accessible) C had been removed, the older and more resistant carbon fraction became dominant. 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 (Fig. 6).

Our findings also imply that a portion of SOM-derived C is absorbed by the roots, transferred to the stems and leaves, and finally occluded into phytoliths. In the bulk plant organs,
the old SOM-derived C amount is far too small to be $^{14}$C detected in tissue clippings, as it is masked by the large amounts of photosynthetic atmospheric carbon tissue (bulk stems and leaves averaged $\sim 41$ % carbon; Tables S1–S3). On the other hand, in phytoliths, the old SOM-derived C becomes over-represented when the most labile-accessible phytC starts to be oxidized. It should be noted that 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 C that may have been absorbed. These drawbacks prevent precise quantification of the old SOM (probably diluted by the young SOM)-derived C contribution to phytC. The impossibility of quantifying precisely the amounts of soil C and associated $^{14}$C signatures in phytC precludes application of any correction that would allow phytC to be used as a reliable dating material. As in any other heterogeneous carbon pool, the phytC continuum can be similarly partitioned differently by distinctive chemical extractions. For instance, in Piperno (2016) the entire data set of post-bomb Neotropical plant phytolith extracts were neither accurate nor precise. While $^{14}$C offsets reached discrepancies as high as 4.4 kyr between expected calendar ages and phytC, two pairs of phytolith extracts obtained by distinct chemical treatments (sulfuric vs. nitric) yielded a 50 % reproducibility rate (Table 1, Santos et al., 2016).

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 microscopic internal cavities and within the silica network. Rapid opening of internal cavities during the dissolution 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 heterogeneity of phytC accessibility to oxidation.

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

Our experiments and data set 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 would trend in a single direction, rather than being both positive and negative (Figs. 2b and 4). In addition, aliquots of SiO$_2$ blank yielded $^{13}$C values in close agreement with the expected results, giving no indication of the presence of unusual contaminants. Second, natural- or spectrometer-produced anomalous $^8$C shifts of phytC were not observed here (Figs. 3 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 (Sect. 3.2). 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) (Corbíneau et al., 2013), a method superior to microscopic evaluation alone (Figs. S1 and S2 in Supplement) (Kameník et al., 2013; Santos et al., 2012a). Moreover, our extracts were consistently reproducible regarding phytC yields across all labs involved (Tables S1–S3) and thermal decomposability properties (Fig. 6). Since it has been established that plants do not photosynthesize all carbon found within their tissues (details in Sect. 3.8), 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.

3.6 Implications for the use of phytC as a proxy of plant C

Since plants (and more specifically phytoliths) contain a broad continuum of C with a complex mixture of chemical compounds of different turnover times as evidenced here (Figs. 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 (Fig. 7).

While pure phytoliths produced from a less aggressive protocol (e.g., 1a) may minimize $^{14}$C offsets to some de-
gree, 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, 2012a, 2016; Sullivan and Parr, 2013; Piperno, 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 (Fig. 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 kyr 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 in phytolith concentrates (Fig. 2 in Santos et al., 2012a), thus demonstrating that even very small amounts of particulate 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 kyr (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 ranging from several decades to 4.4 kyr (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 concentrations 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 in the light of the present findings.

### 3.7 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, 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 (Kamenik 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), and 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 4.1 × 10⁹ tC yr⁻¹, which is roughly one hundred times lower than the 3.7 × 10⁶ tC yr⁻¹ value reported elsewhere (Song et al., 2014 and references therein). This amount is insignificant when compared to the 2.6 × 10⁹ tC yr⁻¹ estimate for the land C sink (IPCC, 2007), or to the 0.4 × 10⁹ tC 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.

3.8 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 1–3% of total leaf-fixed CO₂ (Foord et al., 2007; Ubierna et al., 2009). However, as DIC is expected to be in equilibrium with soil CO₂ respired from autotrophic and heterotrophic sources, its ¹⁴C signature should reflect an average of SOM ¹⁴C signatures, close to contemporary. Assuming soil DIC as the soil end-member in Fig. 2, the phytC samples from ambient CO₂ experiments would plot along mixing lines with lower slopes than the actual ones. The ¹³C age of several thousand years systematically measured for the most resistant phytC, rather suggests that an older SOM fraction supplies the SOM-derived C absorbed 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 the detection in roots, stems and shoots of polycyclic aromatic hydrocarbons (PAH) (Gao et al., 2010; Yu et al., 2013), and soil amino acids (AA) (Puafgfo-Lonhienne et al., 2008; Warren, 2012; Whiteside et al., 2009, 2012). Although reported PAH concentrations were three orders of magnitude below phytC concentrations (e.g., 10⁻⁵ g g⁻¹ 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 a percent 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 belowground physical, chemical and biological processes in the rhizosphere (e.g., microbial priming effect or N and C cycle interactions) have also been proposed (Heimann and Reichenstein, 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). Common sources of dissolved Si for plants are clay minerals and amorphous silicates (allophane, imogolite). Due to 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). Further studies are needed to investigate whether dissolution of Si-bearing forms during active uptake of Si (Ma et al., 2006) may also promote old SOM mobilization, ready to be chelated with Si, absorbed by the roots and translocated to the stems and leaves.

4 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 ¹⁴C ages found in the literature. Moreover, 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 CO₂. Revisited estimates of atmospheric CO₂ biosequestration by phytoliths led to values that are insignificant compared to the total land C or soil C sinks. All in all, 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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