Plant and Soil

Limited carbon inputs from plants into soils in arid ecosystems: a study of changes in the delta-13C in the soil-root interface

--Manuscript Draft--

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Abstract:	Background and aims. The tracing of C assimilation and the subsequent partitioning among plant organs has been a central focus of studies utilising Free Air CO2 Enrichment (FACE) facilities. The approach makes use of the fossil origin of this carbon, which is depleted in 13C. However, there is little data for desert environments. The Nevada Desert FACE Facility (NDFF), located in the Mojave Desert, has been one of the main facilities for the study of C dynamics in arid ecosystems and how they respond to rising atmospheric CO2 concentrations. In this experiment, we studied the incorporation of fixed CO2 during the previous two years (detectable by its lower 13C) in the soil fraction surrounding roots. Methods. The soil was collected monthly in direct vicinity to the roots during a complete growth season, at two depths (5 and 15 cm). Soil samples were dried and fractionated by size (> 50 µm and < 50 µm) by wet sieving, and both size fractions were then analysed for the d13C of their organic matter and their carbonates. Results. In the coarse fraction (> 50 µm), d13C values ranged between –1 and –2‰ for carbonates and between –23 and –25‰ for soil organic matter. These values did not significantly change throughout the experiment and were not affected by depth (5 or 15 cm). In contrast, d13C values for both organic and inorganic carbon in the fine fraction (< 50 µm) were much more variable than in the coarse fraction (> 50 µm). The d13C values for organic C ranged mostly between –20‰ and –27‰, and were roughly maintained throughout the sampling period. For inorganic C, the d13C values were mostly between 0‰ and –15‰, and tended to become less negative during the course of the sampling period. Overall the effect of [CO2] on d13C values of either organic or inorganic carbon was not significant for any experimental condition (plant species, depth, fraction).

	Conclusion. Little or no signs of recently fixed CO2 (13C-depleted) were detected in the soils close to the roots, in the coarse fraction (> 50 µm), the fine fraction (< 50 µm), the organic matter, or in carbonates. This indicates a slow C turnover in the studied soils, which can result from a highly conservative use of photoassimilates by plants, including a very low release of organic matter into the soil in the form of dead roots or root exudates, and from a conservative use of available C reserves.
Response to Reviewers:	The 'responses to reviewers' have been uploaded as a separate file.

From: Pere Rovira
Forest Sciences Centre of Catalonia (CTFC)
Solsona
Spain

June 11, 2018

Dear Sirs,

Herewith, we send you the corrected version of our paper 'Limited carbon inputs from plants into soils in arid ecosystems: a study of changes in the $\delta^{13}C$ in the soil-root interface', which we want to submit for your consideration, to be published in **Plant and Soil.**

We took into consideration all criticisms of the two reviewers. Following the criticism of reviewer #2, somewhat surprised by our short 'Results' section, in this new version we give substantially more information. Specifically, we give data about total OC and IC, not given in the previous version. However, as a result of the need of properly comment all these additional results, the paper has suffered substantial changes: the 'Results' section has been almost completely rewritten, and the 'Discussion' has been notably enlarged. The main conclusions do not change, however.

We sincerely expect you will consider this second version as a substantial improvement of the previous one. At any rate, owing to the big changes in the manuscript, we assume that a new round of revision will be necessary, before you consider the paper good enough to be published in **Plant and Soil**.

Yours sincerely,

PERE ROVIRA

(on behalf of all authors)

COMMENTS FOR THE AUTHOR:

Please check online for possible reviewer attachments.

Editor Comments: I have received two reviews, which consider that this manuscript is an valuable contribution. Both reviewers have a number of suggestions to improve the manuscript and once these have been incorporated the manuscript will be assessed again for publication.

GENERAL RESPONSE (for the editor and the two reviewers)

Dear Sirs,

We sincerely acknowledge your efforts as reviewers and your criticisms about our work. Below we answer all your queries. Nevertheless, there are some additional comments to do.

Reviewer #2 was a bit surprised about the short 'Results' section. In our previous version of this paper we wanted to focus on the isotopic compositions, avoiding a too long description of our dataset. Nevertheless, eventually we agreed that it was worth to give a larger set of results, for some of them could be interesting for readers.

Thus, we added a more detailed description of our data, more specifically the total OC and IC contents of the obtained fractions. Owing to the need of making our previous texts consistent with these new data, we decided to rewrite the 'Results' section, almost completely.

The Tables, which in the previous version were put erroneously as 'Supplementary material', have been moved to the main corpse of the paper. In addition, all but one (table 1) have been changed and re-designed.

Additional statistical information has been given as 'Supplementary material'.

Three new figures have been added. To compensate for this, the last figure of the old version (Figure 4) has been suppressed.

Also, substantial additions have been made to the 'Discussion', in order to include the newly added information. Some of these data add some nuances to our previous conclusions, but they do not substantially alter the main ones.

Thus the paper has considerably changed, and the reviewers will probably add new substantial comments and criticisms, not just add some comments to the previous ones. We assume that a new round of revision will be necessary.

We acknowledge, at any rate, the detailed and extensive comments made by both reviewers, which helped us to substantially redesign our paper.

Yours sincerely,

Pere Rovira
(on behalf of all co-authors)

Reviewer #1: Manuscript number: PLSO-D-17-01573

Title: Limited carbon inputs from plants into soils in arid ecosystems: a study of changes in the delta-13C in the soil-root interface

General comment:

This study assessed the incorporation of recent root derived C in the soil surrounding the roots, employing a 13C tracer approach using a FACE system. In contrast with most 13C tracer studies, this study considered not only the organic C pool in soil, but also carbonates in soil. In addition, the soil was analysed targeting root debris derived-C and exudate derived-C separately by studying the coarse soil fraction (>50 ?m) and the fine soil fraction (<50 ?m), respectively. In combination, this experimental design is novel. The findings of this study also adds to our understanding of the contribution of root derived-C in soil in desert plants. The article is well structured and well written, and is suitable for Plant and Soil. However, the authors should address a few issues listed in specific comments below.

Specific comments:

L157-164: Although both plants are shrubs, the authors should consider adding text to provide a brief description of their root systems (whether similar or different), such as rooting depth that may be important for understanding the depth data in L248-254.

Unfortunately we do not have this information. For the sampling, we took advantage of an excavated trench and we took samples of the soil around the visible roots. Several parameters about the root systems were investigated (and published later: Clark et al 2010), but the rooting depth was not investigated then. Thus we do not have this information. We agree with you that having this information would be of help in our interpretations.

L190-194: The article does not describe how the soil in close contact with the roots was sampled. I suggest this information should be included here, considering that the method of collecting the soil surrounding roots is crucial to account for the recent root derived C particularly exudate C. For instance, collecting the soil surrounding roots by washing and subsequently decanting excess water may lead to loss of exudate C and thus underestimation of the root derived C in soil.

The soil around the roots was collected manually, with a spatula, and put immediately in a plastic vessel. No water leaching occurred at the sampling. This detail has been added to the text.

L209-211: If the separate trials that were used to verify the effectiveness of the method for analysis of 13C signature of carbonates are published work, the authors should include the citations here.

No, the trials were done in our laboratory (University of Barcelona), for a previous work (Rovira & Vallejo 2008, Geoderma, mentioned in the 'References' section) in which this problem was also settled, and we needed to verify that the method was fine, i.e., that calcination does not affect carbonate amount nor isotopic composition. We did not publish these results.

L240: Change "results" to "resulted".

Done.

L268: Remove the comma from "was not significant, either" to read "was not significant either".

Done.

L291: Change "derived from" to "was derived from".

Done.

L376: Change "affect" to "effect".

Done. Sorry, it was a stupid error.

L401: Change "(Evans et al. (2014)" to "(Evans et al. 2014)".

Done.

L423: Change "thanks" to "thank".

Done.

Fig. 1 caption: "the 3d harvest" should be "the 3rd harvest".

Done.

Figs. 2 and 3 captions: "soil depth" should be changed to "soil depths".

Done.

Fig. 4 caption: "in coarse (> 50 μm) and fine fraction (< 50 μm)" should be changed to "in coarse (> 50 μm) and fine (< 50 μm) fractions".

Done.

Figs. 1 to 4: Consider indicating that 'Harvest numbers' on the horizontal axes of all four figures represent monthly samplings, also quoting the months (such as April-August). This information can be added to the captions.

The months have been added to the abscissa axes. We consider that the letters ‘A M J J A’ are obvious enough. Note also that the design of all figures has changed (and, we expect, improved).

Tables 1 to 4: It looks like all four tables have been submitted as Supplementary material, which is fine. However, consider making it clear in the text; for example, by writing "Table S1" instead of "Table 1" in L239.

Putting the tables as ‘supplementary material’ was an error when uploading the files. Tables should be a part of the main text. In this corrected version, tables are no longer ‘supplementary material’. We apologize for our mistake.

Note also that substantial changes in the tables have been made. And the information we give in them, too. Now we consider they give more relevant information.

Note also, finally, that in spite of all this, we added a ‘Supplementary Material’, with additional statistical information. We did it like this in order to avoid an overwhelming statistical data, which could distract readers from the core of the paper. However, we give this information because we suppose that some readers may be interested in specific points about our results.

We sincerely acknowledge your effort and your interest in our paper.

Reviewer #2: Review of the ms:

Limited carbon inputs from plants into soils in arid ecosystems: a study of changes in the delta-13C in the soil-root interface submitted by Pere Rovira, Iker Aranjuelo, Robert S. Nowak, Salvador Nogués for Plant and Soil

The ms presents an important study of the incorporation of C from roots and rhizodeposition into organic and inorganic C under elevated CO2 provided in FACE in an arid ecosystem. There are only very few FACE experiments in the arid ecosystems, and the results from them were published extremely seldom. So, despite the tradeoff in plants between water losses and CO2 assimilation is especially extreme under arid conditions, we have nearly no studies in these ecosystems under elevated CO2. The other advantage of the study is that not only the organic C was investigated (as nearly in all other studies), but also incorporation of 13C from roots into incorganic C war traced. To my knowledge, this is the first FACE study considered the incorporation of C from roots into CaCO3.

The authors showed that already after 2 years of FACE application it is possible to trace the root-derived C in soil, but not in the bulk soil - only in the fine fraction.

Nevertheless, I recommend very strong revision and various additional calculations that will strongly increase the quality and the resonance of the paper.

General comments

- For the papers based on d13C natural abundance or very low enrichment / depletion of 13C, the words "increase" or "decrease" or "higher" or "lower" - are always not clear. So, write better: got more negative or less negative or were more / less depleted. e.g. L41: the value -15‰ is actually more negative than the control, but you write "higher" - not really clear what you mean.

You are right that we must be careful at this point. The text has been revised according to your advice. Sometimes we added small texts ('more negative', 'less negative') to avoid any confusion when we say 'higher' or 'lower'. Nevertheless, we did not detect any error; actually we consider that the text was very clear at this point.

- Keywords are missing

Keywords added.

- The authors provided nice data about the changes of d13C - all Figures present this in various C pools. However, - actually this are the raw data. The Authors need to make an important step for quantification of the amounts of SOC and SIC that was exchanges during the 2 years of FACE. So, the Authors should multiply the d13C changes with the stocks of SOC and SIC and present - How much C from roots was exchanged in soil. They can also estimate the Turnover rate of C pools based on this data. This would bring the ms on a much higher process level and understanding, and surely, will attract more people to the paper!

Not possible, unfortunately. As you mention in your comment, such a calculation involves differences in d13C values, and precisely one of our main results is that CO2 treatment did not affect d13C values overall. Only in a few cases we detect differences between ambient and elevated CO2 as to the obtained d13C values of either OC or IC, and these few moments were possibly spurious (see figures 5 and 6). ^{13}C-labelling started in 2003, and our sampling was in 2005: after only two years, not enough labelling was transferred from roots to the soil to make such a calculation with a minimum of confidence.

- Statistics: despite a session about statistics is presented in M&M, not statistical results are presented on graphs. This is strange. - I recognized later - the Tables were not included in the main text - please check by the revision!

We apologize for this. When submitting the paper, I (Pere Rovira) did not realize that tables had been added as 'supplementary electronic material', which of course was not my aim. In the corrected version this has been changed, and the main tables are included in the main text corpse.

In the figures, some statistical results are presented, focused in (i) the presence of significant differences between ambient and elevated CO2, for pairs of data, and (ii) the significance of the 'harvest' effect, in order to check whether significant changes with time occurred.

-Further, 4-way ANOVA is surely possible, but considering huge number of interactions between the 4 factors, the uncertainty of the conclusions is very high. May be it make

sense to think how to reduce the number of the factors and provide specific conclusions (excluding at least one of the factors).

We agree with your comment. In this new version, ANOVAs have been designed in order to analyze data carefully, avoiding possible dilution effects. In the new ANOVAs, the harvest has been suppressed as a factor. And ANOVAs have been done first for the whole dataset, but next for each species separately.

The 'harvest' factor has been studied further, when we studied the (possible) temporal changes along the sampling period, in both OC, IC, and d13C of either OC and IC (see figures 3-6).

- The Results sections is very short - just one page. As mentioned above, the Authors should present not only their d13C data (from the IRMS), but should recalculate considering the pool size etc. and should try to make ecological conclusions and that to the exchange rates of C in soil.

We added more data in our revised version, mainly about total OC and IC. The addition of all these new data is the reason of the substantial changes in the manuscript. We sincerely expect you will consider the manuscript considerably enriched, relative to the previous version.

But as above mentioned, the lack of significant differences in d13C between ambient and elevated CO2 hampers making too much calculations about exchanges and turnovers. A main exigence for these calculations is that, for a given condition, the d13C values of either OC or IC must differ between ambient and elevated CO2 rings. And in all cases (see particularly Table 3) we obtained no significant differences. This is a result in itself. But it has a consequence: that we do not have any solid basis to calculate turnover rates nor exchange rates. Unfortunately.

- The most of the literature cited to the results of FACE studies is rather old. It looks that there were nothing new in the last 5-10 years.

We added more references in many places. Even though we gave priority to recent papers, sometimes the best papers to cite were older (say, 2009 or 2005). Note that the classical FACE facilities were all closed. New facilities, re-designed, are going to be available. But it is quite logical that the publication of papers about FACE experiments is in a kind of impasse: we are in a transition stage.

Specific remarks

L24 These are not very low d13C, but just a little lower than ambient. Actually, depleted is enough.
The sentence '(very low d13C values)' has been supressed. The word 'highly' has been supressed, too. The overall text has been revised in order to avoid words such as 'highly' when referred fo CO2 concentrations.

L30 Not clear: Soil can be collected monthly - is better

The sentence has been rewritten in this way: ‘The soil was collected monthly in direct vicinity... (etc)’.

L35 Barely is not clear: were these changes significant or not? If not - write were identical or similar or …

The sentence has been changed to ‘These values did not significantly change throughout the experiment and were not affected by depth (5 or 15 cm)’. We expect you will find it ok. Note, however, that the whole ‘Results’ section has been rewritten.

L35 variable is not clear: high variation does not mean significant changes.

In the next lines of the abstract we precise a bit more what do we mean. Nevertheless, when comparing figures 5 and 6, the word that in my view reflects better the difference between both is that in the fine fraction the d13C values are more variable.

L41 what means higher here - it is actually lower compared to -1 … -2‰ in the bulk soil

It means higher (less negative) than the values for organic carbon in the fine fraction. In the fine fraction, the d13C values for organic carbon are between -20 and -27 per mil. Those for inorganic carbon are between 0 and -15 per mil, and thus are higher (i.e., less negative). However we corrected a bit the text in order to avoid these confusions, and we used the term ‘more negative’ or ‘less negative’ when appropriate. We agree than in the context of delta values the words ‘higher’ or ‘lower’ may be confusing.

Note also that this part of the abstract has been rewritten, however, in order to put more emphasis in the comparison of elevated- versus ambient [CO2] rings.

L45 "highly conservative use …" this is not correct. May be low C amounts were allocated belowground, but this does not mean conservative use. Rewrite.

Sorry but we do not agree. To retain the photosynthates within the plant and avoiding its release to the surrounding medium (e.g., soil) is to make a conservative use of them. We suppressed in these sentences the word ‘highly’, which is always debatable and often unnecessary. But the word ‘conservative’ has been maintained.

L51 biocoenoses - this word will be very seldom used. Ecosystems - is much more common and clear for broader communities

Changed as requested.

L51 what is "plant structure"? Community structure or what?

Plant structure refers to its anatomy at a ‘macro’ scale. Ramification, height, number and dimension of leaves, etc. Of course, this result also in changes in community structure (changes in spatial competition, for instance). Perhaps the term ‘plant structure’ was not the best for this

concept. We replaced it by the word ‘anatomy’, taking into account that further in this sentence we used the term ‘tridimensional structure’. Also, we considered that adding the word ‘Elevated’ before [CO2] improved the sentence.

L51 only one function?

‘Function’ in the sense of the way a plant runs. Perhaps ‘functions’ (in plural) is better.

L51-52 the sentence is not clear! IMPROVE!

After replacing ‘structure’ by ‘anatomy’ the sentence is clear.

L59… not clear what you mean specifically

It refers to the interactions between diversity (simple versus complex micro- or mesocosms), increased CO2, temperature, etc. Perhaps this could be written in a different way. Because the main feature of these complex relationships is the presence of fauna, and its interaction with a plant + soil system, the sentence ‘in particular when fauna is involved’ has been added. We whish you find the sentence improved in this way.

L66 again: it is NOT very low. Just slightly lower than ambient

‘A very low $\delta^{13}C$ value’ has been replaced by ‘a lower $\delta^{13}C$ value’. The whole text has been revised to account for this misuse of ‘very low’. Actually the word ‘very’ has been suppressed almost everywhere.

L76 Rasse et al has not written anything about the exudates

The paper of Rasse et al is a classical citation about the relevance of roots as a source of soil organic matter. But it is true that he has not papers about exudates. We solved this by suppressing any mention to root exudates in this sentence, and also the cite of Baptist et al.

L78 May be they observed slight increase of the C stocks, but surely not in ALL depths! And, there are very many papers in which C stocks remained unchanged.

Of course this refers to the depths sampled. This depends on each study, of course. To make it simple, we deleted the words ‘at all depths’. Also, we added two references in which no net sequestration was observed.

L119-126 the reason for the dilution should be clearly mentioned: The reason is fully correct, just present more clearly.

Sorry, but in our opinion lines 123-126 explain this very well. But perhaps these lines were not perfectly connected to the previous ones. We added some small additions (‘because...’, ‘and thus...’) to make more obvious such a connection.

L129 The aim of the study should be more clearly justified in the Introduction.

We added some lines to make our aim more explicit. Also, we improved (I expect) the two paragraphs immediately before this one. We expect you will find ok these amendments.

L131 It is not clear: the study was done 2005 - more than 10 years ago. Why it was not published before 2018?

Leaving aside Dr. Nowak, none of us had a linear and sure career; we changed of university, we did post-docs, etc. Often the day-to-day imposes above everything else, and the unsure status of young scientists affects too. Sorry. But most particularly, we (Pere Rovira, Iker Aranjuelo and Salvador Nogués) were not able to put our analyses within a scientific context until the paper of Evans et al was published. Then we were able to properly discuss the meaning of our results, put it on place (like a piece of a jigsaw) and go on with our paper.

L153 why the SIC content is not presented here?

We added a number (15% carbonates), which is what we obtained from our samples. But actually (and very surprisingly to me), throughout the studies generated in the NDFF facility I did not detect any paper in which the soils had been thoroughly described, including carbonate content. In most works the organic matter was the focus, thus carbonates are destroyed with acid before C or 13C analyses, but that's all. It's a pity, no doubt.

L239 Sorry, I have not found Table 1, 2, 3

As mentioned before, I put the tables (erroneously) as 'supplementary electronic material'. Now tables have been added to the main core of the paper. Note however that tables have completely changed, and – we expect it – improved.

L257 it is not a theory, but experimental conclusions!

Perhaps the word 'theory' was not the appropriate one. We replaced it by 'view'. We expect you will find this term more acceptable.

L297 it could be also - extreme fast rates of decomposition!

This is well explained further in the text. See 'b) Root exudates may not be rapidly stabilised by SOM...'.

L310 root exudates will be decomposed much faster than the sieving procedure come. Their decomposition rates are hours (see Jones DL, Gunina A, …)

We understand that you refer to the fact that during our procedure (wet sieving, etc) root exudates remaining in the soil sample may suffer decomposition. In our view, this explanation is already included in our text, in lines 310-313 of the previous version. Nevertheless, to account a bit more for this, we added a sentence within the point a). We added '- and, owing to their lability, rapidly decomposed -' after the word 'removed'. We expect you will find ok this solution.

L319 for the phenomena of hydraulic conductivity close to the roots - see studies of Carminati A

We knew the excellent work of Andrea Carminati about water absorption by root hairs (do you refer to his paper in New Phytologist, 2017?). However, we do not add it here because our discussion is already a bit long; we actually considered suppressing some parts, not enlarging it.

L355 this is probably correct that CaCO3 has very slow "turnover" time. Nevertheless, you can calculate how much CaCO3 was renewed over the 2 years of FACE - based on d13C changes and the pool size.

See our previous comments about this problem. We can not do this if the d13C values of carbonates in ambient and elevated CO2 rings can not be distinguished. Since we did not detect significant differences, we have no solid basis for such a calculation. The turnover time of carbonates in this experiment is below the detection limit.

Fig 1 Low and high CO2 sounds very poor. Ambient and Elevated are common! It is very hard here to differentiate between squares and circles.

The figure has been corrected. Both circles and squares are now bigger. Also, the design has been revised.

In addition, the word ‘elevated’ has been applied everywhere in the text to account for the ‘high CO2' treatment.

Fig 2 make separate scales for SOC and SIC, or: put separately SIC on one fig, and SOC on another fig. You should think how to present the differences between CO2 treatments and not between SOC and SIC! Put the legend on one of the figures.

The legend has been added to all figures, as a part of the re-design of these figures.

As to the rest of recommendations, sorry but we disagree. The way figures were setup is, in our view, the right one to make evident the main features of our results, including the comparison, for any experimental condition (organic or inorganic carbon, coarse or fine material, 5 or 15 cm depth) of the samples obtained from ambient CO_2 with those obtained from elevated CO_2 rings. Also, we do not agree with your request of making a separate scale for organic and inorganic carbon: using the same scale their comparison is much more obvious, and it is also clear to the eye the contrasted behaviour of coarse (> 50 µm) and fine material (< 50 µm). A difference that is in our view one of the most interesting results of our work.

Fig 4 Check the d13C of you replications for SIC for harvest 1. It is not clear: did d13C of ambient SIC has any variation (SE)?

All numerical data have been checked, and also the statistical study. Yes, the d13C of ambient SIC has standard deviation, but much lower than that observed for elevated [CO2]. Note

however that this figure has been suppressed in the new version.

We sincerely acknowledge your effort as a reviewer, and your detailed comment about our paper. We sincerely expect you will find this revised version substantially improved relative to the previous one.

Figure captions revised changes visible

Tables 2nd version changes visible

Title

Limited carbon inputs from plants into soils in arid ecosystems: a study of changes in the $\delta^{13}C$ in the soil-root interface

Authors

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Abstract

Background and aims. The tracing of C assimilation and the subsequent partitioning among plant organs has been a central focus of studies utilising Free Air CO_2 Enrichment (FACE) facilities. The approach makes use of the fossil origin of this carbon, which is depleted in ^{13}C. However, there is little data for desert environments. The Nevada Desert FACE Facility (NDFF), located in the Mojave Desert, has been one of the main facilities for the study of C dynamics in arid ecosystems and how they respond to rising atmospheric CO_2 concentrations. In this experiment, we studied the incorporation of fixed CO_2 during the previous two years (detectable by its lower $\delta^{13}C$) in the soil fraction surrounding roots.

Methods. The soil was collected monthly in direct vicinity to the roots during a complete growth season, at two depths (5 and 15 cm). Soil samples were dried and fractionated by size (> 50 µm and < 50 µm) by wet sieving, and both size fractions were then analysed for the $\delta^{13}C$ of their organic matter and their carbonates.

Results. In the coarse fraction (> 50 µm), $\delta^{13}C$ values ranged between –1 and –2‰ for carbonates and between –23 and –25‰ for soil organic matter. These values did not significantly change throughout the experiment and were not affected by depth (5 or 15 cm). In contrast, $\delta^{13}C$ values for both organic and inorganic carbon in the fine fraction (< 50 µm) were much more variable than in the coarse fraction (> 50 µm). The $\delta^{13}C$ values for organic C ranged mostly between –20‰ and –27‰, and were roughly maintained throughout the sampling period. For inorganic C, the $\delta^{13}C$ values were mostly between 0‰ and –15‰, and tended to become less negative during the course of the sampling period. Overall the effect of $[CO_2]$ on $\delta^{13}C$ values of either organic or inorganic carbon was not significant for any experimental condition (plant species, depth, fraction).

Conclusion. Little or no signs of recently fixed CO_2 (^{13}C-depleted) were detected in the soils close to the roots, in the coarse fraction (> 50 µm), the fine fraction (< 50 µm), the organic

matter, or in carbonates. This indicates a slow C turnover in the studied soils, which can result from a highly conservative use of photoassimilates by plants, including a very low release of organic matter into the soil in the form of dead roots or root exudates, and from a conservative use of available C reserves.

Introduction

In addition to its well-known greenhouse effect, atmospheric CO_2 ($[CO_2]$) has direct effects on terrestrial ecosystems. Elevated $[CO_2]$ affects plant anatomy and functions to an extent that the tridimensional structure of the whole plant community may become affected (Billès et al. 1993; Moscatelli et al. 2001). Root growth is often enhanced (Yang et al 2008; Jin et al 2012). Elevated $[CO_2]$ also affects the biochemical quality of plants (Porteaus et al 2009) and the decomposability of plant debris (De Angelis et al. 2000; Gifford et al. 2000; Gorissen et al. 1995; Gorissen and Cotrufo 2000), the dynamics of soil N, which is often the limiting factor for the dynamics of soil organic C (SOC) (Cannell and Thornley 1998), several aspects of soil biochemistry such as the soil solution (Hagedorn et al. 2002), the composition of the soil microbial community (O'Neill 1994; Panikov 1999; Frey et al 2008; He et al. 2010; Puissant et al 2015), and even ecological interactions whose effect on the overall C cycle may be extremely difficult to predict, in particular when fauna is involved (e.g., Barbehenn et al. 2004; Coûteaux et al. 1991, 1996; Coûteaux and Bolger 2000).

Free Air CO_2 Enrichment (FACE) experiments have been the framework to assemble a substantial knowledge about the effect of CO_2 enrichment on ecosystems. FACE experiments often use ^{13}C-depleted CO_2 (of fossil origin) to achieve elevated $[CO_2]$. The carbon recently incorporated into the ecosystem should be detectable as a different isotopic signature, namely, a lower $\delta^{13}C$ value. Carbon allocation and partitioning can be studied in plants (Körner et al. 2005; Kodama et al. 2010, Aranjuelo et al. 2011) and in soils. Thus, Van Kessel et al. (2000)

applied this method to the study of SOC turnover in temperate grasslands in Switzerland, Matamala et al. (2003) applied it to experimental *Pinus taeda* forests in Durham, NC USA, and Jastrow et al. (2005) to sweetgum plantations in Oak Ridge, TN USA. In Europe, Hagedorn et al. (2003) also detected enrichments in newly added CO_2 in the dissolved organic carbon of forest soils in Switzerland, which suggested that new and recent CO_2 may account for most of this compartment.

Root production has been a major focus of research using FACE techniques. An increase in the production of fine roots has been observed (Matamala and Schlesinger 2000). Because roots are the main source of soil organic matter (Rasse et al. 2005), an increase in SOC storage should be expected. Thus, Jastrow et al. (2005) and Prior et al. (2008) observed increases in SOC stocks. Martens et al (2009), using a pulse-labelling technique, concluded that under elevated [CO_2] the influx of C and potential sequestration in soil is enhanced, in wheat fields of Germany. Although this enhancement of the role of soils as C sinks helps ecosystems retain C, it is unclear whether this effect persists for a long time and leads to C sequestration. Examples of lack of net sequestration of C in soils can be found, too (Xie et al 2005, Lenhart et al. 2016).

These examples come from ecosystems where primary production may be very high, at least in some seasons, and therefore inputs of dead roots or leaf litter to the soils may be noteworthy. Environments in which plant production is severely constrained may be more difficult to study under such an approach. Desert environments cover more than one third of the Earth's surface, and their area is increasing (Dregne 1991; Kassas 1995; Reynolds 2001). Despite the relative importance of deserts, there have been few FACE experiments undertaken in desert environments.

The Nevada Desert FACE Facility (NDFF) (Jordan et al. 1999) is one of the rare examples and underscores how desert environments respond to [CO_2] increases. Specifically,

no effect of [CO_2] increase has been observed in the Mojave Desert on the biochemical quality of green tissues or the dead litter (Billings et al. 2003), and thus no effect was observed on the decomposition rate of the litter of any given species (Weatherly et al. 2003), which contrasts with results obtained for temperate areas. Increases in aboveground biomass and net photosynthesis due to elevated [CO_2] have been observed in the NDFF (Smith et al. 2000; Housman et al. 2006), but no significant increases in root production (Phillips et al. 2006) or root respiratory activity have been recorded (Clark et al. 2010). In contrast, soil microbial activity seems enhanced (Jin and Evans 2007).

The trends above refer to experimental work carried out in the first few years after starting elevated [CO_2] treatments. Not all of these trends were maintained over the following years, as shown in the paper of Evans et al. (2014), which summarises the changes in total C and isotopic composition after ten years of CO_2-enriched atmospheric supply. Relative to the control plots, those with elevated [CO_2] supply showed an increase in C stock from 1.03 to 1.17 kg C m^{-2} that extended down to a 1 m depth, as measured at a whole-ecosystem level (SOC + plant biomass, including above- and belowground components). This increase was entirely due to SOC; no increases in plant biomass were detected in either the above- or belowground compartments.

Previous research carried out in the NDFF (Ferguson and Nowak 2011) established that elevated [CO_2] enhances soil respiration just below shrubs, without any significant increase in fine root production or turnover. Thus, an increased release of dead fine roots into the soil cannot be the reason for the increased C sequestration in the soil. An enhancement in rhizodeposition might be the alternative explanation (Evans et al. 2014). Indeed, in semiarid steppes, elevated [CO_2] increases aboveground production by 33%, but it doubles rhizodeposition (Pendall et al. 2004). Nevertheless, after 10 years of [CO_2] enrichment in the NDFF, a reliable quantification of the new, ^{13}C-depleted C in the total SOC was not possible

120 because the $\delta^{13}C$ values of SOC in CO_2-enriched plots were lower than those of control plots,
121 although the differences were very small and not significant (Evans et al. 2014).

122 There are two main explanations for this. First, SOC turnover could be very slow: thus
123 the lack of significant change in SOC $\delta^{13}C$ would merely reflect a true lack of relevant inputs
124 of fresh organic matter into the soil. However, a second explanation – which does not exclude
125 the previous one – could be a dilution effect. Evans et al. (2014) excavated pits of 0.5 × 0.5 m
126 down to 1 m, in 20 cm- increments. The entire soil from each pit and depth increment was
127 homogenised and sieved to 2 mm. Such an approach gives a precise measure of the average
128 soil $\delta^{13}C$, but it implies a strong dilution of the ^{13}C label, because the new, ^{13}C-depleted C
129 released into the soil, via root exudates or root turnover, is expected to be concentrated close
130 to the roots: thus, if root density or the amount of newly released C are low, the ^{13}C label may
131 become diluted enough after whole soil sampling and sieving to be undetectable.

132 Such a dilution within the whole soil may be avoided by searching for the new, ^{13}C-
133 labelled C where it is primarily released: in the vicinity of the fine roots, either as dead tissue
134 fragments or organic exudates. The aim of our study was thus to see whether the lack of a
135 detectable ^{13}C-depletion in soil organic matter is really the consequence of (i) an extremely
136 small input of root-derived organic matter, or (ii) a dilution effect, due to the homogenisation
137 of the whole sampled soil in the pit. To this end, we focussed our work on two main points:

138 A. We searched for the ^{13}C-label in the SOC in 2005 (after 2 years of CO_2 enrichment),
139 studying the coarse soil fraction (> 50 µm) and the fine fraction (< 50 µm) separately. Dead
140 roots and root fragments were expected in the coarse fraction as part of the particulate organic
141 matter (POM), but root exudates were expected in the fine fraction, where fine silt and clays
142 are found.

143 B. We searched for the ^{13}C-label not only in the SOC, but also in carbonates, a
144 frequently ignored part of the C cycle. Root respiration may affect the carbonates in the root

vicinity: the carbonate dissolution-precipitation events may result in detectable shifts in its $\delta^{13}C$, which should be also taken as a sign of new C released into the soil.

Materials and methods

Site

The experiments were performed in the Nevada Desert FACE Facility (NDFF), located within the Nevada Test Site (36º39'N, 122º55'W, 960 m altitude). The mean annual precipitation is around 140 mm, but extremely variable; for example, annual precipitation values of just 29 mm have been recorded. Diurnal (maximum) temperature may rise above 45ºC in summer months, compared to about 20ºC in winter; diurnal variation is strong, of about 20ºC, so that night temperature may drop to about –10ºC in January or February (Jordan et al. 1999). The soils, derived from calcareous alluvium, are classified as Aridisols (Soil Taxonomy 1999). Soil texture ranges from loamy sand at soil surface (A_1 horizon: uppermost 16 cm) to sandy in the subsoil, with a dominance of coarse sand. At soil surface (0 to 20 cm), where our study was performed, carbonate content was about 15% (thus, about 1.84% of inorganic carbon). No caliche layer was developed in the subsoil. Soil organic carbon varied from 1.80% at soil surface to 0.18% at 1 m depth. Soil total nitrogen ranged from 0.08% at soil surface to 0.01% at 1 m depth. Soil pH was between 8 and 9 at all depths.

The vegetation of the area involves shrub species with short growing periods, concentrated to the months where water availability is enough to support primary production. Our research focussed on two shrubs, the creosote bush *Larrea tridentata* (Zygophilaceae) and the white burrobush, *Ambrosia dumosa* (Asteraceae). New leaves on *Larrea* at the NDFF emerge in late April or early May, with the majority of new growth occurring between mid-May and mid-June (Housman et al. 2006). *Ambrosia* initiates a leaf canopy in early spring and then loses all its leaves during the hot, dry summer months and remains deciduous until the

next year (Ackerman et al. 1980). These shrubs are the dominant ones in the vegetation of the area and represent two distinct survival strategies as *Larrea* is an evergreen shrub, whereas *Ambrosia* is a deciduous plant that loses its leaves when water stress is at its peak.

FACE experiment

Six circular plots (diameter: 23 m) were equipped with a full FACE system, including standpipes and blowers. In three of the plots, plants were continuously exposed to elevated [CO_2] atmosphere (521 µmol mol^{-1}); the other three were exposed to ambient [CO_2] (380 µmol mol^{-1}). CO_2 was supplied by BOC Gases (Murray Hill, NJ, USA). The exposure to elevated [CO_2] was continuous, except for brief periods in which it was interrupted, i.e. when wind speed was > 7 m s^{-1}, or when air temperature was < 4ºC.

The source of elevated [CO_2] changed during the experiment: from April 1997 until February 2003 it came from a geological source. From that date onwards, CO_2 supply was from fossil origin. Thus, a strong change in isotopic composition occurred: $\delta^{13}C$ of –5.4‰ until February 2003, and –32.0‰ from that date on. When mixed with the ambient CO_2, whose $\delta^{13}C$ was –8.0‰, CO_2 in the CO_2-elevated rings had a $\delta^{13}C$ value of –7.3‰ until 2003, but a value of –18.2‰ from February 2003 onwards (Schaeffer 2005).

Sampling

Sampling was performed in 2005, two years after the shift from unlabelled- to ^{13}C-labelled CO_2. Leaves, shoots and roots that had emerged during the current year were sampled monthly. Leaf and shoot samples were collected from April to August in the case of *Larrea* and from April to July in the case of *Ambrosia* (which reached its dormancy period in August, after which the plants lost all their leaves). In the case of roots, no sampling was conducted in either species during July and August because roots that had developed during the experimental

year had died by the end of June. Root samples were collected from root boxes located at the base of each shrub species (Clark et al. 2010). Roots from two plants per ring were harvested (i.e., 6 plants per species per treatment), at 5 and 15 cm depths.

Using a small spatula, a portion of the soil in close contact with the roots was also sampled alongside the roots: this soil sample, owing to its direct contact with the roots, is expected to reflect the direct effect of roots on the soil biochemistry in the vicinity of the roots. Because priority was given to sampling soil in immediate contact with the roots, the amount of soil sampled was very small (2-3 g). The soil was placed immediately in polypropylene vials and air-dried.

Sample treatment

Soil samples were sieved through a 2 mm mesh. Materials > 2 mm (gravel and some organic fragments, always in very small amounts) were discarded. The fine materials (< 2 mm) was used for analyses.

A sample of material < 2 mm (about 2 g) was placed in a glass beaker, and 20 ml of deionised water was added. Then the sample was dispersed by an intense agitation in an end-over-end shaker for 30 minutes, and sieved through a 50 µm mesh under magnetic stirring and water flushing. Both the coarse (> 50 µm) and the fine fraction (< 50 µm) were recovered by centrifugation, dried at 60ºC to constant weight, weighed, and finely ground for analyses.

In both fractions we analysed the $\delta^{13}C$ in organic matter and in carbonates, using an elemental analyser Flash 1112 coupled to an isotope ratio mass spectrometer Delta C with CONFLO III interface (Thermofisher Scientific):

–The $\delta^{13}C$ of carbonates (henceforth, d_I) was analysed in subsamples calcinated at 550ºC for 6 hours. We verified in separate trials that such a treatment does not result in any loss of carbonates or in any detectable change in their d_I. We verified also that after such a

220 treatment, organic carbon content is undetectable.

221 – The $\delta^{13}C$ of organic carbon (henceforth, d_O) was quantified as follows. A subsample
222 of the coarse fraction was placed in a vessel and treated with cold dilute HCl to destroy
223 carbonates. The sample was dried at 60ºC in the same vessel to ensure that all hydrosoluble
224 organic compounds released remained in the sample. After this treatment, the remaining
225 sample (organic matter only) was analysed for $\delta^{13}C$.

226 – In the fine fraction the above procedure was not successful because the remaining
227 material after the HCl treatment had a doughy consistency and was impossible to transfer into
228 standard vessels for mass spectrometry. In this case, the $\delta^{13}C$ for the whole sample (organic
229 plus inorganic carbon) was analysed. Then d_O was calculated by the following equation:

230 $$d_T = f\, d_I + (1 - f)\, d_O \qquad (1)$$

231 which may be re-arranged in this way,

232 $$d_O = (d_T - f\, d_I) / (1 - f) \qquad (2)$$

233 where d_T is the $\delta^{13}C$ of the whole sample, d_I the $\delta^{13}C$ of the carbonates, d_O the $\delta^{13}C$ of the
234 organic carbon, and f is the fraction (0 to 1) of the total C in the sample that is in carbonate
235 form.

236

237 Statistics

238 The studied variables (total OC and IC for coarse and fine fractions, and their $\delta^{13}C$
239 values) were analysed with ANOVAs. The factors under study were (i) plant species (*Larrea*
240 of *Ambrosia*), (ii) [CO_2] (elevated or ambient), and (iii) depth (5 or 15 cm).. This study was
241 carried out separately for d_O and d_I, and for the coarse (> 50 μm) and fine fractions (< 50 μm).
242 The effect of harvest (1, 3, 5) was tested for each experimental condition, to verify whether
243 temporal changes in the studied variables were significant. All statistical analyses were
244 performed using SPSS v. 11.0.

Results

Stable isotopes in plant organs

For each of the studied organs (leaves, stems, roots), the $\delta^{13}C$ values were significantly affected by the [CO_2] treatment (ambient vs. elevated [CO_2]), by the species (*Larrea* vs. *Ambrosia*) and by harvest number (Table 1). As shown in Fig. 1, elevated [CO_2] always resulted in lower $\delta^{13}C$ values, owing to the low $\delta^{13}C$ of the added CO_2. Species effects were also obvious: $\delta^{13}C$ values of *Larrea* were almost always higher than those of *Ambrosia*. Variability among harvests was also obvious, sometimes strong (e.g., for roots), but without a clear pattern.

Carbon content in the fractions

The soil material is dominated by the coarse fraction: the > 50 µm material accounts on average for 86.7% of the total weight, while just 13.3% is recovered in the fine fraction. The two fractions differ in their composition. Whereas the coarse fraction has a mean OC content of 2.01 g kg^{-1}, the fine fraction has a concentration of 11.14 g kg^{-1}, more than five times higher. In carbonates (IC: inorganic carbon), the opposite is found: 20.18 g kg^{-1} in the coarse fraction, but just 4.10 in the fine fraction. As a result, the fine material, being just 13% of the total weight, stores 84.11% of the total OC, but only 3.26% of the total IC.

Table 2 summarizes the results of statistical tests. We did not detect any significant effect of depth. A species effect is clear for OC, for both the coarse and the fine fraction: OC contents were higher under *Larrea* than under *Ambrosia*. None of the studied factors significantly affected OC content in the coarse fraction. In the fine fraction, in contrast, OC content was affected by [CO_2], but the effect reached significance only for *Ambrosia*. The behaviour of IC was different. Even though we did not detect a significant effect of [CO_2] on

IC content overall, the effect was clearly species-dependent, for it was significant under *Ambrosia* for both the coarse and the fine fraction, and null under *Larrea*.

These results are developed in more detail in figure 2, specially focused on the effect of [CO_2] on OC and IC contents in soil. As to the OC content, in the coarse fraction no pattern was detected, and the effect of [CO_2] was not significant in any case. In contrast, in the fine fraction a consistent pattern was observed: elevated [CO_2] increases OC content, at both depths (5 and 15 cm) and under both species (*Ambrosia* and *Larrea*), even though under *Larrea* the increases did not reach significance, owing to the huge variability.

A different behaviour is observed for IC. Under *Ambrosia*, elevated CO_2 consistently decreased IC contents, at both depths (5 and 15 cm) and in both fractions (coarse and fine). Thus a decarbonation is induced. Under *Larrea*, in contrast, no effect of elevated CO_2 is detected in any case.

Overall, in the vicinity of roots, elevated CO_2 affected OC and IC in different ways. The effect on OC depends on the fraction: increased OC content, but only in the < 50 µm fraction. In contrast, the effect of elevated CO_2 on IC is species-dependent: consistent loss of IC under *Ambrosia*, no effect under *Larrea*.

Temporal changes in total OC and IC

Figures 3 and 4 show how the average contents summarized in figure 2 change with time, along the sampling season (harvests 1, 3 and 5). In the coarse fraction, few changes are detected (Fig. 3). The harvest factor was significant in one case only (*Ambrosia*, OC, 5 cm, elevated CO_2), meaning that, overall, in the coarse fraction no significant temporal changes occurred along the studied season. In the fine fraction, in contrast, both OC and IC concentrations seem very unstable (erratic dynamics) and highly variable (error bars are often noteworthy) (Fig. 4). The harvest factor was significant in 6 out of 8 experimental conditions,

meaning that in the fine fraction temporal changes are much stronger than in the coarse fraction. It is noteworthy, however, that as to the OC the harvest factor was significant in only one case (*Ambrosia*, 15 cm, elevated CO_2), thus suggesting that the changes in OC content were actually smaller than it is suggested by Fig. 4, and in most cases not significant. However, this cannot be applied to the changes in IC content, for which the harvest factor was significant in 5 out of 8 cases. The carbonate content of the fine fraction seems quite variable with time, highly erratic.

Isotopic composition of the obtained fractions

Coarse fraction (> 50 μm) had d_O values around -24.5‰ on average, and dI values around -1.6‰. In the fine fraction (< 50 μm) the values were less negative for d_O, with an average value of -22.3‰, but more negative for d_I, with a mean value of -7.97‰. Table 3 gives a panoramic view of the values obtained for the several experimental conditions (fraction, species, depth, CO_2 treatment).

From the statistical analysis of these data relevant issues arise (Table 4). In the coarse fraction, a significant species effect was detected for d_O values: −24.81‰ under *Ambrosia*, −24.32‰ under *Larrea*. This was the only significant effect detected. For the rest of cases (d_O of fine fraction, d_I of both coarse and fine fractions) no significant effect was detected for species or depth.

Most relevant, we failed to detect any significant effect of [CO_2], even though in one case (*Ambrosia*, fine fraction, d_O) the [CO_2] effect was very close to signification ($p = 0.051$). Same result was obtained when the comparison between ambient and elevated [CO_2] was carried out for each experimental condition separately: in Table 3, none of the pairwise comparisons (ambient / elevated CO_2) reached significance.

Temporal dynamics of the isotopic compositions

Figures 5 and 6 summarize the changes in d_O and d_I in the several species (*Ambrosia* / *Larrea*), depths (5 or 15 cm) and fractions (coarse and fine). The significance of the 'harvest' factor, labelled with a cross (+) at the left of each series, was taken as indicator of the significance of the temporal changes. Also, the [CO_2] treatment was evaluated for each harvest individually, and labelled with an asterisk (*) when significant.

In the coarse fraction, both d_O and d_I values were very stable overall (Fig. 5). Only in two cases significant temporal changes were detected (*Ambrosia*, 5 cm, d_I, ambient [CO_2]; *Ambrosia*, 15 cm, d_O, elevated [CO_2]).

The isotopic composition of the fine fraction was less stable than that of coarse fraction, and more variable for a given condition (Fig. 6). An overall trend to a temporal increase in $\delta^{13}C$ values (i.e., to become less negative) persistently appears, even though the trend did not always reach significance. Temporal changes were noteworthy for d_I values (harvest effect significant in 6 out of 8 cases), not so for d_O (significant in 2 out of 8 cases).

The most relevant issue of figures 5 and 6 is the lack of significant effects of [CO_2] treatment. In the coarse fraction, no effect was observed in any case. As to the fine fraction, only in one case a significant effect of [CO_2] was detected (*Ambrosia*, 15 cm, harvest 1, d_I). The effect was in the expected sense, i.e., lower $\delta^{13}C$ value (more negative) under elevated [CO_2].

Discussion

In summary, our data suggest that in the Mojave Desert ecosystem there is a true effect of elevated [CO_2] on C dynamics, driven by roots. Such an effect is both fraction-dependent and species-dependent (Fig. 2): net increase in OC in the fine fraction under both species (*Ambrosia* and *Larrea*), net decrease in IC in both coarse and fine fractions but only under Ambrosia. Nevertheless, we failed to detect significant effects of elevated [CO_2] on d_O or d_I.

These changes are the result of nine years of elevated [CO_2], but after only two years of ^{13}C-labelling (^{13}C-depleted CO_2). Thus we were able to detect medium-term changes (shifts in total OC and IC), but not short-term ones (shifts in d_O or d_I). This points to a very slow C turnover, as discussed below in more detail.

Methodological constraints

Our primary aim was to search for ^{13}C-depleted carbon derived from the elevated [CO_2] atmosphere provided in the NDFF facility, where it should be primarily found: at the very vicinity of roots. The need of avoiding the dilution of such a label, as explained in the introduction, implies that the obtained soil samples must be very small. This may represent a handicap for some studied parameters, because soils are heterogeneous mixtures, and a too small sample could be not representative of the whole soil surrounding the roots. The risk is especially high for the fine fraction (< 50 µm), which accounts for about 13% of the total soil samples, in weight.

The comparison of figures 3 and 4 illustrates this problem. For both OC and IC concentrations, we obtained a consistent pattern in the coarse fraction, but a chaotic pattern in the fine fraction. In the later, the variability is huge (large error bars). Being a small fraction, any addition/subtraction of either OC or IC may translate to a noticeable change in OC or IC concentrations. On the other hand, both OC and IC concentrations show a high small-scale heterogeneity, which may be not well reflected if the soil sample taken is very small, as in our case.

These considerations suggest that complex and chaotic dynamics such as that shown in Fig. 4 for OC and IC concentrations in the fine fraction may be spurious, rather than reflecting true changes in OC and IC content in the fraction, along the sampling season. This is an obvious explanation for the erratic and inconsistent dynamics of OC in this fraction. The wide error

bars, particularly when some values are high (e.g., harvest 1, *Larrea*, OC under elevated CO_2), are in favour of this hypothesis. In contrast, for the coarse fraction no such erratic trends appear (Fig. 3), and the error bars are comparatively smaller, a fact probably explained by the fact that the coarse fraction accounts for more than 85% of the soil samples, and thus their OC and IC concentrations are less sensitive to small inputs/outputs from dead roots or root exudates.

Isotopic values for organic carbon

In contrast with classical views about soil organic matter dynamics, which proposed a dominant role for leaf litter as the main source, today it is widely assumed that soil organic matter originates mostly from roots (Rasse et al. 2005). According to this view, the amount and biochemical quality of dead roots should be determinant for SOC turnover, and $\delta^{13}C$ of the dead roots should be determinant for the $\delta^{13}C$ of soil organic matter (d_O values). In the NDFF experiment, the majority of root tissue was already composed of new carbon when our sampling was performed: in both *Larrea* and *Ambrosia* roots more than 90% of the C was from new C, i.e. supplied after February 2003 (Aranjuelo et al. 2011). The presence in the soil of recent inputs of dead roots or recent root exudates should have resulted in more negative d_O values in the soil samples from the elevated [CO_2] rings, in both the coarse and fine fractions.

Evidence from experiments conducted across the world with ^{13}C-labelled materials suggests that root turnover may be very active, and root-derived tissues may be quickly detectable in SOC. In Pyrenean grasslands, for instance, during the decomposition of ^{13}C-labelled roots, the ^{13}C label was detectable in the coarse fraction (> 50 µm) at the end of the first year, and the fine fraction (< 50 µm) becomes more important as a sink for root-derived material as decomposition proceeds (Garcia-Pausas et al. 2012). In FACE experiments, Van Kessel et al. (2000) observed in Swiss grasslands that after six years of exposure to elevated [CO_2], up to 25-30% of the total SOC in the uppermost 10 cm was derived from newly added

395 CO_2, and it was up to 57% in sand-size fractions. Interestingly, below a depth of 10 cm the
396 input of newly added CO_2 was almost undetectable. In the study of Jastrow et al. (2005), after
397 5 years of elevated [CO_2] exposure, about 30% of the organic C in the soil surface was recent.
398 Our results differ from those observations. In the NDFF, the lack of ^{13}C-label in soil
399 organic matter, even very close to the roots, suggests an extremely low rate of release of root-
400 derived material into the soil. The results were particularly clear for the coarse fraction, whose
401 d_O values did not show any change related to CO_2 treatment. In the fine fraction, the variability
402 was higher than in the coarse fraction, but the CO_2 treatment also had no significant effects on
403 the d_O values. Thus, recent inputs of root-derived materials in the SOM (< 2 years old) must be
404 small, not detectable from d_O values. On the medium-term (up to 8 years), however, inputs are
405 detectable, as shown in Fig. 2.
406 Our results contrast with reports showing substantial increases in root production driven
407 by elevated [CO_2] (Agathokleous et al. 2016, Hao et al 2016), but agree with previous results
408 obtained in the NDFF facility. Thus, Ferguson and Nowak (2011) did not detect, after 10 years
409 of elevated [CO_2], a significant increase in fine root biomass, nor any shift in their depth
410 distribution, thus concluding that fine roots are unlikely to be a source of net C sequestration
411 in soils. Of special interest in that study is the fact that this lack of a long-term effect on fine
412 root production does not hamper sporadically detectable differences between plants grown
413 under elevated [CO_2] and ambient [CO_2]; but these differences are transient and do not persist
414 on the long-term. However, some nuance must be added to the above findings. Our results (Fig.
415 2) suggest that under elevated [CO_2] fine roots do really release extra organic matter to the soil,
416 via root debris or exudates; but that this input accumulates in the fine fraction (< 50 μm), which
417 in the Mojave Desert soil accounts for a small part of the soil mass (< 15%), and thus may
418 become undetectable when the whole soil, unfractionated, is studied.
419 Root exudates have been described to increase upon CO_2 enrichment (Pendall et al

2004). Evans et al. (2014) hypothesized that enhanced rhizodeposition may be one explanation of increased SOC stock under elevated [CO_2]. Our failure to detect a signal of ^{13}C-depleted organic material in the fine fraction (< 50 µm), where root exudates should become stabilised, does not necessarily contradict Evans et al.'s hypothesis, for this failure refers to the very recent C (< 2 years), as above explained. Also, three additional reasons (or the combination of some of them) could explain why we did not detect ^{13}C-depleted organic material in the fine fraction:

a) Methodological constraints: root exudates could have been removed – and, owing to their lability, rapidly decomposed – during the fractionation between coarse and fine fractions by wet sieving. This would only affect those compounds not yet stabilised by association with fine silt and clays, i.e. water-extractable compounds, which are the most labile of all soil organic matter fractions.

b) Root exudates may not be rapidly stabilised by SOM transformation or by their association with active soil mineral components such as clays or Fe or Al oxyhydroxides. A possible reason for slow stabilization rates is that in desert soils, low contact between fine roots and fine earth (< 2 mm) is likely to occur, particularly in dry periods: drought results in a retraction of soil structure, which increases the voids between roots and the soil around them, decreases hydraulic conductivity, and decreases the transfer of liquids from roots to the soil and vice-versa. The phenomenon has been studied in *Opuntia* (North and Nobel 1997), but likely affects many desert species. Because root exudates are highly labile *per se* (Hütsch et al, 2002), the reduction of their stabilization by soil components then allows rapid biodegradation by the root-associated microflora.

c) The quantitative relevance of root exudation may be much lower than expected. A detailed discussion of this matter is beyond the scope of our paper, but several facts must be mentioned. The review of Hütsch et al. (2002) states that root exudates account for 14-18% of the total net photosynthetically fixed C. Nevertheless, these estimates are mostly based on

445 experiments carried out under optimal conditions, in which roots were watered intensively,
446 precisely to avoid water stress. Root exudation under non-optimal conditions may be much
447 lower. For example, the theoretical analysis by Luo et al. (2001) suggested that root exudates
448 account for a very small part of the soil C budget and fluxes in the Duke Forest (North Carolina,
449 USA). Recent work in temperate forests (Hagedorn et al. 2016) showed that drought
450 substantially reduces rhizodeposition in beech trees. Because even moderate water stress has
451 diverse effects on root exudation (Sanaullah et al. 2012), it is doubtful that the quantitative
452 results obtained under optimal conditions can be extrapolated to the extremely dry conditions
453 of the Mohave Desert. Although sound experimental data about root exudation in desert plants
454 under field (or, at least, realistic) conditions is sorely needed, our results suggest that root
455 exudation under natural conditions could be much lower than often assumed, and be one of the
456 reasons of the lack of detection of new, ^{13}C-depleted organic matter at the root vicinity.
457
458 Isotopic values for inorganic carbon (carbonates)
459 A relevant result from our work is the lack of significant differences between controls
460 and elevated [CO_2] rings, regarding the evolution of $\delta^{13}C$ of carbonates (d_I values), indicating
461 that the input of recent C was not detectable in inorganic C. In our experiments, atmospheric
462 CO_2 (which diffuses within the soil) apparently did not play any role in the changes in d_I values.
463 We must note that, while in control rings the atmospheric CO_2 had a $\delta^{13}C$ value of –8.0‰, it
464 was –18.02‰ in elevated [CO_2] rings, i.e. a difference of about 10‰. Therefore, had
465 atmospheric CO_2 played any role in the generation of the new carbonates, we should have
466 detected differences in d_I, at least in the < 50 μm fraction. To a lesser extent, this can also be
467 applied to the root-respired C: the differences in root $\delta^{13}C$ between controls and elevated [CO_2]
468 rings were smaller than for atmospheric CO_2 (between 4 and 6‰), but still enough to result in
469 carbonate $\delta^{13}C$ values consistently lower (i.e., more negative) in elevated [CO_2] rings.

This result was expected, owing to the overall slow turnover of soil carbonates, which makes it unlikely to detect new (^{13}C-depleted) carbon in soil carbonates after just two years of ^{13}C-labelling. The replacement rate for carbonates in the vicinity of roots increases with temperature. Gocke and Kuzyakov (2011) working with *Zea mays* submitted to ^{14}C-CO_2 labelling, calculated that a complete replacement of old by new, ^{14}C-labelled carbonates would occur in 5740 years at 10ºC, in 4330 years at 20ºC, and 1060 years at 30ºC. The experiments of Gocke and Kuzyakov were performed with well-watered plants, and thus the replacement rates of carbonates under real field conditions must be much slower. It is often assumed that changes in the isotopic composition of carbonates are extremely slow, and become detectable only after centuries or millennia (Pendall et al. 1994). Carbonates have been proposed as a very stable pool within the C cycle, with turnover times much longer than those of soil organic matter. The δ^{13}C values of pedogenic carbonates, for instance, have been used as indicators of ancient shifts in vegetation type (from C3 to C4 or vice-versa) (Ding and Yang 2000; Kelly et al. 1991; Wang et al. 1993).

Nevertheless, fast changes in carbonate δ^{13}C values have also been observed, linked to episodes of strong CO_2 production, such as in the presence of decomposing plant residues (Rovira and Vallejo 2008) or in the vicinity of highly active roots (Li and Wang 2001; Li et al. 2002). If intense enough, the release into the soil of CO_2 from microbial or root respiration results in solubilisation of carbonates, which may re-precipitate further, either totally or partially. Solubilisation-precipitation cycles increase the d_I values (Salomons and Mook 1976, 1986), and it is worth stressing that substantial changes in d_I may happen without a massive effect (solubilisation + precipitation) on the overall carbonate pool. As shown in a previous paper (Rovira and Vallejo 2008), a few solubilisation-precipitation events, each one affecting a small fraction of the existing carbonates, are enough to result in substantial increases in d_I.

The changes we observed in d_I for the fine fraction are probably of this kind, i.e.,

associated with episodes of increased microbial activity (and, therefore, increased pCO_2). The lack of significant effects of the [CO_2] treatment on the d_I values compels us to assume that the source of CO_2 for these changes was neither root respiration nor atmospheric CO_2 diffused down into the soil. Rather, the origin must be native soil organic matter, whose $\delta^{13}C$ was the same in control and in CO_2-enriched rings. Because the changes occurred mainly in the fine fraction, the most likely driver of these changes was the microbial decomposition of the organic matter associated with microaggregates.

A detail to stress is that the observed increase in d_I (2-3 ‰ in just 5 months), if sustained over time, should have resulted in much higher d_I values. The NDFF was established in plots chosen for their degree of ecological conservation: rings were placed at points where the surface crust remained intact, a sign of lack of perturbation by regular human visitation (Jordan et al. 1999). In addition, the setup of the experiments specifically avoided any effect on the natural vegetation within the rings. It seems logical to assume that any process occurring in the NDFF experiments (at least in the control rings) has been happening in the soil for a long time. The dynamics shown in Fig. 3 for the d_I values do not reflect the activities of a complete year. A seasonal cycle could be present: after the observed increase, a decrease may occur. However, this remains a hypothesis to be tested in future studies.

An overall view

Our data confirm the very slow SOC turnover in the NDFF soils (Evans et al. 2014), but in addition our results indicate that such a slow turnover is not solely the result of a dilution effect. Furthermore, our results indicate that the release of root-derived materials into the soil is low not only on a per surface basis (i.e., per square metre), but also for a given individual root.

These observations point to an ecological adaptation of desert plants (at least, the two

studied species, *Ambrosia dumosa* and *Larrea tridentata*). Where primary production is largely constrained, a conservative management of photoassimilates is to be expected. This involves a low release of organic matter into the soil, in the form of dead roots or root exudates.

The lack of an effect of root respiration (as deduced from the lack of detectable differences due to CO_2 enrichment) may be related to a conservative management of respirable C reserves. The theory of respiratory physiotypes (Nogués et al. 2014) provides a framework to understand these results. In highly productive environments, where plants may grow without drastic constraints, conservative strategies in the use of photoassimilates may not make too much sense. This is not so in environments where strong limitations to water or nutrient availability seriously limit growth. Under these constraints, a so-called *parsimonious* strategy for plant respiration (Nogués et al. 2014) must ensure survival: in these plants the respirable C pool has a slow turnover. This translates into a slow CO_2 release, not enough to increase soil CO_2 to levels capable of generating detectable shifts in soil $\delta^{13}C$.

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Table 1 Overall significance (P > F) of the several factors considered on the $\delta^{13}C$ values of plant organs.

Factor	**Leaves**	**Stems**	**Roots**
A. CO_2 (elevated / ambient)	< 0.001 ***	< 0.001 ***	< 0.001 ***
B. Species (*Larrea* / *Ambrosia*)	< 0.001 ***	< 0.001 ***	< 0.001 ***
C. Harvest (1-5)	0.003 **	0.001 ***	0.001 ***
A × B	0.254	0.025 *	< 0.001 ***
B × C	0.192	0.254	< 0.001 ***
A × C	0.020 *	< 0.001 ***	0.226
A × B × C	0.377	0.003 **	< 0.001 ***

Table 2 Significance of the several factors on the OC and IC content of the soil samples. Data are the p > F values obtained in the several ANOVAs. ANOVAs were carried out (i) for all samples altogether, or (ii) for soil samples close to roots of either *Ambrosia* or *Larrea*, separately. The significance is labelled: (*) at p = 0.05; (**) at p = 0.01; (***) at p = 0.001. If no asterisk is given, the factor had no significant effects.

	OC content			IC content		
Factor	All	*Ambrosia*	*Larrea*	All	*Ambrosia*	*Larrea*
			a) Coarse fraction (> 50 µm)			
A. Species	0.016 *			0.386		
B. Depth	0.653	0.462	0.910	0.991	0.824	0.826
C. CO_2	0.577	0.752	0.262	0.005 **	<0.001***	0.876
A × B	0.543			0.754		
A × C	0.312			0.009 **		
B × C	0.063	0.224	0.158	0.782	0.464	0.763
A × B × C	0.910			0.473		
			b) Fine fraction (< 50 µm)			
A. Species	0.044 *			0.463		
B. Depth	0.387	0.180	0.657	0.782	0.667	0.993
C. CO_2	0.004 **	<0.001***	0.085	0.212	0.010 **	0.529
A × B	0.995			0.771		
A × C	0.671			0.028 *		
B × C	0.759	0.334	0.523	0.853	0.257	0.459
A × B × C	0.352			0.191		

Table 3 Isotopic composition ($\delta^{13}C$) of organic and inorganic carbon (carbonates), in the several fractions isolated in our work. Numbers are averages ± standard deviations. For a given condition (fraction, carbon type, species, depth), all harvests have been pooled. No significant differences between ambient and elevated CO_2 were detected for any pair of means, in any case.

				[CO_2]		
Fraction	$\delta^{13}C$	Species	Depth	Ambient	Elevated	All
> 50 µm	d_O	*Ambrosia*	5	-24.70 ± 0.45	-24.95 ± 0.98	-24.83 ± 0.77
			15	-24.66 ± 0.50	-24.92 ± 0.65	-24.80 ± 0.59
		Larrea	5	-24.30 ± 1.49	-24.28 ± 0.53	-24.29 ± 1.09
			15	-24.37 ± 0.54	-24.34 ± 0.71	-24.35 ± 0.62
	d_I	*Ambrosia*	5	-1.69 ± 0.41	-1.65 ± 0.31	-1.67 ± 0.36
			15	-1.51 ± 0.39	-1.58 ± 0.56	-1.55 ± 0.48
		Larrea	5	-1.76 ± 0.59	-1.60 ± 0.60	-1.68 ± 0.59
			15	-1.60 ± 0.46	-1.63 ± 0.58	-1.61 ± 0.51
< 50 µm	d_O	*Ambrosia*	5	–22.81 ± 1.97	-21.04 ± 3.21	-21.98 ± 2.73
			15	-22.53 ± 1.87	-21.77 ± 2.97	-22.17 ± 2.44
		Larrea	5	-22.14 ± 0.98	-23.36 ± 4.76	-22.71 ± 3.32
			15	-21.94 ± 2.51	-22.79 ± 2.32	-22.38 ± 2.41
	d_I	*Ambrosia*	5	-8.24 ± 6.95	-8.50 ± 7.69	-8.37 ± 7.21
			15	-6.01 ± 3.86	-9.93 ± 7.20	-8.08 ± 6.12
		Larrea	5	-6.04 ± 3.70	-8.13 ± 6.48	-7.08 ± 5.30
			15	-8.47 ± 6.56	-8.20 ± 7.66	-8.34 ± 7.00

Table 4 Significance of the several factors on the $\delta^{13}C$ of OC and IC of the soil samples (d_O and d_I, respectively). Data are the p > F values obtained in the several ANOVAs. ANOVAs were carried out (i) for all samples altogether, or (ii) for soil samples close to roots of either *Ambrosia* or *Larrea*, separately. Values are enhanced when significant: (*) at p = 0.05; (**) at p = 0.01; (***) at p = 0.001.

	d_O			d_I		
Factor	All	*Ambrosia*	*Larrea*	All	*Ambrosia*	*Larrea*
			a) Coarse fraction (> 50 µm)			
A. Species	<0.001***			0.650		
B. Depth	0.894	0.846	0.758	0.267	0.238	0.628
C. CO_2	0.398	0.122	0.916	0.791	0.856	0.631
A × B	0.715			0.725		
A × C	0.310			0.625		
B × C	0.999	0.981	0.985	0.377	0.595	0.480
A × B × C	0.977			0.821		
			b) Fine fraction (< 50 µm)			
A. Species	0.286			0.676		
B. Depth	0.863	0.733	0.605	0.699	0.800	0.416
C. CO_2	0.817	0.051	0.168	0.174	0.188	0.553
A × B	0.536			0.453		
A × C	0.020*			0.592		
B × C	0.741	0.431	0.809	0.764	0.247	0.444
A × B × C	0.484			0.173		

Figure captions

Fig. 1 Carbon isotope composition ($\delta^{13}C$) of leaves, stems and roots for *Ambrosia* and *Larrea* plants, under control conditions or elevated CO_2. Means are averages, vertical bars are standard deviations. In *Ambrosia*, no new leaves nor stems appeared in the 5th harvest. In both *Ambrosia* and *Larrea*, no new roots appeared beyond the 3rd harvest.

Fig. 2 Organic and inorganic carbon content, in coarse (> 50 µm) and fine (< 50 µm) fractions of the soil samples collected at 5 and 15 cm depth, from rings under ambient and elevated [CO_2]. Data in g per kg of the fraction. Wide bars are averages; thin lines are standard deviations. For each experimental condition (species × depth × size fraction) the significance of [CO_2] effect has been tested: ns, not significant; *: significant at p = 0.05; **: significant at p = 0.01.

Fig. 3 Changes with time in organic carbon (OC) and inorganic carbon (IC: carbonates) in the coarse fraction (> 50 µm). Data in g C per kg of the fraction. Dots are averages, vertical bars are standard deviations. An asterisk (*) at the top of a pair of dots indicates that the effect of [CO_2] was significant at p = 0.05. A cross (+) at the left of a temporal series indicates a significant effect of harvest, and thus significant temporal changes in this series.

Fig. 4 Changes with time in organic carbon (OC) and inorganic carbon (IC: carbonates) in the fine fraction (< 50 µm). Data in g C per kg of the fraction. Dots are averages, vertical bars are standard deviations. An asterisk (*) at the top of a pair of dots indicates that the effect of [CO_2] was significant at p = 0.05. A cross (+) at the left of a temporal series indicates a significant effect of harvest, and thus significant temporal changes in this series.

Fig. 5 Carbon isotope composition ($\delta^{13}C$) of organic carbon (OC) and inorganic carbon (IC: carbonates), in the coarse fraction (> 50 µm) for samples collected at 5 cm (upper panels) and 15

cm (lower panels) soil depths. Dots are averages, vertical bars are standard deviations. An asterisk (*) at the top of a pair of dots indicates that the effect of [CO_2] was significant at p = 0.05. A cross (+) at the left of a temporal series indicates a significant effect of harvest, and thus significant temporal changes in this series.

Fig. 6 Carbon isotope composition ($\delta^{13}C$) of organic carbon (OC) and inorganic carbon (IC: carbonates), in the fine fraction (< 50 µm) for samples collected at 5 cm (upper panels) and 15 cm (lower panels) soil depths. Dots are averages, vertical bars are standard deviations. An asterisk (*) at the top of a pair of dots indicates that the effect of [CO_2] was significant at p = 0.05. A cross (+) at the left of a temporal series indicates a significant effect of harvest, and thus significant temporal changes in this series.

Figure 1 revised

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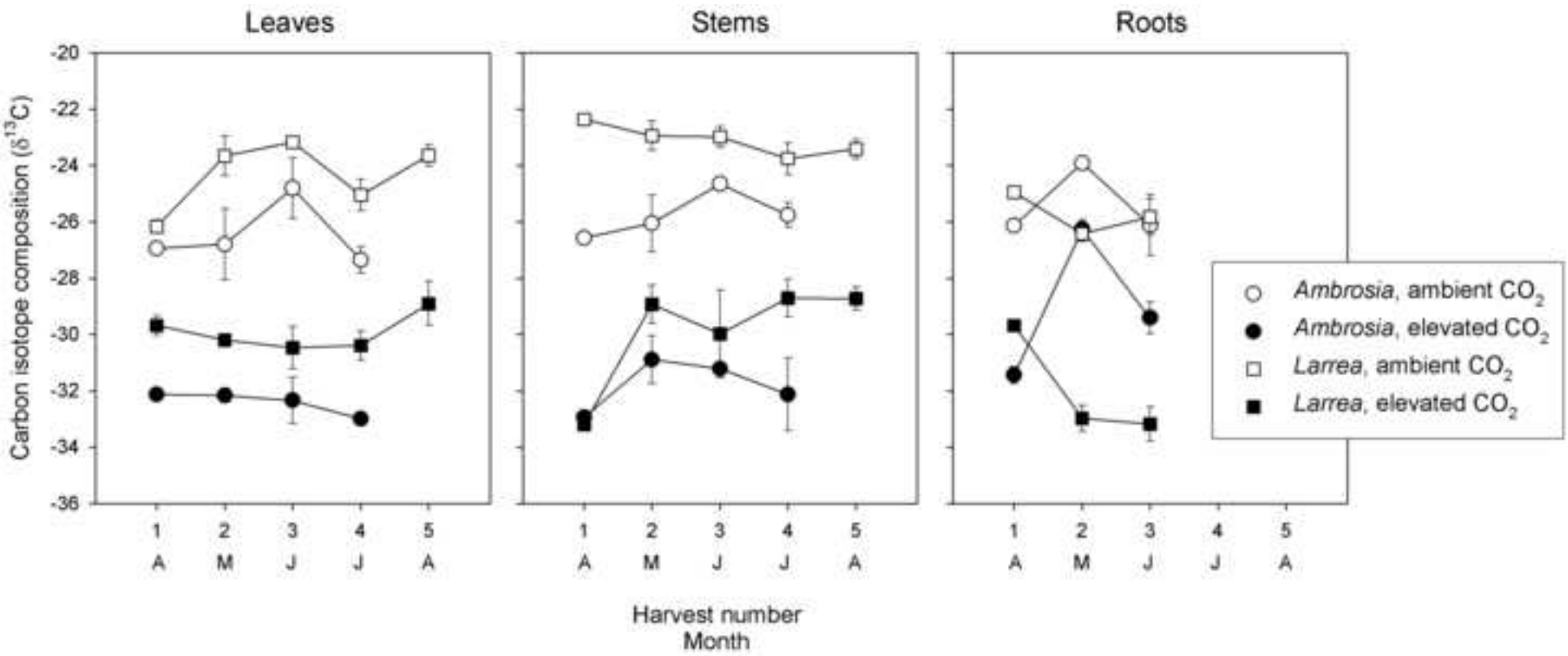

Figure 2 new

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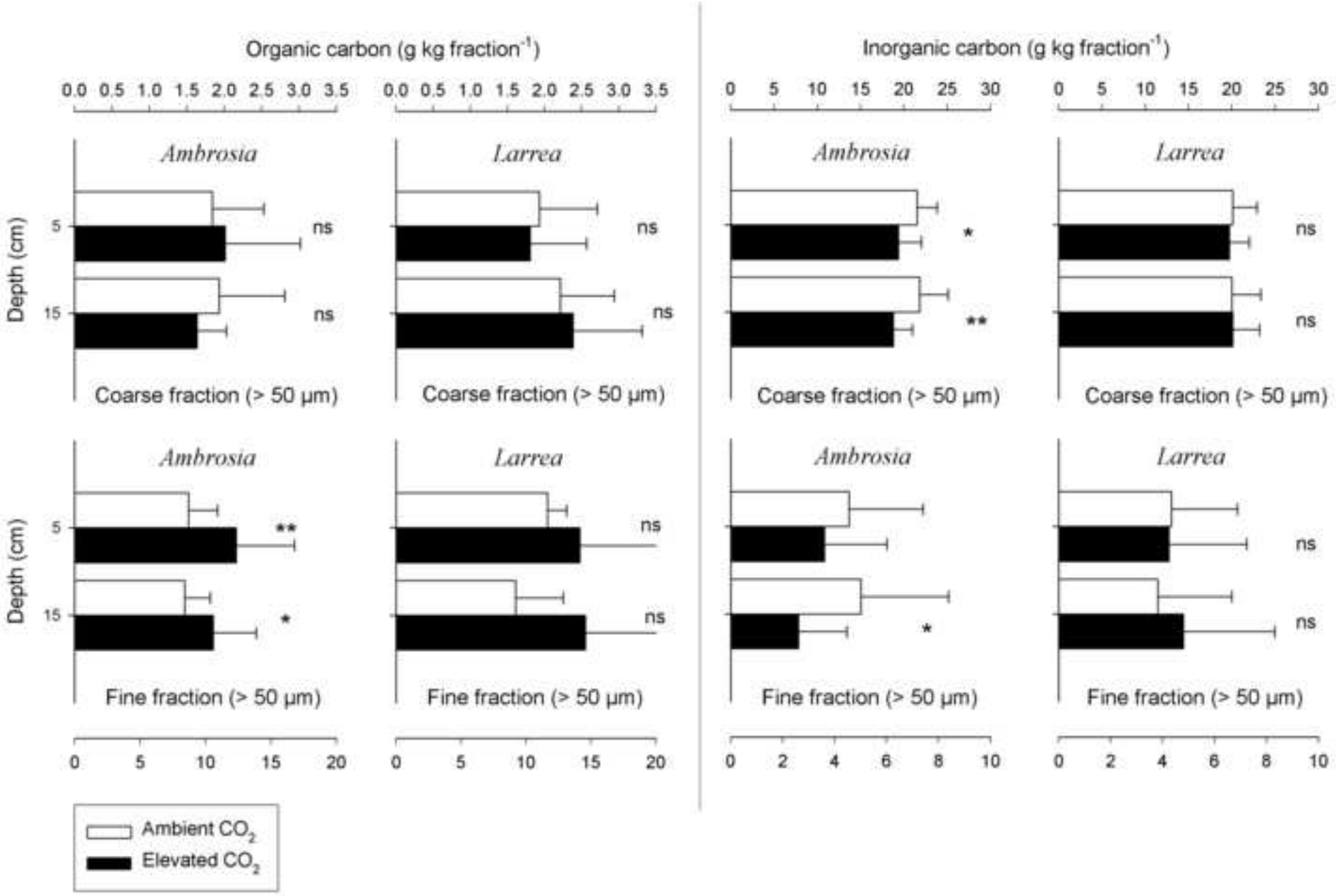

Figure 3 new

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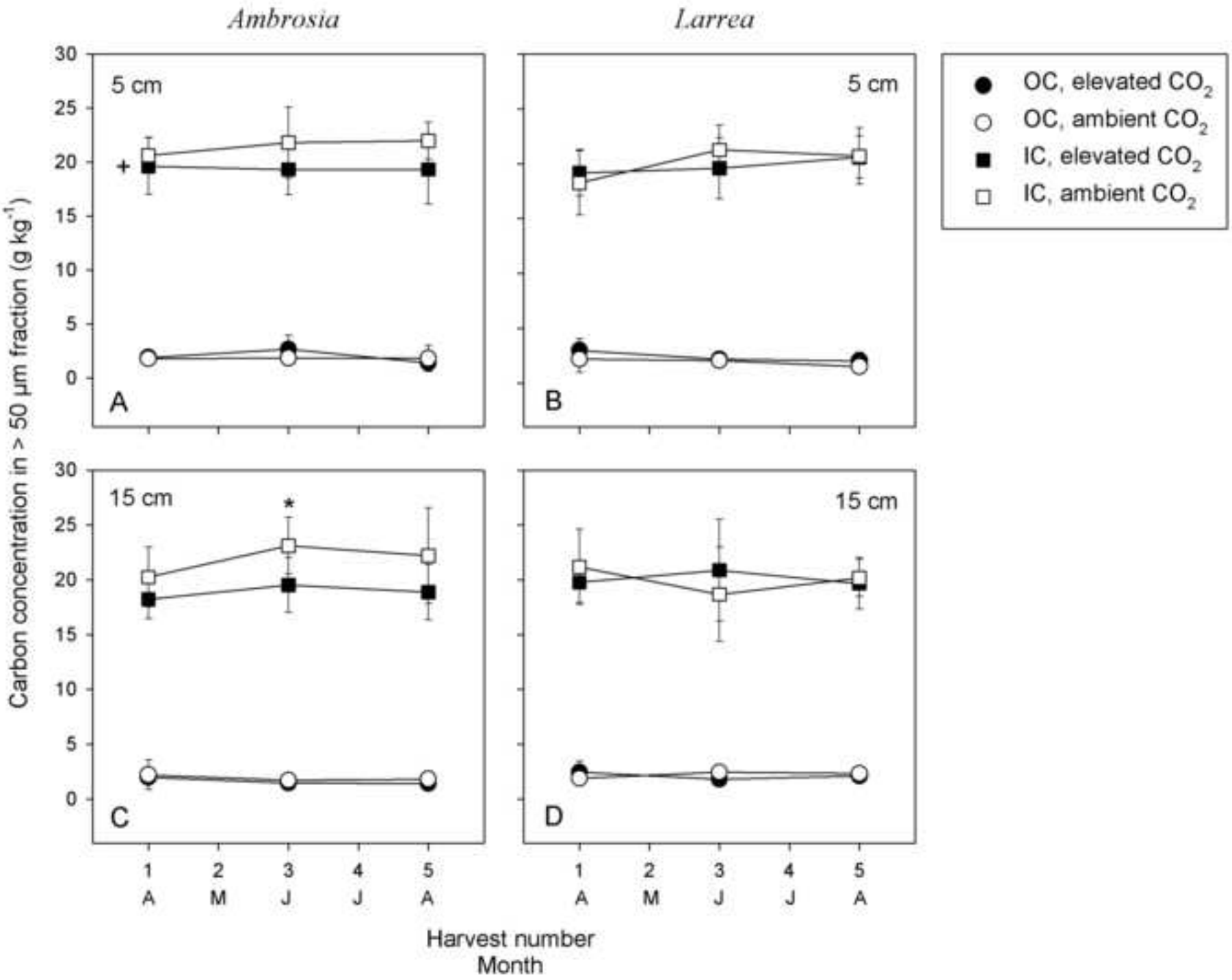

Figure 4 new

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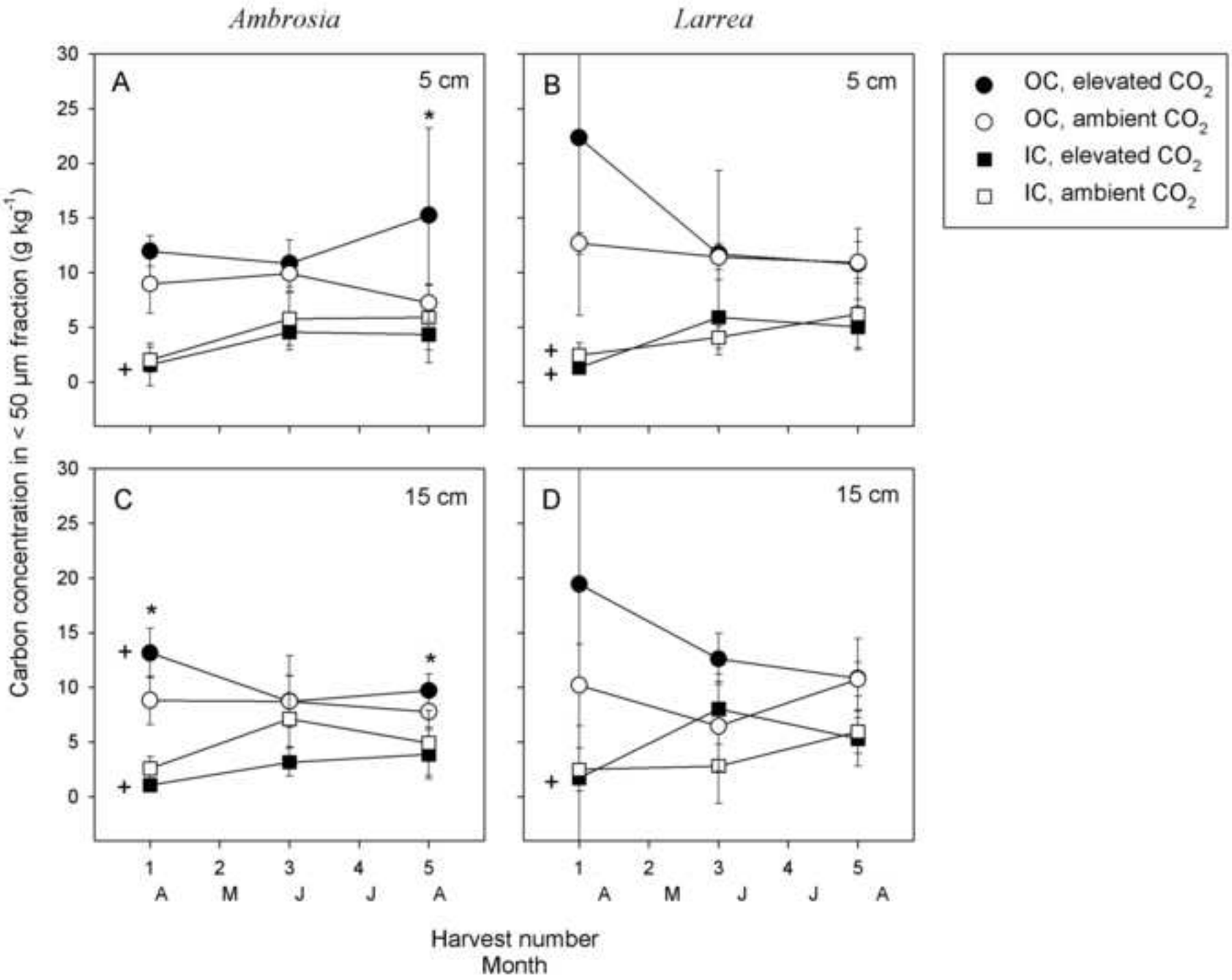

Figure 5 revised

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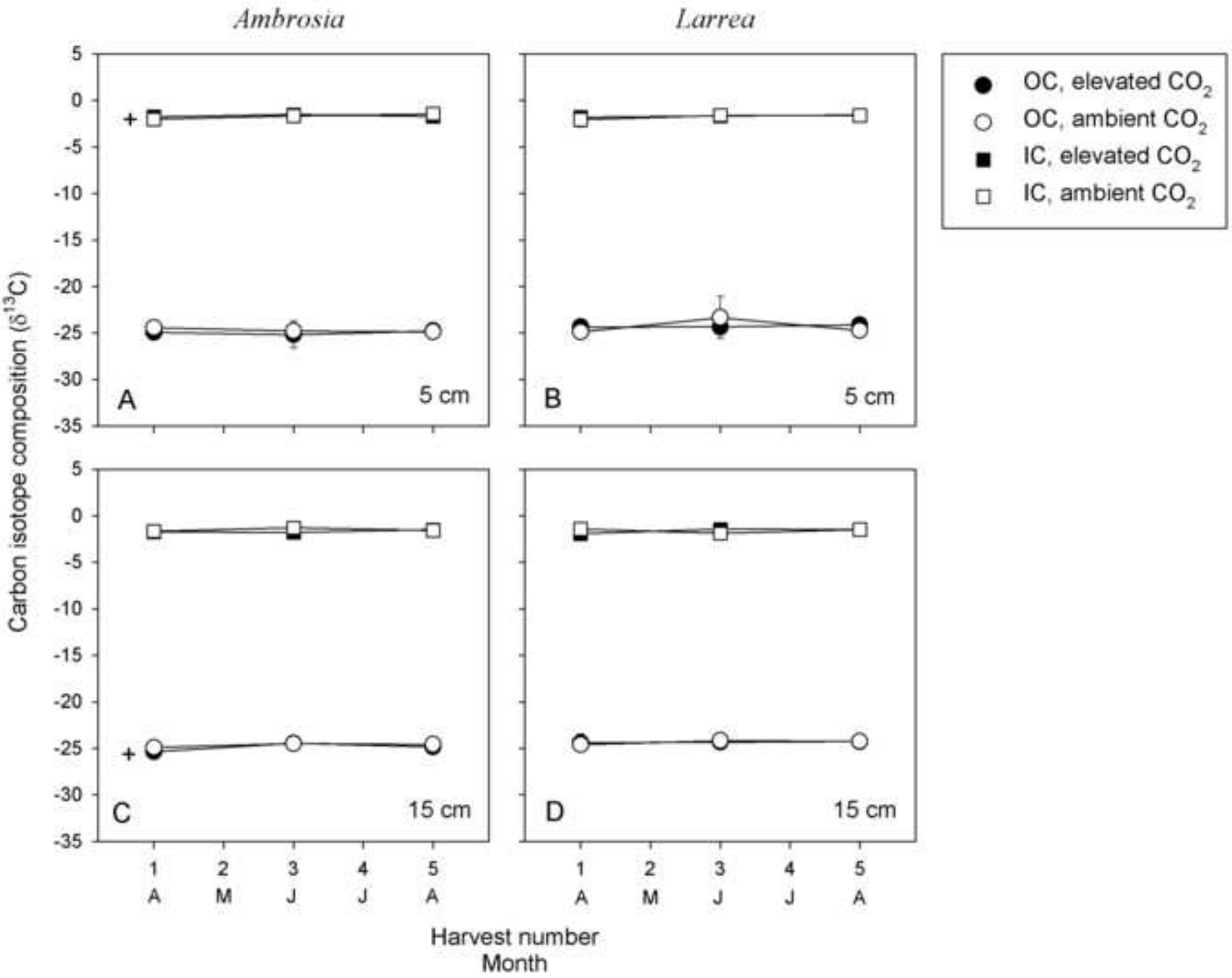

Figure 6 revised

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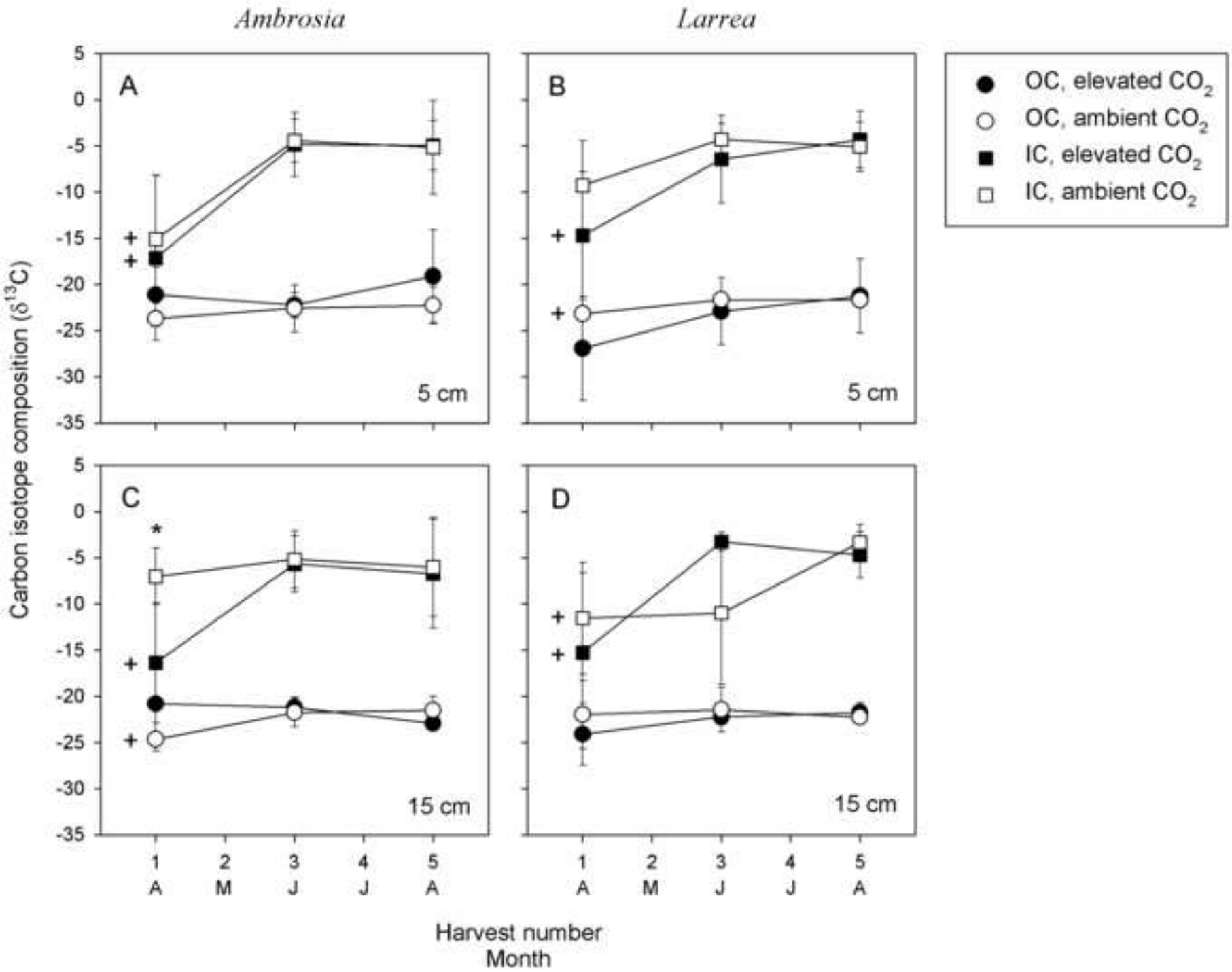

ANNEX. Additional numerical and statistical information

Part 1. General data about the soil samples

Table 1.1. Weight of fine material (< 50 μm), as percent of total weight. Data are averages ± standard deviations.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	14.62 ± 2.34	11.68 ± 3.08	13.19 ± 3.07
	15	15.17 ± 2.31	11.80 ± 3.30	13.44 ± 3.30
	All	14.89 ± 2.31	11.74 ± 3.15	13.32 ± 3.17
Larrea	5	15.09 ± 2.71	11.81 ± 4.47	13.45 ± 4.01
	15	14.20 ± 2.11	12.45 ± 4.11	13.33 ± 3.34
	All	14.65 ± 2.44	12.13 ± 4.25	13.39 ± 3.67
All	5	14.85 ± 2.51	11.74 ± 3.80	13.32 ± 3.55
	15	14.69 ± 2.24	12.11 ± 3.68	13.38 ± 3.30
	All	14.77 ± 2.36	11.93 ± 3.72	13.35 ± 3.41

Table 1.2. ANOVA results. Relevance of the several factors in the experiment as to the above data.

Factor	p > F
A. Species	0.890
B. Depth	0.840
C. CO_2	< 0.001 ***
A × B	0.665
A × C	0.546
B × C	0.604
A × B × C	0.353

Table 1.3. Organic carbon (OC) content in fine material (< 50 μm), as percent of total OC. Data are averages ± standard deviations.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	84.71 ± 6.09	86.44 ± 8.27	85.55 ± 7.16
	15	83.20 ± 5.93	81.00 ± 22.68	82.10 ± 16.36
	All	83.93 ± 5.96	83.55 ± 17.44	83.75 ± 12.85
Larrea	5	87.83 ± 3.33	86.32 ± 4.21	87.13 ± 3.78
	15	78.03 ± 21.54	85.34 ± 7.10	81.81 ± 15.95
	All	83.26 ± 15.44	85.81 ± 5.80	84.51 ± 11.71
All	5	86.27 ± 5.08	86.38 ± 6.52	86.33 ± 5.76
	15	80.87 ± 15.06	83.04 ± 17.12	81.97 ± 16.05
	All	83.61 ± 11.40	84.63 ± 13.20	84.11 ± 12.28

Table 1.4. ANOVA results. Relevance of the several factors in the experiment as to the above data.

Factor	p > F
A. Species	0.807
B. Depth	0.047 *
C. CO_2	0.547
A × B	0.665
A × C	0.479
B × C	0.581
A × B × C	0.152

Table 1.5. Inorganic carbon (IC: carbonates) in fine material (< 50 μm), as percent of total IC. Data are averages ± standard deviations.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	3.91 ± 2.88	2.68 ± 2.15	3.30 ± 2.58
	15	3.81 ± 3.12	1.87 ± 1.61	2.81 ± 2.62
	All	3.86 ± 2.96	2.26 ± 1.91	3.05 ± 2.59
Larrea	5	3.82 ± 2.62	3.06 ± 3.09	3.45 ± 2.84
	15	2.96 ± 2.10	4.13 ± 4.28	3.53 ± 3.34
	All	3.39 ± 2.38	3.59 ± 3.71	3.49 ± 3.07
All	5	3.86 ± 2.71	2.86 ± 2.61	3.37 ± 2.69
	15	3.38 ± 2.65	2.93 ± 3.31	3.16 ± 2.99
	All	3.62 ± 2.67	2.90 ± 2.97	3.26 ± 2.84

Table 1.6. ANOVA results. Relevance of the several factors in the experiment as to the above data.

Factor	p > F
A. Species	0.382
B. Depth	0.720
C. CO_2	0.156
A × B	0.558
A × C	0.068
B × C	0.530
A × B × C	0.176

Part 2. Contents of total organic and inorganic carbon in the fractions

2.1) COARSE FRACTION. Organic carbon

Table 2.1.1. Organic carbon in the coarse fraction (g / kg). Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	1.84 ± 0.69	2.01 ± 1.01	1.92 ± 0.86
	15	1.93 ± 0.88	1.64 ± 0.39	1.78 ± 0.68
	All	1.86 ± 0.78	1.81 ± 0.76	1.85 ± 0.77
Larrea	5	1.93 ± 0.73	2.39 ± 0.92	2.17 ± 0.85
	15	2.21 ± 0.52	2.15 ± 0.72	2.18 ± 0.62
	All	2.06 ± 0.64	2.27 ± 0.82	2.17 ± 0.74
All	5	1.88 ± 0.70	2.20 ± 0.97	2.05 ± 0.86
	15	2.06 ± 0.74	1.88 ± 0.62	1.97 ± 0.68
	All	1.97 ± 0.72	2.04 ± 0.82	2.01 ± 0.08

Table 2.1.2. ANOVA results for OC content in the coarse fraction: Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.016 *		
B. Depth	0.653	0.462	0.910
C. CO_2	0.577	0.752	0.262
A × B	0.543		
A × C	0.312		
B × C	0.063	0.224	0.158
A × B × C	0.910		

Table 2.1.3. Effect of harvest (H1, H3, H5) on total OC content in the coarse fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	1.80 ± 0.27	1.87 ± 0.44	1.84 ± 1.21	0.989
		Elev	1.92 ± 0.54	2.68 ± 1.29	1.40 ± 0.60	0.076
	15	Amb	2.22 ± 1.36	1.73 ± 0.31	1.83 ± 0.73	0.633
		Elev	1.99 ± 0.41	1.47 ± 0.22	1.42 ± 0.21	0.006 **
Larrea	5	Amb	2.17 ± 1.18	2.05 ± 0.19	1.50 ± 0.16	0.301
		Elev	2.97 ± 1.05	2.18 ± 0.41	2.02 ± 0.75	0.160
	15	Amb	1.90 ± 0.34	2.46 ± 0.40	2.32 ± 0.68	0.174
		Elev	2.45 ± 0.99	1.82 ± 0.61	2.13 ± 0.38	0.371

Table 2.1.4. Effect of CO_2 treatment (ambient /elevated) on OC content in the coarse fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.672	0.172	0.451	0.582
	15	0.676	0.113	0.209	0.207
Larrea	5	0.243	0.678	0.170	0.112
	15	0.228	0.086	0.579	0.809

2.2) COARSE FRACTION. Inorganic carbon (carbonates)

Table 2.2.1. Inorganic carbon in the coarse fraction (g / kg). Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	21.51 ± 2.31	19.41 ± 2.59	20.46 ± 2.64
	15	21.84 ± 3.29	18.81 ± 2.18	20.28 ± 3.13
	All	21.70 ± 2.80	19.10 ± 2.38	20.37 ± 2.89
Larrea	5	20.07 ± 2.79	19.75 ± 2.26	19.91 ± 2.51
	15	20.01 ± 3.29	20.11 ± 3.05	20.06 ± 3.13
	All	20.04 ± 3.01	19.94 ± 2.66	19.99 ± 2.82
All	5	20.77 ± 2.64	19.58 ± 2.40	20.18 ± 2.57
	15	20.90 ± 3.37	19.46 ± 2.70	20.17 ± 3.11
	All	20.83 ± 3.00	19.52 ± 2.54	20.18 ± 2.85

Table 2.2.2. ANOVA results for IC content: Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.386		
B. Depth	0.991	0.824	0.826
C. CO_2	0.005 **	< 0.001 ***	0.876
A × B	0.754		
A × C	0.009 **		
B × C	0.782	0.464	0.763
A × B × C	0.473		

Table 2.2.3. Effect of harvest (H1, H3, H5) on total IC content in the coarse fraction

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	20.62 ± 1.68	21.80 ± 3.29	21.97 ± 1.70	0.616
		Elev	19.62 ± 2.61	19.33 ± 2.35	19.32 ± 3.23	0.980
	15	Amb	20.25 ± 2.73	23.12 ± 2.58	22.20 ± 4.37	0.325
		Elev	18.24 ± 1.79	19.52 ± 2.50	18.87 ± 2.53	0.633
Larrea	5	Amb	18.25 ± 2.94	21.25 ± 2.28	20.70 ± 2.56	0.140
		Elev	19.17 ± 2.08	19.58 ± 2.79	20.60 ± 1.91	0.567
	15	Amb	21.18 ± 3.44	18.68 ± 4.29	20.17 ± 1.69	0.443
		Elev	19.76 ± 1.84	20.88 ± 4.65	19.68 ± 2.36	0.771

Table 2.2.4. Effect of CO_2 treatment (ambient /elevated) on IC content in the coarse fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.491	0.166	0.106	0.018 *
	15	0.140	0.044 *	0.148	0.003 **
Larrea	5	0.547	0.284	0.978	0.718
	15	0.395	0.414	0.692	0.925

2.3) FINE FRACTION. Organic carbon.

Table 2.3.1. Organic carbon content (g / kg) in the fine fraction. Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	8.70 ± 2.22	12.39 ± 4.39	10.43 ± 3.84
	15	8.41 ± 1.96	10.63 ± 3.24	9.52 ± 2.87
	All	8.55 ± 2.07	11.46 ± 3.86	9.96 ± 3.38
Larrea	5	11.66 ± 1.48	14.17 ± 10.15	12.88 ± 7.12
	15	9.21 ± 3.67	14.61 ± 14.28	11.91 ± 10.62
	All	10.44 ± 3.02	14.40 ± 12.25	12.38 ± 9.01
All	5	10.14 ± 2.40	13.28 ± 7.74	11.63 ± 5.79
	15	8.80 ± 2.90	12.56 ± 10.24	10.68 ± 7.71
	All	9.47 ± 2.72	12.90 ± 9.07	11.14 ± 6.83

Table 2.3.2. ANOVA results for OC content in the fine fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.044 *		
B. Depth	0.387	0.180	0.657
C. CO_2	0.004 **	< 0.001 ***	0.085
A × B	0.995		
A × C	0.671		
B × C	0.759	0.334	0.523
A × B × C	0.352		

Table 2.3.3. Effect of harvest (H1, H3, H5) on total OC content in the fine fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	8.97 ± 2.69	9.93 ± 1.64	7.24 ± 1.70	0.098
		Elev	11.97 ± 1.40	10.84 ± 2.13	15.24 ± 7.99	0.310
	15	Amb	8.82 ± 2.20	8.71 ± 2.37	7.77 ± 1.40	0.635
		Elev	13.15 ± 2.27	8.73 ± 4.16	9.70 ± 1.51	0.042 *
Larrea	5	Amb	12.68 ± 0.98	11.42 ± 1.17	10.94 ± 1.87	0.154
		Elev	22.31 ± 16.23	11.70 ± 7.65	10.80 ± 3.24	0.175
	15	Amb	10.20 ± 3.75	6.46 ± 4.07	10.76 ± 1.56	0.123
		Elev	19.44 ± 23.47	12.60 ± 2.34	10.83 ± 3.61	0.599

Table 2.3.4. Effect of CO_2 treatment (ambient /elevated) on OC content in the fine fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.058	0.428	0.040 *	0.005 **
	15	0.011 *	0.991	0.044 *	0.021 *
Larrea	5	0.220	0.932	0.935	0.337
	15	0.363	0.019 *	0.971	0.153

2.4) FINE FRACTION. Inorganic carbon

Table 2.4.1. Inorganic C in the fine fraction (g / kg). Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	4.56 ± 2.84	3.62 ± 2.39	4.10 ± 2.63
	15	5.01 ± 3.37	2.62 ± 1.85	3.75 ± 2.90
	All	4.78 ± 3.07	3.09 ± 2.15	3.92 ± 2.76
Larrea	5	4.34 ± 2.53	4.26 ± 2.98	4.30 ± 2.72
	15	3.81 ± 2.85	4.81 ± 3.51	4.29 ± 3.18
	All	4.07 ± 2.67	4.53 ± 3.21	4.30 ± 2.93
All	5	4.45 ± 2.66	3.94 ± 2.68	4.20 ± 2.66
	15	4.41 ± 3.13	3.62 ± 2.91	4.01 ± 3.03
	All	4.43 ± 2.88	3.78 ± 2.78	4.10 ± 2.84

Table 2.4.2. ANOVA results for inorganic carbon in the fine fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.463		
B. Depth	0.782	0.667	0.993
C. CO_2	0.212	0.010 **	0.529
A × B	0.771		
A × C	0.028 *		
B × C	0.853	0.257	0.459
A × B × C	0.191		

Table 2.4.3. Effect of harvest (H1, H3, H5) on total IC content in the fine fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	2.03 ± 1.16	5.77 ± 2.40	5.88 ± 2.96	0.017 *
		Elev	1.59 ± 1.93	4.56 ± 1.65	4.35 ± 2.59	0.067
	15	Amb	2.60 ± 1.06	7.11 ± 3.93	4.91 ± 2.98	0.077
		Elev	1.08 ± 0.38	3.17 ± 1.26	3.87 ± 2.25	0.008 **
Larrea	5	Amb	2.45 ± 1.11	4.07 ± 1.00	6.19 ± 3.28	0.036 *
		Elev	1.32 ± 0.63	5.93 ± 3.43	5.04 ± 1.89	0.016 *
	15	Amb	2.48 ± 1.97	2.82 ± 3.43	5.96 ± 2.00	0.058
		Elev	1.71 ± 1.20	8.03 ± 3.22	5.31 ± 2.49	0.003 **

Table 2.4.4. Effect of CO_2 treatment (ambient /elevated) on IC content in the fine fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.651	0.336	0.363	0.296
	15	0.005 **	0.041 *	0.508	0.012 *
Larrea	5	0.083	0.231	0.473	0.932
	15	0.433	0.038 *	0.642	0.373

Part 3. Data about the isotopic composition ($\delta^{13}C$) of the studied samples

3.1) COARSE FRACTION: $\delta^{13}C$ of organic carbon

Table 3.1.1. Organic carbon $\delta^{13}C$ in the coarse fraction. Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	-24.70 ± 0.45	-24.95 ± 0.98	-24.83 ± 0.77
	15	-24.66 ± 0.50	-24.92 ± 0.65	24.80 ± 0.59
	All	-24.68 ± 0.47	-24.94 ± 0.81	-24.81 ± 0.68
Larrea	5	-24.30 ± 1.49	-24.28 ± 0.53	-24.29 ± 1.09
	15	-24.37 ± 0.54	-24.34 ± 0.71	-24.35 ± 0.62
	All	-24.33 ± 1.12	-24.31 ± 0.61	-24.32 ± 0.89
All	5	-24.49 ± 1.12	-24.60 ± 0.84	-24.55 ± 0.98
	15	-24.52 ± 0.53	-24.65 ± 0.73	-24.59 ± 0.64
	All	-24.51 ± 0.86	-24.63 ± 0.78	-24.57 ± 0.82

Table 3.1.2. ANOVA results for $\delta^{13}C$ of organic carbon in the coarse fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	< 0.001 ***		
B. Depth	0.894	0.846	0.758
C. CO_2	0.398	0.122	0.916
A × B	0.715		
A × C	0.310		
B × C	0.999	0.981	0.985
A × B × C	0.977		

Table 3.1.3. Effect of harvest (H1, H3, H5) on the $\delta^{13}C$ of organic carbon in the coarse fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	-24.43 ± 0.57	-24.79 ± 0.34	-24.86 ± 0.41	0.286
		Elev	-24.93 ± 0.83	-25.16 ± 1.46	-24.76 ± 0.53	0.799
	15	Amb	-24.92 ± 0.68	-24.49 ± 0.29	-24.59 ± 0.42	0.314
		Elev	-25.37 ± 0.67	-24.46 ± 0.63	-24.87 ± 0.24	0.032 *
Larrea	5	Amb	-24.88 ± 0.34	-23.34 ± 2.26	-24.74 ± 0.47	0.147
		Elev	-24.36 ± 0.76	-24.34 ± 0.32	-24.13 ± 0.48	0.737
	15	Amb	-24.62 ± 0.48	-24.17 ± 0.58	-24.26 ± 0.57	0.381
		Elev	-24.41 ± 0.90	-24.34 ± 0.51	-24.27 ± 0.76	0.946

Table 3.1.4. Effect of CO_2 treatment (ambient /elevated) on the $\delta^{13}C$ of organic carbon in the coarse fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.303	0.556	0.729	0.358
	15	0.258	0.931	0.196	0.187
Larrea	5	0.155	0.307	0.063	0.959
	15	0.639	0.649	0.992	0.902

3.2) COARSE FRACTION. $\delta^{13}C$ of inorganic carbon (IC: carbonates)

Table 3.2.1. Organic carbon $\delta^{13}C$ in the coarse fraction. Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	-1.69 ± 0.41	-1.65 ± 0.31	-1.67 ± 0.36
	15	-1.51 ± 0.39	-1.58 ± 0.56	-1.55 ± 0.48
	All	-1.60 ± 0.41	-1.62 ± 0.46	-1.61 ± 0.43
Larrea	5	-1.76 ± 0.59	-1.60 ± 0.60	-1.68 ± 0.59
	15	-1.60 ± 0.46	-1.63 ± 0.58	-1.61 ± 0.51
	All	-1.68 ± 0.53	-1.61 ± 0.58	-1.65 ± 0.55
All	5	-1.72 ± 0.50	-1.62 ± 0.48	-1.67 ± 0.49
	15	-1.56 ± 0.42	-1.61 ± 0.57	-1.58 ± 0.50
	All	-1.64 ± 0.47	-1.61 ± 0.52	-1.63 ± 0.49

Table 3.2.2. ANOVA results for $\delta^{13}C$ of inorganic carbon in the coarse fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.650		
B. Depth	0.267	0.238	0.628
C. CO_2	0.791	0.856	0.631
A × B	0.725		
A × C	0.625		
B × C	0.377	0.595	0.480
A × B × C	0.821		

Table 3.2.3. Effect of harvest (H1, H3, H5) on the $\delta^{13}C$ of inorganic carbon in the coarse fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	-2.03 ± 0.31	-1.65 ± 0.48	-1.44 ± 0.19	0.042 *
		Elev	-1.74 ± 0.18	-1.52 ± 0.22	-1.72 ± 0.45	0.444
	15	Amb	-1.66 ± 0.22	-1.30 ± 0.43	-1.59 ± 0.45	0.249
		Elev	-1.75 ± 0.41	-1.48 ± 0.82	-1.49 ± 0.46	0.629
Larrea	5	Amb	-2.07 ± 0.80	-1.59 ± 0.43	-1.62 ± 0.43	0.301
		Elev	-1.80 ± 0.46	-1.68 ± 0.52	-1.32 ± 0.77	0.380
	15	Amb	-1.42 ± 0.43	-1.91 ± 0.53	-1.47 ± 0.26	0.120
		Elev	-1.93 ± 0.74	-1.45 ± 0.50	-1.50 ± 0.40	0.300

Table 3.2.4. Effect of CO_2 treatment (ambient /elevated) on the $\delta^{13}C$ of inorganic carbon in the coarse fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.102	0.548	0.194	0.771
	15	0.608	0.634	0.718	0.652
Larrea	5	0.490	0.741	0.434	0.434
	15	0.172	0.158	0.869	0.864

3.3) FINE FRACTION: $\delta^{13}C$ of organic carbon

Table 3.3.1. Organic carbon $\delta^{13}C$ in the fine fraction. Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	–22.81 ± 1.97	-21.04 ± 3.21	-21.98 ± 2.73
	15	-22.53 ± 1.87	-21.77 ± 2.97	-22.17 ± 2.44
	All	-22.67 ± 1.89	-21.41 ± 3.06	-22.07 ± 2.57
Larrea	5	-22.14 ± 0.98	-23.36 ± 4.76	-22.71 ± 3.32
	15	-21.94 ± 2.51	-22.79 ± 2.32	-22.38 ± 2.41
	All	-22.04 ± 1.85	-23.06 ± 3.61	-22.54 ± 2.88
All	5	-22.49 ± 1.58	-22.16 ± 4.13	-22.34 ± 3.03
	15	-22.25 ± 2.17	-22.30 ± 2.66	-22.27 ± 2.41
	All	-22.37 ± 1.88	-22.23 ± 3.42	-22.30 ± 2.72

Table 3.3.2. ANOVA results for $\delta^{13}C$ of organic carbon in the fine fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.286		
B. Depth	0.863	0.733	0.605
C. CO_2	0.817	0.051	0.168
A × B	0.536		
A × C	0.020 *		
B × C	0.741	0.431	0.809
A × B × C	0.484		

Table 3.3.3. Effect of harvest (H1, H3, H5) on the $\delta^{13}C$ of organic carbon in the fine fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	-23.72 ± 0.83	-22.61 ± 2.57	-22.26 ± 1.99	0.477
		Elev	-21.13 ± 2.98	-22.25 ± 1.34	-19.12 ± 5.03	0.342
	15	Amb	-24.64 ± 0.38	-21.76 ± 1.52	-21.53 ± 1.54	0.003 **
		Elev	-20.81 ± 5.11	-21.20 ± 1.13	-22.94 ± 0.44	0.483
Larrea	5	Amb	-23.17 ± 0.72	-21.66 ± 0.61	-21.69 ± 0.82	0.007 **
		Elev	-26.95 ± 5.64	-22.93 ± 3.64	-21.26 ± 4.03	0.182
	15	Amb	-21.99 ± 3.68	-21.44 ± 2.41	-22.26 ± 0.62	0.900
		Elev	-24.12 ± 3.35	-22.25 ± 0.81	-21.75 ± 1.07	0.205

Table 3.3.4. Effect of CO_2 treatment (ambient /elevated) on the $\delta^{13}C$ of organic carbon in the fine fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.098	0.770	0.198	0.066
	15	0.134	0.549	0.057	0.386
Larrea	5	0.175	0.413	0.819	0.325
	15	0.320	0.499	0.379	0.331

3.4) FINE FRACTION: $\delta^{13}C$ of inorganic carbon (IC: carbonates)

Table 3.4.1. Inorganic carbon $\delta^{13}C$ in the fine fraction. Averages ± standard deviations for all harvests pooled.

		CO_2 treatment		
Species	Depth	Ambient [CO_2]	Elevated [CO_2]	All
Ambrosia	5	-8.24 ± 6.95	-8.50 ± 7.69	-8.37 ± 7.21
	15	-6.01 ± 3.86	-9.93 ± 7.20	-8.08 ± 6.12
	All	-7.16 ± 5.70	-9.26 ± 7.36	-8.22 ± 6.63
Larrea	5	-6.04 ± 3.70	8.13 ± 6.48	-7.08 ± 5.30
	15	-8.47 ± 6.56	-8.20 ± 7.66	-8.34 ± 7.00
	All	-7.25 ± 5.38	-8.16 ± 6.97	-7.70 ± 6.18
All	5	-7.17 ± 5.64	-8.31 ± 7.00	-7.73 ± 6.33
	15	-7.24 ± 5.45	-9.14 ± 7.36	-8.20 ± 6.51
	All	-7.20 ± 5.51	-8.73 ± 7.14	-7.97 ± 6.40

Table 3.4.2. ANOVA results for $\delta^{13}C$ of inorganic carbon in the fine fraction. Relevance of each factor, either considering all samples altogether, or each species separately.

	Values for p > F		
Factor	All samples	*Ambrosia*	*Larrea*
A. Species	0.676		
B. Depth	0.699	0.800	0.416
C. CO_2	0.174	0.188	0.553
A × B	0.453		
A × C	0.592		
B × C	0.764	0.247	0.444
A × B × C	0.173		

Table 3.4.3. Effect of harvest (H1, H3, H5) on $\delta^{13}C$ of inorganic carbon in the fine fraction.

Species	Depth	CO_2	H1	H3	H5	p > F
Ambrosia	5	Amb	-15.13 ± 6.90	-4.44 ± 2.34	-5.16 ± 5.06	0.004 **
		Elev	-17.14 ± 8.97	-4.85 ± 3.44	-4.94 ± 2.70	0.003 **
	15	Amb	-7.02 ± 3.09	-5.16 ± 3.07	-6.00 ± 5.37	0.754
		Elev	-16.36 ± 6.50	-5.66 ± 3.03	-6.72 ± 5.88	0.004 **
Larrea	5	Amb	-9.26 ± 4.81	-4.31 ± 1.71	-5.09 ± 2.68	0.052
		Elev	-14.71 ± 6.92	-6.44 ± 4.74	-4.33 ± 3.07	0.011 *
	15	Amb	-11.53 ± 6.05	-10.97 ± 7.75	-3.32 ± 1.92	0.045 *
		Elev	-15.25 ± 8.61	-3.25 ± 0.99	-4.70 ± 2.48	0.006 **

Table 3.4.4. Effect of CO_2 treatment (ambient /elevated) on $\delta^{13}C$ of inorganic carbon in the fine fraction. Analysed per experimental condition (species, depth) and per harvest. Values are the p > F.

Species	Depth	H1	H2	H3	All
Ambrosia	5	0.683	0.816	0.929	0.919
	15	0.014 *	0.784	0.831	0.053
Larrea	5	0.186	0.324	0.657	0.257
	15	0.407	0.058	0.327	0.915

Review of Rovira et al. (2017)

The manuscript of Rovira et al., assesses soil carbon cycling in arid environments by tracing the 13CO2 signal from a Free Air CO2 Enrichment Experiment in organic and inorganic C. My general comments are:

1. The study makes an important contribution to our knowledge due to the following reasons:

i. While there are a number of studies in temperate and humid environments, there is a clear lack of studies in dry ecosystems especially 13C tracer studies. The results show a very small (non-detectable) input of C from recent assimilates into soils. Although the result is not very surprising, it is still important to quantify the dynamics of new C in soils (and hence C cycling rates) under these conditions for both: understanding the functioning of arid ecosystems and interpreting other results of the desert FACE experiment.

ii. The study also assesses the dynamics in inorganic C. Most studies focus on soil organic matter but especially in arid environments carbonate dynamics may be equally important. However, data is rather scarce.

2. The study is conducted within a highly sophisticated set-up the Nevada FACE experiment adding 13C depleted CO2 on relative large plots. It applies novel soil fractionation methods and uses stable isotopes as a powerful technique

3. The interpretation of the data is very good and reflects our current knowledge and the existing literature

In summary, I am recommending the manuscript to be reviewed by Plant and Soil and as it examines and provides data on an important process, I am confident that it becomes accepted.

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