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New highlights on phytolith structure and occluded carbon location: 3-D X-ray microscopy and NanoSIMS results

Dear Dr. Jack Middelburg, BG Editor,

Please, find below the list of changes that were made in the revised manuscript. We tried to take into account all referees'comments. Changes were underlined in grey in the main text and abstract files.

Sincerely yours,

Anne Alexandre

Response to Anonymous Referee #1

... I do not completely agree with the authors that they provide a big step forward in the understanding of big discrepancies in the phytC pool. These discrepancies have been linked to different location of carbon in the phytolith structure, e.g. in cavities and in holes on the surface of the phytolith. Authors using microwave digestion to remove organic matrices have claimed that underestimates result from using aggressive dissolution protocols for isolating the phytoliths (see this paper and references therein). As I understand from the results and discussion in this paper, cavities and holes have been filled with air and/or epoxy resin during the applied procedures, making it impossible to quantify or qualify a cavity related carbon pool. While the authors acknowledge this throughout the paper, they still conclude that they provide new discussion material for these discrepancies, to re-assess the paleo-environmental meaning of the phytC. I think this conclusion is overly strong: the authors should refrain more to the conclusions they can perfectly draw from their research, on the distribution of C withinthe silica structure.

In the revised manuscript, we further distinguished conclusions, clearly supported by the SEM, 3D-Xray microscopy and NanoSIMS data, from suggestions we made for reliable assessments of the significance of phytC in atmospheric CO_2 sequestration as follows:

In the discussion section we changed L330: "At the end of the cell silicification, residual cell organic compounds that were not already occluded may gather in a remaining space and delimitate the micrometric central cavities. This second pool of phytC should be rapidly oxidized when phytoliths start to dissolve after their deposition in litter, soil or sediment (fig.09). This suggests that this phytC pool participates in a limited extent to long term atmospheric CO_2 sequestration. These considerations rise the need to further estimate the respective contributions to C contents measured from bulk phytolith concentrates of (i) phytC in the silica structure, of (ii) phytC in the central cavities, and of (iii) extraneous C that may

remain on porous phytolith surfaces. This is a prerequisite for reliable assessment of the significance of phytC in atmospheric CO_2 sequestration."

In the conclusion and abstract sections we changed, L35 and L364: These findings constitute a basis to further characterize the origin, occlusion process, nature and accessibility of phytC, necessary for assessing its significance in the global C cycle."

They should provide discussion on how this approach can be improved to allow better understanding of cavity related carbon pools, rather than current, not-fully-supported, generalities like "These findings provide strong bases necessary to further characterize the nature, occlusion process, accessibility and origin of phytC.They also should help to reappraise the significance of phytC in the global C cycleand reassess the paleo-environmental meaning of phytolith features observed by NL microcopy".

In the discussion section we added L338: "For that purpose, phytC contents measured from phytolith assemblages characterized by 3D X-Ray microscopy as dominated by phytoliths with closed internal cavities or by phytoliths with open cavities should be compared."

Further, the authors should better describe the amount of microscopical analyses they

have performed, and whether conclusions could be generalized among all observations.

SEM, 3D-Xray microscopy and NanoSIMS images illustrated the main features commonly observed on Grass Short Cell phytolith types. The extent of these observations was reported in the "Material and method" and "Results" sections of the modified manuscript as follows:

L187: Three morphological categories of phytoliths, commonly found in grasses, constituted the bulk sample. SEM pictures of phytoliths placed on the aluminum mount illustrate these categories on figure 01. SEM pictures of cross sections of the same categories are shown on figure 02.

L219: Two examples of reconstructed 3D X-ray microscopy volumes are presented in figures 04 and 05. The observed patterns were common to the five analyzed GSC particles.

L231:The NanoSIMS results, common to the dozens of analyzed phytolith thin sections, are illustrated in Figures 06, 07 and 08.

However one may notice that each volume reconstruction from 3D-Xray microscopy required more than 20 hours of analyze which limited the number of particles that can be visualized (5 in the present study). This was precised in the revised manuscript, L124:

A few phytoliths, randomly selected from the bulk sample, were deposited on the inner surface of a bevel-cut Kepton tube of 50µm of internal diameter. Five individual GSC phytoliths were recognized by optical microscopy at 200X magnification and their position located for 3D visualization.

and L140: After 20 hours of analysis, reconstruction of the phytolith volume was performed using XMReconstructor

- The authors mention in the introduction that aggressive chemical methods could remove some phytC hypothesized to exist on the surface of phytoliths. How can they test whether phytC persists in holes on the phytolith surface, as earlier hypothesized, if they used aggressive extraction methods?

Precision was given on that matter in the introduction section as follows:

L77: Chemical extractions leading to high purity phytolith concentrates are indeed difficult to implement. Although the absence of organic particles can be checked by Scanning Electron Microscopy (SEM) coupled with EDX (Corbineau et al., 2013), the presence of extraneous organic remains on the phytolith surface cannot be accurately detected.

On page 14707, the authors mention such cavities open to the surface, filled with air. If the cavities connect to the surface, can epoxy resin be incorporated in the cavities? The authors even mention this in the last part of the results section. How can they then discuss that they can quantify potential Si in cavities by comparing fossil to recent phytoliths? Can the C from the cavities not have been lost during the extraction?

There may be a misunderstanding here. As we wrote in the result section, C in the central cavities is indentical as C in the epoxy resin, L248: Carbon was present in the cavities and in the silica structure itself. However when values of $[^{12}C]^-$ intensity were similar in the cavities and in the Epoxy resin, they were 10 to 20 times lower in the silica structure than in the Epoxy resin (fig06, 07).

The approach we propose is further described in the revised section:

L338: For that purpose phytC contents measured from phytolith assemblages characterized by 3D X-Ray microscopy as dominated by phytoliths with closed internal cavities or by phytoliths with open cavities should be compared.

- It is currently impossible to assess how many microscope pictures were taken, and how representative the samples from the Triticum durum were. How representative are the microscopical analysis for the sampled leaves? Why was this plant species chosen? How many leaves were used for the phytolith extraction? How many replicates of extraction? How many replicate pictures? Was their variability among observed patterns across (assumed) replicates?

This was precised in the revised version.

L109: Hundreds grams were made available to us for phytC investigation. Phytoliths were extracted from 50g of dry leaves

L119: Several NL microscopy and SEM pictures, illustrating the composition of the TD-F-L phytolith assemblage, were taken. For the purpose of morphological comparison, pictures of fossil GSC and bulliform phytoliths from available soil assemblages described in previous papers, were additionally taken.

L124: A few phytoliths, randomly selected from the bulk sample, were deposited on the inner surface of a bevel-cut Kepton tube of 50µm of internal diameter. Five individual GSC phytoliths were recognized by optical microscopy at 200X magnification and their position located for 3D visualization.

L143: NanoSIMS analyses were performed on cross sections of TD-F-L phytoliths embedded in epoxy resin.

L149: Embedded samples were taken off the tubes and polished with diamond paste up to 0.1 µm, until the PTFE filter was completely removed and cross sections of phytoliths were visible in NL microscopy.

L151: Dozens of GSC phytoliths cross sections to be analyzed with the nanoSIMS were located by SEM.

L187: Three morphological categories of phytoliths, commonly found in grasses, constituted the bulk sample. SEM pictures of phytoliths placed on the aluminum mount illustrate these categories on figure 01. SEM pictures of cross sections of the same categories are shown on figure 02.

Minor comments

14706, line 13: wide, not width? Corrected

14707, line 15: the structure Corrected

14700, Line 24: take up. Corrected

14701, line 5: Upon plant decay, or when plants decay. Corrected

14701, line 25: remove bracket. Corrected

14701, line 26: taken up. Corrected

14703, line 2: measurements Corrected

Response to Anonymous Referee #1

We tried to be as exhaustive as possible when considering the related works.

The references list was checked carefully. Laue et al. is 2007. That was corrected.