

Dear Editor,

We thank the reviewers for their useful comments on the manuscript. We have now fully revised the manuscript taking into account all the comments of the three reviewers. We feel the manuscript is greatly improved and we have thoroughly address all the concerns of the reviewers. Because of the extent of the revision, we have submitted the revised text without “track changes” as this would made it impossible to read. We have also reordered the Tables & Figures.

Our detailed responses to each the reviewers’ comments are given below.

Your sincerely,

On behalf of all co-authors,

Wafa Abaker

#### Editor comments

Although the three reviewers have recognized that your manuscript has merits, at least two identified major deficiencies which should be addressed before the ms. can be published. In particular, I want to stress the following:

#### NOVELTY:

\* Reviewers 2 and 3 noticed the similarity between the present manuscript and previous paper by the same authors (Abaker et al. 2016 and Abaker et al. in press). If parts of Figure 3 and Table 2 are identical to SOC results reported in the former paper, these results should be excluded and simply referred to in the discussion only, removing the emphasis in the current manuscript on the SOC results. More generally, the novelty/added value of the present ms. should be presented very clearly, and already published data recognized as such.

The aim of the paper is show how soil nutrients (N, P, K) are linked to SOC. We therefore feel that the SOC results need to be included in this manuscript: This enables the reader to see how the N,P,K results compare to SOC results without having to access a separate paper for such results. This comment also somewhat contradicts the comment of reviewer3 who asks for more SOC results! We therefore wish to keep the SOC results (included in Fig. 3 (became Fig 2) and Table 2) in this manuscript, but have now acknowledged that they are taken from our earlier paper (Abaker et al. 2016) dealing specifically with plantation age related C sequestration.

#### METHODOLOGY:

\* As raised by reviewer 1, the experiment is pseudo-replicated (plots for each treatment located together spatially); this needs to be acknowledged, i.e. you need to discuss the possibility that the changes in SOC and nutrients with age of plantation could have been inherited from the sites and acknowledge this in the interpretation of the results.

This would be the case with all such experimental plot based studies. The idea that the plots are located in same vicinity is that they would be as similar to each other in terms of climate, soils and land-use history and that any differences related to the age of the plantation would overwhelmingly likely to be caused by age. Furthermore – and unlike most other such published studies - we have replicated the plot layout at two separate locations. We therefore feel this comment unwarranted, something surely the reader would be aware of and not in need of addressing in the paper.

The authors should read Hurlbert 1984 Ecological Monographs 54: 187-211. Although there are two locations in which there are trees of different ages, the statistical analyses were conducted “for each site separately” (line 234/5). You have assumed that the sites were similar to start with, but have given no evidence to support that claim. What was the prior land use? Was it a grassland or cropping lands into which Acacia trees were planted? Were there older trees that were removed before new ones were planted? Is there any data on elevation, drainage etc to justify your assumption that the sites were similar to start? The satellite photographs indicate some substantial local patterning in the landscape reflecting variations in underlying soil types or hydrology or growing of trees. It appears to be a region of old roughly N-S dunes, with inherent underlying spatial patterning. How were the sample points chosen? Is the 0-age or grassland site a grassland for any particular reason? Is it naturally a grassland and the other areas naturally treed? The grassland has trees within it, albeit at a low density. Why is that? How did you take those trees into account? Is that why the special sampling was different? Why are there plantations of different age? Could it be that the most fertile sites were planted first, then less productive sites? Is it a rotation? What happened on the 15 year site before those trees were planted? This is all critical information to give some degree of interpretation in an experiment that is not properly replicated.

The statistical analysis for each location cannot be used to draw a general conclusion about the response of nutrients to age of plantation. The two locations taken together give a better indication, but not one that is statistically robust. This is not to say that the changes are not occurring, but that this analysis cannot justify it as is.

The finding that nutrients are higher in plantations than in grasslands is really a statement that under trees is richer than between trees. It would have been good to have some samples along a gradient away from a tree.

\* If soil samples were actually dried at 105 degrees prior to analysis of organic matter, it is needed to discuss the implications for the results (see reviewer 2).

While drying samples at 105 °C can affect inorganic N analysis, it is less likely to affect the determination of total elemental contents (Bremner & Mulvaney. 1982). Methods of soil analysis. Part 2). We therefore consider the drying to have no significant effect on our results. Furthermore, our results would be directly comparable with those published in many other papers (e.g. El Tahir et al. 2009. J. Arid Environ. 73: 499-505; a reference now included in the revised manuscript). We have addressed this issue in the detailed response to the reviewer 2 (see below) and in the manuscript as well.

\* The laboratory packing method for bulk soil density assessment should be detailed, and an adequate reference given. Similarly, more details on the root sampling method should be given.

More details regarding the determination of bulk density have been given in the methods along with a reference. We have also included a description of root sampling, although the results in this manuscript only deal with above-ground biomass, as has now been clearly indicated in the revised methods section of the manuscript.

\* I would like you to seriously address the concern of reviewer 2 that comparison of soil nutrient and carbon stocks between different land uses may be expressed in terms of soil mass in addition to soil volume.

Because of time restrictions and to keep our results consistent with our other publications, we have not recalculated the stocks using the equivalent mass correction method as suggested. In any case, most of the results presented in the manuscript relate to concentrations. Although our stocks

calculated using the conventional fixed depth procedure (Table 2) may not be considered as accurate as values calculated using the equivalent mass correction method, we consider them sufficiently so, and since they were calculated in the same way, the relationships between the stocks will be unaffected. We have therefore decided to only discuss this issue in the discussion of the revised manuscript and hope this is acceptable.

It should not take long to do these calculations. They are important to be done.

\* Use the right taxonomic name for the tree species (you can indicate what was the old name).

We have addressed this issue in the detailed response to the reviewer 2 (see below) and in the manuscript as well.

#### DATA INTERPRETATION:

\* Reviewer 1 also identified kind of self-contradiction in the following reasoning: (i) you argue that there has been negligible N fixation, but (ii) as the plantations age, the N cycle becomes more open with enhance N volatilization. So (iii) how does N increase with time, and how the increasing N is coming from N leaf litter cycling if it is not initially derived from N fixation (what would be the source then)? The reviewer encourages you to use previous papers for addressing this paradox.

This is a good point and we have now addressed this paradox in the revised manuscript. Studies (Boddey et al. 2000; Hedin et al. 2009) have shown that there is active  $N_2$  fixation when the trees are young resulting in an accumulation of N-rich organic matter in the soil. The resulting increase in N availability then begins to inhibit further  $N_2$  fixation and the older trees/plantations become more dependent on N recycling and the amount of biomass. To account for the additional soil N in the plantations, we have now speculated in the discussion about two possible sources: deposition (unlikely as regional studies indicate N deposition is too low) and inputs of excreted N brought in by grazing animals).

#### OTHER MANUSCRIPT IMPROVEMENTS:

\* Keep the order of the main issues addressed in this study consistent between the introduction; methods and results.

This has been addressed.

\* I also recommend that you seriously consider the recommendation of referee 3 to provide a summary table of soil carbon stocks and rates of carbon sequestration in relation to other studies.

If the intention of the paper was to compare our SOC results with those reported in other studies, i.e. a review paper, then such a table would indeed be useful. However, as our intention is not to make a review of C stocks and sequestration rates and limit the size of the manuscript, we have only included our C stocks and sequestration rates in the manuscript. Furthermore, we have compared our SOC results, including stocks and sequestration rates, with those reported in other studies in our earlier C paper (Abaker et al. 2016) and do not wish to repeat this in the paper. However, we have now referred the reader to this earlier manuscript for such a comparison.

The reviewers have made a range of other suggestions that will help you to improve the ms.

When you will submit the revised version, please carefully address each point raised by the reviewers in your response letter and manuscript.

best regards, Xavier LE ROUX

## **Reviewer: Garry Cook**

### **Basic reporting**

This paper is written in clear professional English. It is of a good standard with adequate referencing, and professional handling of data, tables and figures.

### **Experimental design**

The experiment is pseudo-replicated, in that the plots for each treatment are located together spatially. This limits the applicability of the findings. The problem is not fatal, but needs to be acknowledged in the interpretation of the results. The experimental layout leaves open the possibility that the differences (increases) in SOC and nutrients with age of plantation could have been inherited from the sites. This possibility needs to be discussed.

This would be the case with all such experimental plot based studies. The idea that the plots are located in same vicinity is that they would be as similar to each other in terms of climate, soils and land-use history and that any differences related to the age of the plantation would overwhelmingly likely to be caused by age. Furthermore – and unlike most other such published studies - we have replicated the plot layout at two separate locations. We therefore feel this comment unwarranted, something surely the reader would be aware of and not in need of addressing in the paper.

### **Validity of the findings**

In Lines 260 - 276, the authors argue that there has been no N fixation, but as the plantations age, the N cycle becomes more open with enhance N volatilization. So how does N increase with time. This query also applies to the Conclusions in section 5. If N fixation is not occurring, how can you argue that the increasing N is coming from N cycling in leaf litter? Where is the N ultimately coming from? It was low to start with - so what was the source? If it was leaves going into leaf litter, then where ultimately was the source for the leaves? Could there be something wrong with the logic. Have a read of Cook and Dawes-Gromadzki 2005 Landscape Ecology 20: 649-660 who looked at some of these issues in an Australian acacia landscape. This paradox needs to be addressed. At the moment it is self-contradictory.

This is a good point and we have now addressed this paradox in the revised manuscript. Studies (Boddey et al. 2000; Hedin et al. 2009) have shown that there is active N<sub>2</sub> fixation when the trees are young resulting in an accumulation of N-rich organic matter in the soil. The resulting increase in N availability then begins to inhibit further N<sub>2</sub> fixation and the older trees/plantations become more dependent on N recycling and the amount of biomass. To account for the additional soil N in the plantations, we have now speculated in the discussion about two possible sources: deposition (unlikely as regional studies indicate N deposition is too low) and inputs of excreted N brought in by grazing animals).

### **Comments for the author**

Line 118: Eragrostis (no "e")

Has been corrected.

## **Reviewer: Anna Richards**

### **Basic reporting**

I have no major comments with regards to basic reporting (although I have included some minor concerns in the general comments below). The manuscript was fairly clear and generally well structured.

### **Experimental design**

See comments under general with regard to methodology queries

### **Validity of the findings**

See general comments below

### **Comments for the author**

The manuscript by Abaker et al. describes nutrient (N, P, K) and soil carbon stocks in the soil and vegetation under grassland and Acacia senegal plantations of different ages. This study reports data from a poorly studied region with some very pressing environmental and land management issues. The authors have provided a fairly clear and comprehensive discussion of their dataset and the implications of plantation establishment for recovering soil fertility.

I have a number of concerns with some of the methodology descriptions and analyses that I have presented below.

#### **1. Soil sampling and analyses:**

The authors mention that soil samples were dried prior to analysis at 105 degrees. This is unfortunate, as it is likely that some organic matter was volatilised at this high temperature (see Reuter, D.J., Robinson, J.B., Peverill, K.I., Price, G.H. and Lambert, M.J. (1997). Guidelines for collecting, handling and analysing plant materials. In: 'Plant Analysis: An Interpretation Manual, 2nd Edition', pp. 53-70, (D.J. Reuter and J.B. Robinson Eds) (CSIRO Publishing: Collingwood)). It might be helpful to discuss the implications of this for your results.

While drying soil samples at 105°C can affect inorganic N analysis, it is less likely to affect the determination of total elemental contents (see Bremner & Mulvaney. 1982. Methods of soil analysis. Part 2). We therefore consider the drying to have no significant effect on our soil total elemental results. Furthermore, our results are directly comparable with those published in many other papers (e.g. El Tahir et al. 2009. J. Arid Environ. 73: 499-505; a reference now included in the revised manuscript). The extractable K and available P were determined from air-dried samples and therefore unlikely to be affected by volatilisation (this has now been clarified in the revised text). The suggested Reuter et al. 1997 reference deals with the analysis of plant material, not soils. Our plant samples were dried at 60°C, as was stated in the manuscript. This drying temperature, which is less than that suggested by Reuter et al., is typical for plant material. We therefore feel volatilisation to have no significant effect on our results.

Secondly, the authors mention on line 153-154 that the apparent bulk density of composited soil samples was determined using the 'laboratory packing method'. I am unfamiliar with this method and I would like to see a more detailed explanation of what it entails. My main concern is that by compositing soil samples much of the sample structure has been lost, making accurate bulk density measurements quite difficult. This may then have a substantial impact on your nutrient stock calculations presented in Table 1. However,

I may be mis-interpreting this paragraph and perhaps bulk density determinations were performed on individual intact soil samples?

More details about the determination of bulk density have now been given along with a reference. The analysis was done on the individual soil samples, including the grassland samples (the sample was taken from the plot centre of one of the grassland plots at both sites). New text has been added to the soil sampling section to clarify that separate sample (i.e. not a composite sample) was collected from grasslands for determination of bulk density. The “tapped bulk density” method was used rather than taking volumetric samples as this method is recommended for loose sandy soils with no structure (Tan, 2005), as was the case with our soils.

My final comment is in regard to comparison of soil nutrient and carbon stocks between different land uses that may vary considerably in terms of bulk density. When SOC and STN stocks are being compared across sites where bulk density varies and soils are sampled to a fixed depth, SOC and N stocks will need to be re-calculated to an equivalent soil mass before comparisons are valid, otherwise differences between sites in nutrient content may be solely caused by differences in volume of soil sampled. Re-calculation of SOC and STN stocks to an equivalent soil mass (usually calculated from the site with the lowest bulk density) is performed by determining the relationship between cumulative soil mass and cumulative C or N stock at each site (see Gifford and Roderick 2003; Lee et al. 2009). It might be helpful to discuss this potential issue in your dataset, particularly if there was substantial soil impacts or compaction during plantation establishment.

Gifford RM, Roderick ML (2003) Soil carbon stocks and bulk density: spatial or cumulative mass coordinates as a basis of expression? *Global Change Biology* 9, 1507–1514

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J (2009) Determining soil carbon stock changes; Simple bulk density corrections fail. *Agriculture, Ecosystems and Environment* 134, 251-256.

Because of time restrictions and to keep our results consistent with our other publications, we have not recalculated the stocks using the equivalent mass correction method as suggested. In any case, most of the results presented in the manuscript relate to concentrations. Although our stocks calculated using the conventional fixed depth procedure (Table 2) may not be considered as accurate as values calculated using the equivalent mass correction method, we consider them sufficiently so, and since they were calculated in the same way, the relationships between the stocks will be unaffected. We have therefore decided to only discuss this issue in the discussion of the revised manuscript and hope this is acceptable.

## 2. Sample types

The authors mention that above and belowground samples of ground vegetation were taken and analysed for nutrient content. I was curious as to how root samples were removed – eg. was the main root ball dug up, was it rinsed prior to drying etc.? There was also an indication on line 146 that these samples were analysed separately, but only a single value was reported in Table 4- is this a combination of below and aboveground biomass samples or just aboveground samples? If below ground samples were combined with aboveground, it is unclear then why root nutrient contents were not sampled for the trees as well.

During ground vegetation biomass sampling, plants were manually uprooted (the annual grasses and herbs present had small and shallow root systems) and above and belowground were separated. However, for logistics and to reduce analysis costs, only the aboveground part was analysed for nutrients. Therefore the results in this manuscript are only for the aboveground biomass. We have not sampled tree roots. We have revised the text to make this clear.

My other concern was the similarity between this manuscript and a previous manuscript by Abaker et al (2016) that also reports soil organic carbon (SOC) stocks and contents under the same plantations. Parts of Figure 3 and Table 2 are identical to SOC results reported in this publication, and this should perhaps be noted or these results excluded and referred to in the discussion only. This would also remove any emphasis in the current manuscript on the SOC results, as the implications of this dataset have already been discussed and published elsewhere.

The aim of the paper is show how soil nutrients (N,P, K) are linked to SOC. We therefore feel that the SOC results need to be included in this manuscript. This enables the reader to see how the N,P,K results compare to SOC results without having to access a separate paper for such results. We therefore have gone for the first option, i.e. the SOC results (included in Fig. 3 (became Fig 2 after reordering) and Table 2) are kept in this manuscript. But have now acknowledged that they are taken from our earlier paper (Abaker et al. 2016).

A number of minor concerns are presented below:

Abstract line 43: the 'nutrient uplift' effect was not directly measured in this manuscript so it may be best to rephrase this sentence as "We speculate that the higher mineral nutrient concentrations...."

Sentence has been revised.

Introduction line 71: the wording of this sentence is a little unclear in terms of how SOM contents are related to increased nutrient contents and cycling under tree canopies (it would seem that these processes result in higher SOM contents rather than the other way around).

Sentence has been revised.

Introduction line 78: It might be helpful to start a new paragraph focused on N fixation here.

Done

Introduction line 104: change hypothesized to hypothesize

Don't agree and not done

Results line 186: The reference to Fig. 2 should be removed as this does not show nutrient concentrations with depth and age.

Done.

Results line 190: Remove "as could be expected"

Done.

Discussion line 235 to 238: Reference and re-iteration of the results should be removed.

Text has been revised



Discussion line 246-255 and 340-342: It might be helpful to indicate that you speculate that 'nutrient uplift' may be the mechanism enhancing nutrient contents in surface soils

Done.

Discussion line 346: I was not entirely clear how improving soil fertility under Acacia plantations would benefit the local community. Is it because they will then be cleared and used for food production? Or is it because it improves the diversity and palatability of ground vegetation that is used for grazing? It might be helpful to either remove this sentence or place the context of plantations and soil fertility and benefits to local communities into the introduction.

We have decided to delete this.

Figure 1: It would be helpful to include a map of the study sites that includes their location in reference to the whole of Sudan (similar to the Abaker et al. 2016 reference).

Done

Figure 3: Would it be possible to include error bars here

We had tried before to include error bars in the figure, but the figure was difficult to read. In order to make the figure simple and readable we prefer not include error bars.

Table 1 to 5: Could you include number of replicates (where applicable) for values where standard deviations have been calculated in the Table labels e.g. "Mean soil stocks ( $\text{g m}^{-2}$ ; 0-50cm  $\pm$  (standard deviation),  $n = 3$ )..."

Done.

Table 2: the formatting of the superscript letters in the SOC column is a little confusing.

That happened during building of the PDF, the original table does not show superscript letters. We will pay attention to this when submitting the revised version.

Best wishes in addressing these concerns,  
Dr Anna Richards

## Reviewer: Alastair Potts

### Basic reporting

This study builds on Abaker et al. (2016) — which investigated biomass and soil carbon in various aged plantations of *Acacia senegal* — by exploring potential co-correlates with important plant nutrients.

This is a very well-written and well-structured article that clearly explains the importance of the research. Figures are relevant, correctly labelled etc. There is one issue that the authors need address: contextualising the other studies on the same system (i.e. Abaker et al. 2016 and Abaker et al. In Press). There are hints throughout the manuscript about these studies, but the reader is expected to piece them together to figure out that these studies share the same study system, and I assume the same research program. Please tell the reader up front in the introduction that this study system has been explored along different avenues and summarise those avenues. That helps contextualise this contribution in relation to the other two. (I continuously had this nagging feeling that the other two studies may have looked at some other area).

New text has been added to the introduction explaining the links between this manuscript and the previous two papers, and that all of them were carried out at same sites.

There is an issue with the species name used: *Acacia senegal*. The new scientific name for this species is *Senegalia senegal* (L.) Britton. Although I strongly dislike the manner in which the name change for African *Acacia* occurred, we do need to maintain the correct taxonomic links. Officially, I should ask the authors to change the name throughout, but I think they could make a convincing argument that the old name has local importance and association (e.g. with government officials?) and so the old name is more accessible. If the authors can provide a convincing argument for using the old name, then the new name does need to be included in the abstract and text at some point, else the new name should be used throughout.

We thank the reviewer for raising this issue. Now we have considered the use of the new name *Senegalia senegal* in the abstract and the first mention of *A. senegal* in the introduction. We also have argued why we want to retain the use of the old name.

My last major comment is that keeping track of samples sizes from the methods to the results is tiresome and difficult. Please include the sample sizes in the tables (even if this is simply in the table heading).

We have clarified sample numbers throughout text and in tables.

A difficult comment to implement, but there seemed to be much repetition between the results text and tables, so this could be shortened. Please ensure only major main trends are reported in the results text.

This has been done.

In terms of maintaining a predictable order for the reader throughout, the introduction sets up the aims (e.g. L98-100), but the methods and results don't follow this order (e.g. 148-164). It is minor, but keeping the order consistent helps the reader.

The text has been rearranged accordingly.

Minor comments:

Line 111: please explain what is "gum arabic"?

Has been explained.

L118: “Eragrosties” to “Eragrostis”

Has been corrected.

Line 152: Please include model and manufacturer for the spectrometer.

Have been included.

Lines 148-159: These methods are not referenced. One I am not familiar with so I think providing a reference for all is necessary.

Model and manufacturer have been added where applicable and references given for the bulk density, exchangeable K and available P methods.

Line 164: I’ve used carbon and oxygen isotopes before, and these are referenced against international standards. Is there an international standard for nitrogen (atmosphere, maybe?)? Please include.

The stable isotopic composition of nitrogen is expressed relative to atmospheric N<sub>2</sub> (international standard for N). This has now been included in the manuscript.

Please consider including an appendix figure with ground-level photos of the different sites to give international readers further insight into the study system.

Photos of the two study sites have been added as a supplementary file.

Line 319: replace “simply” with “by process of elimination”. (Yes?).

Sentence has been revised

Line 364: “of of”

Repeated preposition has been deleted.

Figure 3. Please include information about replicates.

Values are the average values from three plots. This has now been included in the Figure.

## Experimental design

The experimental design is sufficient to answer the questions raised, and is sufficiently described for experimental reproduction. Although I don’t believe it is important, the shape and size of plots varied across different treatments (e.g. grass vs plantation). Please could a brief explanation be included as to why this was done, and also any potential (or not) influence this might have had. Again, I don’t think this is important, but the change in design was odd.

Square plots were used for the grasslands as it was easier to delineate such large plot in the field and to carry out the sampling while circular plots were preferred in the plantations because of ease of establishment. Now this has been explained in the methods.

Line 204: Although unlikely in such a dry environment, fire can influence SOM rates. Please briefly mention whether fires occur in these landscapes (at least to rule this out as influencing factor, as it does play a role in more mesic environments). If fires do occur, then this needs to be considered...

Unfortunately, specific information about the fire history at the two sites is not available. However, fire in general has been reported to occur within the study area every 5th year on average (Olsson and Ardö 2002). This limited information (along with grazing) has now been included in the site description and the potential impact of fire discussed speculated on in the discussion.

### **Validity of the findings**

The interpretation is consistent with the results, i.e. the findings are valid. No further comments required.

### **Comments for the author**

This was a well-thought out study and well put-together. One way to make your results accessible to a general reader is to provide a summary table of soil carbon stocks and rates of carbon sequestration in relation to other studies. This does not need to be a comprehensive list, but a table with stocks, rates, vegetation type and mean annual precipitation will allow the reader to immediately gauge your values in comparison to other systems. One such study that is at the same MAP is Mills & Cowling (2010; J. Arid Environments, 74: 93-100), but there are many more that can be easily harvested from Google Scholar. This little additional effort will help the reader, and likely increase the citation rate for the article.

We presented C stocks and sequestration rates in our earlier paper (Abaker et al. 2016) and described how they compared to the results from other studies. Therefore, to avoid repeating the results presented in our earlier paper, we have not included the suggested table in this paper.