

ACCEPTED VERSION

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Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings?

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Highlights

- Differences in soil C under N-fixing and non-N-fixing trees were studied
- The ability of N-fixers to increase soil C and N was site specific
- Need to study what site traits affect the potential of N-fixers to increase soil C

[Response to review of Ms. No. AGEE11155 - Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings? Please see our response to the editors and reviewers below in blue.](#)

Editor's comments:

Associate Editor:

The topic of the manuscript is of interest to AGEE, however, there are concerns that the results are somewhat weak or limited. Review #1 has concerns about the adequacy of the sampling design - this observation is also shared by Review #2. I agree with Review #2, that the nature of this manuscript is a short communication - it addresses an important question, but further studies are needed for more conclusive statements.

I would encourage the authors to revise their manuscript as a Short Communication (SC) - the format is the same but a little reduction in length (in Discussion and References) would be beneficial. All the review comments need to be individually addressed.

M.R. Carter

Dear M.R. Carter,

Thank you for your and the reviewers feedback and thoughtful comments. Hereby we would like to resubmit the manuscript as a Short Communication. We have substantially reduced the length of the manuscript, and worked hard to address of the reviewers and editors comments; we think the manuscript is substantially improved in light of these suggestions and so have thanked the reviewers and editor in the acknowledgements section of the manuscript. To achieve the six printed pages limit (as per the authors guidelines for a short communication found on the website) we have shortened the Introduction, and merged the Results Discussion and Conclusion section into one section. In addition we have removed two of the tables and submitted them as online appendices. Please see blow the responses to the reviewers comments.

Reviewer #1:

This manuscript describes a study which evaluates the impact of tree species (non-fixing versus N₂ fixing trees), and landscape position (upland vs. riparian) on the C status of land that was converted from agricultural land to a forested area (afforestation) over 15 years ago. The following questions and comments should be addressed before this paper could be considered for publication.

1. I was surprised that an anaerobic mineralization procedure was used. Were these sites typically saturated? Additional information is required to justify the use of an anaerobic assay.

The PMN method is a standardised method that is widely used as a measure of N cycling in soils (see references cited in the manuscript). Briefly, the reason that the incubation is performed under anaerobic conditions is that if it were conducted under aerobic conditions, any NH₄⁺ generated could be nitrified (i.e. into NO₃⁻), making it impossible to measure the rate at which organic matter in mineralized (i.e. NH₄⁺ production).

2. Soil samples were collected at 0-10 and 10-20 cm depths. Given that the trees were well established, was there an LFH layer present (organic layer over the mineral soils)? If so how was this layer handled as far as sampling and analysis is concerned?

Added sentence in methods (P. 5, L. 54). "Litter or grassy vegetation was removed from the surface before sampling. No pronounced organic layer was present at any of the sites."

3. Additional information is required concerning the trees. The results include measurements of tree breast height diameter but these measurements are not described in the materials and methods section.

Added sentence in methods (P.6, L. 14). "The diameter of the stem of each tree was measured at breast height (approx. 1.3 m)."

4. What percentage of each tree species and for the fixing versus non-fixing species? The tree densities are provided, but these values should also be broken up into the respective tree species. Please include both this analysis and the corresponding description in the materials and methods section.

This information has been added to the table. To meet the page-limit requirements for a short communication, this table is removed from the manuscript but is provided as supplementary online material. (Appendix, table 1A.)

5. There were many instances in which the spaces separating words were missing. Please revise the paper and ensure that words are separated.

This must have been an issue that occurred when uploading the manuscript and I failed to notice it when checking the PDF file that was created. Apologies for this, I will make sure to check the PDF file more thoroughly.

6. The term deep soil layer is misleading as 10-20 cm is not very deep and in an agricultural context is considered the 'top soil' or Ah horizon. I would suggest you just refer to this depth as the 10-20 cm soil layer throughout the paper.

We take this point well and have changed the terms 'shallow' and 'deep' to '0-10 cm', and '10-20 cm', throughout the manuscript.

7. It appears that the data for total C and total N is repeated in figure 1. It is recommended that this data should not be repeated.

Indeed, this was not supposed to be repeated, the columns have been removed from the table. To meet the page-limit requirements for a short communication, this table is removed from the manuscript but is provided as supplementary online material. (Appendix, table 2A.)

8. Only one location chosen for soil sampling (ie. from 0.25 to 1 m from the tree trunk). Is the root geometry similar between N₂ fixing and non-fixing tree species? What impact would this location have on the results and interpretation from an ecological perspective? In particular, if you only observe a localized effect on soil C within 1 m of the tree, would there be a net ecosystem benefit to including N₂ fixing trees if the entire area was considered?

The location for soil sampling was chosen to represent soil of which we could be as certain as possible that it was influenced by the tree, i.e. soil underneath the canopy of the tree that receives organic input from litter of the respective trees. It is indeed important to question how far away from the tree the (potential) benefits of the N-fixing trees reach and whether it will deliver a net ecosystem benefit. I have added a sentence in the conclusions (P.10, L. 50) to acknowledge this: "Furthermore, in the current study, soil was sampled directly underneath the canopies of individual trees. More research is required to determine if the (potential) increases in soil C under N-fixing trees have a significant effect in soil C sequestration on a whole planting and landscape scale."

9. Given that soil mineral N is so transitory as a result of plant uptake, leaching, etc., how much can you say by only sampling each site once?

The reviewer raises an important point. Although measuring mineral N at a single time point is not unusual in such studies (see references cited in manuscript), we agree that a single measurement has its limitations. We have therefore been careful to not comment on potential temporal patterns. Further, in recognition of the temporal variation often seen in mineral N pools we decided to measure PMN in this study as an indication of soil N cycling processes. In our earlier work (see Smith et al., 2012 cited in manuscript) we have found PMN to provide a very reliable measure of N cycling processes in soils in the same region where this study was undertaken. In light of the reviewers comment we do acknowledge these points in the discussion and refer to Smith et al (2012) who found in the same study area that PMN is more temporally stable (see P, 9, L. 9) than NO₃ and NH₄.

Specific comments

Highlights - please revise the third highlights as it appears some words may be missing.

Must have occurred during uploading, I will check the PDF more carefully.

Pg 3 | 10 ... which promotes soil C sequestration, a potentially significant factor in mitigating ...

This sentence is removed in the current version, to meet the page-limit for a short communication.

Pg 3| 39 If tree species influence soil C sequestration differentially, then afforestation...

This sentence is removed in the current version, to meet the page-limit for a short communication.

Pg 4 | 1 ... potentially offering a greater C sequestration potential compared to commercial plantations.

This sentence is removed in the current version, to meet the page-limit for a short communication.

Pg 7 | 49 to 55. This equation is not necessary. Further, if this equation were kept, additional correction factors are required to obtain the t/ha units for the soil chemical properties.

This sentence is removed in the current version.

Pg 9 | 25-27 Total N was marginally higher with N₂ fixers compared to non-fixers in the 10-20 cm soil layer.

Corrected.

Pg 9 | 37, 46 and elsewhere. You often refer to a model but the only model presented is a statistical model.

Please clarify what you mean. If a non-statistical model is used, then additional information is required in the Materials and Methods section and discussion of the model validation and outputs is required.

Clarified by rephrasing as “statistical model” and “analysis”.

Pg 9 | 44. At site R2, the treatments did not significantly affect total C and N between tree types.

This sentence is removed in the current version, to meet the page-limit for a short communication.

Reviewer #2:

This manuscript addresses relevant questions, which are within the scope of AGEE.

However, the conclusions are rather complicated; the question mark in the title of the manuscript reflects this.

Overall, there was a significant increase in subsoil C near N fixing trees compared to subsoil C near non-fixing trees (Table 2). However, in none of the sites there was a significant difference in subsoil C between fixing and non-fixing trees, mainly because the differences in subsoil C were small (~<15%). Moreover, there were relatively large (~20%), significant but contrasting differences in topsoil C between fixing and non-fixing trees within sites. Authors emphasize especially the significant differences in subsoil C, which are small. They also mention that the ability of N-fixers to increase soil C (and N) was site-specific, but do not indicate that these were relative large.

Added sentences to bring attention to these points (P.8 L.12) “[..], though overall levels of soil C and N in this soil layer are low (see Fig. 1c and d.)” and (P9, L. 35): “[..] large significant differences in total C and N were found between tree types in the 0-10 cm soil layer at some sites.”

After reducing the length of the discussion, less emphasis is put on the small differences in subsoil C.

Authors did make an attempt to analyse the total net effect of fixing versus non-fixing trees on soil C in the top soil + sub soil.

The factors controlling the site-specificity of the differences in topsoil are not explained, remain unknown.

While this study was not designed to study the factors responsible for the site-specificity, we do suggest some possible explanations in the discussion. (P.10, L.2)

The sampling design is simple, but no explanation is given about the sampling strategy, unfortunately. Authors selected four sites, but it remains unclear why four sites. Why not 10, 20? At each site, two times 20 trees were selected. Why 20 and not 10? Around each tree, 4 soil samples were taken and then bulked. Why 4, and not 10 or 20? In short, the underpinning of the design of the sampling strategy is not clear.

Additional explanation regarding the sampling design is added in the methods:

(P.5, L. 28) “Sites were selected that had never before been harvested, were regenerated from tube stock on former agricultural pastures, >15 yr of age, had a minimum area of 1 ha and had to contain an adequate number of N-fixing and non-N-fixing trees for replication. This resulted in four study sites.”

(P.5. L38) “At each site, 20 N-fixing trees and 20 non-N-fixing trees were selected randomly. This number was chosen to make sure the whole planting was sampled as evenly as possible.”

(P.5, L. 57) “In the 0-10 cm layer, four subsamples (~100 g) were taken and then bulked to make one composite sample (per tree), in order to try to compensate for spatial heterogeneity. In the 10-20 cm layer, two subsamples (~200 g) were taken to make one composite sample (per tree). Fewer subsamples were taken in this layer as the soil was hard and difficult to sample. “

The M&M and Results sections are concise. The General Discussion is repetitive and can be shortened, in my view, because of the nature of the study and also because of the overlap with the Introduction.

The manuscript is now submitted as a short communication. The introduction and discussion are reduced substantially and overlapping information has been removed. Furthermore the Results, Discussion and Conclusion section are merged and two of the tables are removed and submitted as online supplements, to achieve a length of 6 printed pages, as per the guidelines for a short communication.

The pdf version of the manuscript is not easy to read because spaces between words were deleted. As a consequence, the readability was low and it is difficult to trace typing errors, although I noticed a few. For example on P3, L37: Reference missing

This must have been an issue that occurred when uploading the manuscript and I failed to notice it when checking the PDF file that was created. Apologies for this, I will make sure to check the PDF file more thoroughly.

The missing reference has been added.

The nature of this manuscript is a short communication. It addresses a relevant question. The results of the measurements suggest some differences between tree species, but further studies are needed for more conclusive statements.

We appreciate this comment and have revised this manuscript as a short communication as suggested.

Summarizing, the General Discussion of this manuscript should be revised (and shortened). The whole manuscript must be checked for typing errors.

This has been done as suggested.