Answer to comments from referee #1 and #2.

We thank referees #1 and #2 (J. Rasmussen) for their critical but fruitful comments. We fully agree that several limits associated to our approach should minor our conclusions. Nevertheless, when compared with already published studies on organic ¹³C and ¹⁵N labels supplied to roots, our work contributes (i) to quantify the fate of assimilated carbon (C) through compound-specific ¹³C analysis of plant material, revealing that C absorbed by the roots under the form of intact amino-acid is minor but not nil and that part of it is fixed in roots, stems and leaves, in agreement with Sauheitl et al., 2009; (ii) to quantify the allocation of C and N absorbed by the roots to phytolith-occluded organic matter. We propose therefore to modify the manuscript to further focus on those two conclusions and take into consideration all the referees' comments.

Considering the major comments from referees #1 and #2, we will take them into account in a new version of the manuscript as follows:

This study only set up two replicates which makes the uncertainties calculated on the labeled C concentrations (table 1) subject to caution. The low number of replicates (two to four) is a major weakness of the study. The low number of replicates was a compromise basically constrained by the large amount of matter required to isolate phytolith-occluded C, the experimental/analysis effort and the cost of the experiment. The standard deviations calculated on the two replicates are very narrow. However, in order to strengthen our interpretation of the data, we will evaluate confidence intervals of the mean values and discuss the data within these confidence intervals.

The authors refers in several places to "old soil C" and "microbial metabolites" but there is no justification given how the amino acids used make a fair representation of old soil C or microbial metabilites. We fully agree with this comment. Although evidences of old, soil-derived C contribution to phytolith occluded-C (Reverson et al., 2015) participated to the initiation of the present study, it should not be referred to in the discussion as it may bring confusion. We will drop all considerations on this aspect for further clarity.

This study used a hydroponic experiment, resulting in a completely different rhizospheric environment than in soils. We fully agree that our experiment doesn't mimic the soil environment, mainly because of the absence of complex soil organic matter, and different nutrient availability for plants. This will be further discussed in a new version of the manuscript.

Uptake of intact amino acids could not be quantified. /The authors make no justification of the extent of intact amino acid uptake from the labeling solution. The present labeling experiment does not allow to precisely trace the molecular form under which the amino acid derived-¹³C and amino acid derived-¹⁵N were absorbed and fixed in roots, stems and leaves, as recently done using a position-specific C and N labeling technique (Moran-Zuloaga et al., 2015). However, the fact that phenylalanine and methionine, that were supplied to the nutritive solution, were significantly more ¹³C-enriched than other amino acids in the roots and leaves (Fig. 4) evidences that part of amino acid derived-¹³C was absorbed and translocated in its original phenylalanine and methionine (or ring-C) forms.

Both ¹⁵N and ¹³C can be taken up in their inorganic forms. Uptake of ¹⁵N in its inorganic form was expected, and is clearly evidenced in our dual-label experiment. Regarding ¹³C, we also agree that inorganic ¹³C probably contributed to the uptake of ¹³C by the grass (Rasmussen et al., 2010) and participated to anaplerotic or photosynthetic C fixation. We will further discuss this aspect on the basis of compound-specific amino acid derived-¹³C vs bulk ¹³C data in roots, stems and leaves in a new version of the manuscript.

The form in which AA-13C, and most generally phytC, has been occluded in the silica structure remains unknown. We fully agree with this comment. Further investigations, including the use of spectroscopies relevant for characterizing phytC at the molecular level, are necessary (and we are currently working on that matter, but this is not in the scope of the present paper) to characterize the forms under which C compounds are occluded in phytoliths.

References

- Moran-Zuloaga, D., Dippold, M., Glaser, B., Kuzyakov, Y., 2015. Organic nitrogen uptake by plants: reevaluation by position-specific labeling of amino acids. Biogeochemistry 125, 359–374. doi:10.1007/s10533-015-0130-3
- Rasmussen, J., Sauheitl, L., Eriksen, J., Kuzyakov, Y., 2010. Plant uptake of dual-labeled organic N biased by inorganic C uptake: Results of a triple labeling study. Soil Biol. Biochem. 42, 524–527. doi:10.1016/j.soilbio.2009.11.032
- Reyerson, P.E., Alexandre, A., Harutyunyan, A., Corbineau, R., Martinez De La Torre, H.A., Badeck, F., Cattivelli, L., Santos, G.M., 2015. Evidence of old soil carbon in grass biosilica particles. Biogeosciences Discuss 12, 15369–15410. doi:10.5194/bgd-12-15369-2015
- Sauheitl, L., Glaser, B., Weigelt, A., 2009. Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environ. Exp. Bot. 66, 145–152. doi:10.1016/j.envexpbot.2009.03.009