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Interactive comment on "Nitrogen availability determines the long-term impact of land-use change on soil carbon stocks in grasslands of southern Ghana" *by* John Kormla Nyameasem et al.

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RESPONSES TO REVIEWER COMMENTS

Comment: The paper is generally well-written (with some exceptions, e.g. lines 66-68, 180-181, 223-225), but the discussion goes well beyond the data, is frequently imprecise, and at times irrelevant (some material might be relevant in a thesis but not a research paper). Discussion needs to focus more on the findings, be less speculative, and substantially shortened. An indication of a lack of focus is the broad opening state-

ment that the scope of the study embraces impacts on C cycling (line 10). It doesn't – the researchers measured the outcome (SOC) of C inputs and outputs over 50 years. There are not even estimates of the current biomass production and removal, and no attempt is made to relate biomass production to soil macro-nutrients or accessions of condensed tannins.

Response: 1) We agree that some portions of the manuscript such as lines 10, 66-68, 180-181, 223-225 need revision to improve readability. We will revise the paper accordingly.

2) We have made additional efforts and now have acquired data on the biomass productivity of the species – albeit not from the time of sampling. However, as no general statements about species are made anyways due to the nature of the experiment, and only information on the functional groups is provided, we still believe it will provide valuable information on the variability in biomass production and the general yield potential for the different functional groups. We will add this data to tables 1 and 3 to show the within and between functional group variability and will relate this information to SOC stocks and soil macro-nutrients in the revised paper.

3) Generally, the paper will be shortened and revised to exclude any information that is not justified based on the data. As part of that, the multivariate regressions etc. will be omitted (see later comments) and the paper will be focusing on the data that we (and the reviewer) deem relevant (i.e. SOC stocks across treatments and polyphenol concentrations across the functional groups), while also introducing biomass.

Comment:

This is not a controlled experiment. The paper is based on opportunistic sampling on and around a research farm, from a selection of locations with supposedly different long-term management. There appears to be no classical experimental design with replicated and randomized treatments. Locations that received different management are deemed to be management 'treatments'. It appears there was no site pairing that could have been used to control error. The fact that this is not a traditional experiment is not in itself a concern, but it does mean that extra care is needed to describe the sampling and analysis, and in particular, care is needed in drawing conclusions.

Response:

It is true that the experiment is not a traditional one. However, as there are no long-term experiments on this topic in Western-Africa, it still provides the best possible means to make assumptions on the long term developments of SOC stocks in Sub-Saharan Africa. However, we agree that we need to provide more information on the experiment and describe the sampling and analyses better to convince readers of their validity. Regarding the site-pairing: We agree that this would have been preferable, yet we were constrained technologically as samples had to be flown from Ghana to Kiel for all analysis, which greatly limited our capacity for sample numbers. Unfortunately, this is something we cannot change at this point, but given the large variability we observed within groups, presumably this would not have changed the overall data quality anyways, as the main "issue" (which we cannot solve as well) is the general lack of replicates and hence our only option to group species according to functional traits. So while some of these issues cannot be resolved, we still believe that this is the best possible dataset that we were able to obtain under the given conditions However, we have generally decided to revise our conclusions and be less speculative. Also, we will improve our communication by describing the experimental units better and show what the reference points are.

Comment:

My first concern is that there are no baseline data for SOC or soil fertility in 1966, and presumably no archived soil samples dating from 1966 that could have been analyzed. To calculated changes in SOC and macro-nutrients over 50 years, the authors use a pseudo baseline derived from just three fields of native grassland located somewhere near the research farm. These were sampled on the assumption that the present

native grassland soils and plants are exactly as they were in 1966. Each field was sub-sampled at only four locations and bulked for analysis. The mean of three values (fields) therefore provides the slender basis upon which the whole paper rests. We have no idea how these fields were chosen, no idea why they were not selected for development in 1966 (too poor, too good or?), no details of management and changes in management over 50 years, and no idea if the fields were representative of native grassland back in 1966. There needs to be sufficient evaluation of the assumption in a revised paper to convince readers of its validity.

Response:

1) We have realized that the reasoning for selecting these sites was not clear enough and we will improve upon this in the final paper. The entire site is government owned and was reserved for research activities by the government due to its suitability for agriculture, its proximity to the capital city and its vegetation and climate which are representative for the largest grassland type of Ghana (Guinea Savannah). Ostensibly, the site had a uniform vegetation until parts were converted to agriculture in 1966 and beyond. While some parts were converted to agricultural use, one part was converted to the "exhibition farm" with the collection of species used in this study. Large parts remained protected, unmanaged natural grassland, however. Only these plots and agricultural fields that were converted in 1966 were considered in this study. 2) Consequently, while no baseline data on SOC or soil fertility of the soils in 1966 could be obtained, the conversion of parts of the native grassland to the different agricultural land uses or the experimental "exhibition farm" occurred within the same time frame. Fields that were converted later that 1996 were omitted from the study. Hence, any deviation in soil carbon stocks developments occurred at that time and we assume that the status of the converted fields would be similar to the native vegetation if they had remained unconverted. 3) In the final paper, we will a) improve the explanation on the selection of the plots and the plots/fields themselves, and b) change the presentation/wording to delete the annual losses etc but rather show the soil carbon stocks as

percentages of the natural grassland, which serves as the baseline not for soil carbon stocks of 50 years ago, but the potential soil carbon stocks that the plots/fields would have, had they not been transformed to their current state.

Comment:

Description of the experiment is vague, but it appears that 'treatments' were located on both the research farm and in the surrounding area. Apparently, in 1966 some fields around the research farm were converted from native grassland to field crops and some were sown to pasture and grazed. This allowed the researchers to select three fields of each of (i) native grassland, (ii) field crops and (iii) seeded-grazed land, located outside the research farm. These were the 'treatments'. Readers are not told where fields were located, why they were chosen, whether management remained stable over the 50-year time-frame, or how the authors know about management over this time (was anything documented?). It is quite possible, for example, that the choice of land for field crops in 1966 was based on a perception it was the most fertile land, in which case the real loss in SOC over time may have been greater than reported here. This an important point, because the authors found no change on SOC over 50+ years of arable agriculture, which is very different from the majority of studies that show SOC declining under arable agriculture (one of the driving forces behind 'conservation agriculture').

Response:

1) The tendency to make the paper as concise as possible caused as to hold back certain details. Like we have indicated, we will improve the description of the experiment in the revised paper and provide more information. I.e. we will include a map (Fig. 1) to provide information regarding the location of the fields. The other details as provided below will be added to the revised paper. 2) Field selection was based on two main criteria- (i) that the field was converted in 1966 and (ii) the management remained fairly stable over the 50-year time-frame. Additional details on field selection

has been provided above, which makes the assumption that these fields were particularly fertile initially unlikely. 3) The information regarding the management of the fields were obtained by one of the authors who manages the farm from documents available to him. 4) We do understand the surprise of the reviewer regarding the lack of effect from arable crop production, despite the general tendency of arable crop production to deplete SOC stocks. However, the largest effect in arable crop production on SOC stocks derives from tillage, as the reviewer himself by mentioning conservation agriculture. But especially the tillage is substantially different in low-input agricultural systems, where soil tillage is largely conducted using simple tools. From our study, SOC stocks of arable crop farming did not differ from the native grassland. Among the three arable crop fields considered in this study, only one of them occasionally adopted conventional tillage. On that particular field, however, crop production was on a rotational basis and occurred only occasionally, i.e. tillage occurred only infrequently. On the other crop fields, soils were manually minimum-tilled using simple farm implements such as hoes. Thus, this is of course a much less invasive technique compared to regular ploughing (but nonetheless representative for many farms in that area) and hence it appears that the net effect of the mixed management practices on the arable crop farms did not impact SOC stocks significantly. Again, we will improve the description of this in the final paper.

Comment:

Continuing with the experiment design, the four cut-use treatments were located on the research farm: 59 small co-located plots with different species were allocated to four groups ('treatments') ranging in size from 3 to 46 species (replicates) per 'treatment'. The text says 59 plots altogether, but Fig. 2 indicates 72. Given the potentially large differences between species within groups, one might expect large standard errors when species are replicates, but this is not the case if the analysis is to be believed. I think the analysis needs to be revisited.

Response:

1) The total number of soil samples obtained from the cut-use forages was 72 and was derived from 59 species. The difference in number is a result of some species having several genetic accessions established, resulting in a total of 72 accessions, in which case all accessions were sampled individually. We will make this clearer in the revised paper. 2) Regarding this comment that one might expect large standard errors, we were surprised as we think the error sizes are large, especially in cases where species numbers are high (e.g. cut-use grass in Fig.2. This is one of the main results for the heteroskedacity of the data. We will, however, revisit the analysis again as suggested by the reviewer.

Comment:

Of the 59 (or 72) small plots of cut-use species, 46 are grasses that are treated as replicates of this 'land-use'. We are required to assume that these 59 (72?) plots are managed today just as they were 50 years ago - same species, nil fertilizers, same cutting regime, no differential tillage for re-establishment etc. This may be a valid assumption, but we are given too little information to test it.

Response:

Like we indicated earlier, one of the authors, who happens to manage the research farm, provided information regarding the management of the plots/fields, based on the available information. Management of the selected plots and fields have been fairly uniform over the years. Plots or fields that underwent some changes in management or re-established for any reason were omitted from the study. To put the paper in the right context, this information will be provided in the revised paper.

Comment:

Analysis The apparent lack of experimental design, and the very different numbers of 'replications', present some challenges for the analysis and interpretation of data. The authors appear to have foregone the advantages of good design which allows the

experimenter to make causal inferences about the relationship between independent variables and a dependent variable, to rule out alternative explanations due to the confounding effects of extraneous variables (i.e. control), and to reduce variability within treatments, making it easier to detect differences in treatments. The authors could usefully say a bit more to convince readers that the assumptions underlying an ANOVA have been met: the experimental errors are normally distributed; variances between treatments is equal; and samples are independent (each sample is randomly selected and independent).

Response:

1) Since the experiment was not originally design to answer the questions we set out in this paper, we had to group the experimental units in such a way to make biological sense. Accordingly, we tried to find all possible cluster or covariates that could make the most biological sense. 2) Previously, we analysed the effect of the different land-use types on soil properties, by performing a one-way ANOVA, followed by Tukey's post hoc tests to permit pairwise comparisons of means ($p<0.05$). In cases where data normality (Shapiro-Wilks) or the equality of error variances (Levene's test) required for ANOVA were not confirmed per data set, a non-parametric test (Kruskal-Wallis) was used, followed by Dunnett T3's post-hoc tests to permit pairwise comparisons of means. 3) As suggested by the reviewer, we will re-analyse the data to ensure our inferences are factual. To analyse the effect of the different land-use types on soil properties, we will perform a one-way ANOVA using generalised linear models. P-values will be estimated from type II sum of squares using the 'car' package (due to the unequal sample sizes; Fox and Weisberg, 2011) followed by Tukey's post hoc tests using the 'multcomp' package (Bretz et al., 2011), all in R (R Core Team, 2019), to permit pairwise comparisons of means. Before the ANOVA, data will be checked for normality and homogeneity of variance. If cases of abnormality, the will be log-transformed, and in cases where equality of error variances is not confirmed even after log-transformation, we will set 'white.adjust=T' to deal with heteroskedacity using

White-adjusted heteroskedacity corrected standard errors.

Comment:

Where replicate data from different treatments are combined for regression analysis, the 'treatments' with large rep numbers are over-represented, possibly giving rise to significant relationships that may not otherwise be significant. For example, the cut-use grass group of plots had the greatest TN (inexplicably), and TN is said to be the individual analyte most highly correlated with change in SOC. So, the question is, would TN still be highly correlated with change in SOC if these data were deleted from the analysis. I suggest the authors consider whether the regressions involving all data would be more appropriate if regressed as treatment means to avoid bias from under or over-represented treatments.

Response:

We agree that this bias is a possibility but that seems not to be the case in our case. For example, correlation between TN and C remains highly correlated even without cut-use grass which suggest that the relationship between TN and C is similar across treatments and the correlation between TN and SOC is also very high when pooled across treatments ($r=0.93$, $P<0.0001$). Thus, we are inclined to use the individual samples for regression analyses.

Comment:

The authors refer to a 16% fall in SOC due to land-use change – this appears to the mean decline of all non-grassland plots, not the mean of treatment means, and as such it is heavily biased towards the treatment with most replicates, i.e. the cut-use grasses.

Response:

We agree to this observation. Accordingly, we have re-calculated the change in SOC due to land-use change using the treatment means, which resulted in a 17 % decline in SOC. This will be corrected in the revised paper.

Comment:

Another area of concern is where the authors attribute cause and effect in a correlation when all they have is an association, e.g. lines 15-16, 205 and Fig. 3. Without more information we cannot attribute cause to either x or y, or to an unknown co-variate of either one. All Fig. 3 shows is a fairly tight C:N ratio of about 12:1, as many others have reported. In other words, Fig. 3 reflects the stoichiometry of stable soil organic matter, not cause and effect.

Response:

The reviewer is right to say we cannot attribute cause and effect in a correlation when all we have is an association. We will revise the manuscript and choose the appropriate vocabularies to describe relationships.

Comment:

We might also expect a stoichiometric relationship between P and C, but this is not evident in Table 4, perhaps because the wrong fraction of P was measured (extractable no organic). This leads me to question the reliability of at least some data in Table 2 – why, for example, would cut-use grasses and legume herbs appear to deplete soil P, but not legume of non-legume trees and shrubs (numerically if not statistically) when product is removed from all of them? What could explain the apparent soil acidification under grazed-seeded grassland and cut-use legume-herb, other than errors? Or the depletion of K under legume-herb? Or the rise in K under arable land, unless K-fertilizer is applied quite heavily?

Response:

1) Our intention was to assess the effect of the land use types on soil fertility, which is the reason why we measured available P instead of organic P. In any case, the lack of stoichiometric relationship between P and C, as shown in Table 4, might be because P was regressed on changes in SOC, not SOC. Meanwhile, bivariate regression has

shown a significant stoichiometric relationship between P and SOC. We will revise the regression analyses between SOC and the other measured variables in the revised paper. 2) Cut-use grasses and legume herbs appear to deplete soil P, but not legume of non-legume trees and shrubs (numerically if not statistically). We see this trend as a possibility as grasses and herbs exploit nutrients from the upper horizon of the soil, the trees/shrubs have deeper roots and therefore their effect on nutrient exploitation might not be profound in the 0-30 cm soil depth considered in this study. It would be interesting to see the nutrient status in lower horizons. 3) Legume plants commonly form symbiotic associations with rhizobia and accumulate most of their N through symbiotic nitrogen fixation. During this process, legume plants take up more cations than anions and release more H+ ions from roots to soil, leading to low pH values in both the rhizosphere and bulk soil (Zhao, K. et al. 2009, Environ. Earth Sci. 59,519–527; Yang et al 2016, Scientific Reports, 6:20469, DOI: 10.1038/srep20469). This effect might differ between legume herbs and legume trees/shrubs due to differences root length. 4) Grazing fields are associated with high N and C returns from animal excreta. Meanwhile, C and N cycles are reported to cause acidification in grazed fields (Ridley et al 1990, Australian Journal of Experimental Agricu1ture, 30, 539-44). For example nitrate leaching might increase the concentration of H-ions, hence increasing soil pH. In any way, we would rather not be speculative about the reasons for these variations due to the observed large errors and the lack of statistical significance. 5) Like we indicated in lines 309-310, the relatively high P and K levels observed in the food crop fields may probably be as a result of over-supply through fertilizer application as one of the arable crop fields was fertilized (Table 1).

Comment:

In line 19, a complex multiple regression is said to 'explain' 92% of variation in SOC stocks – the equation might mathematically account for 92% of the variation, but this is very different from a biophysical 'explanation'. The authors saw value in reporting a complex equation to account for 92% of variation in SOC, but skim over the fact that

a single variable, N, accounts for 90% of the variation (Table 4). Does increasing the complexity improve our understanding of the processes?

Response:

In an attempt to avoid potentially misleading equations and clearly indicate the correlation of each individual compound with SOC, we have decided to replace the complex regression equations with bivariate equations (please see the attached fig. 2) between SOC and the other biophysical factors in the revised paper. This should also clearly indicate the (indeed) strong relation between TN and SOC.

Comment:

I suggest the authors take care not to refer to means as being different when statistically they are not. Line 230 refers to a sequestration rate of 31 kg C/ha in legume trees/shrubs, implying this is greater than with other land uses and a strategy worth pursuing to build SOC, but the statistical reality is that after 50 years of different land uses, no treatment differs from native grassland (Fig. 2). All you can say is that trends were evident but they were not statistically significant. Lines 231-234 state your expectation, not what you can statistically support. I think all you can say is that your trends are heading in the expected direction. You can propose a hypothesis worth testing. I recommend the authors review all data to ensure they are reported only to the number of significant digits that can be measured. Fig. 2 shows that although there are numerical differences in SOC, statistically there are NO treatment differences (judging by the letter superscripts – I suggest you check this). Statistically speaking, there are presently no significant differences in SOC. If there are no differences in SOC, there can be no differences in the rate of decline over 50 years. Only non-significant trends.
Response:

The suggestion of the reviewer is well accepted. We will revise the statistical analyses and the text accordingly to ensure our statements reflect the statistical reality.

Comment:

Other When rewriting, give the name of the nearest town to the research site, and in the Introduction give a very brief overview of how livestock are managed, to provide context.

Response:

1) We have decided to provide a location map and site plan in the revised paper. 2) The farm keeps Sanga cattle (adult weight ranging 300-330 kg), which is a cross between the humped Zebu type animal and the local West African Shorthorn known for their resistance against trypanosomiases, and Djallonké sheep (adult weight ranging 25-37 kg). These animals are grazed rotationally on seeded-pastures during the raining season (April – October) and fed on conserved fodder harvested from arable fields and a fodder bank. This information will be added to the revised paper as suggested by the reviewer.

Comment:

Remove Equation 1 from the Methods and include in Results, making any other changes necessary to make this possible. Response:

Once we do away with the multiple regression models, this equation will be completely omitted from the revised paper.

Comment:

I suggest you delete Fig.2 and put the data into Table 2. This will make it easier for readers to view and relate all of the data, and make the paper shorter. Table 2 as it stands does not present 'impacts' (changes), it presents only the status of soils following 50 years of various land-uses. Only if you include the apparent change in SOC does it include an impact.

Response:

We can understand the reviewer's suggestion to delete figure 2. However, since the whole paper is more focused on SOC stocks and since some of the treatments include different species we thought it would be interesting to showcase the variability within the functional groups. Therefore, adding this information to Table 2 might hide the distribution of SOC within the groupings. Attempts will be made anyway to shorten the paper, especially the discussion, avoiding speculations, etc. as suggested by the reviewer.

Comment:

The data in Table 3 and related discussion appears to be the most original and interesting, but it's hard to interpret its significance without knowing the biomass produced.

Response:

Like we indicated earlier, we have biomass productivity data of the species. This information will be added to the revised tables in the form of means and standard

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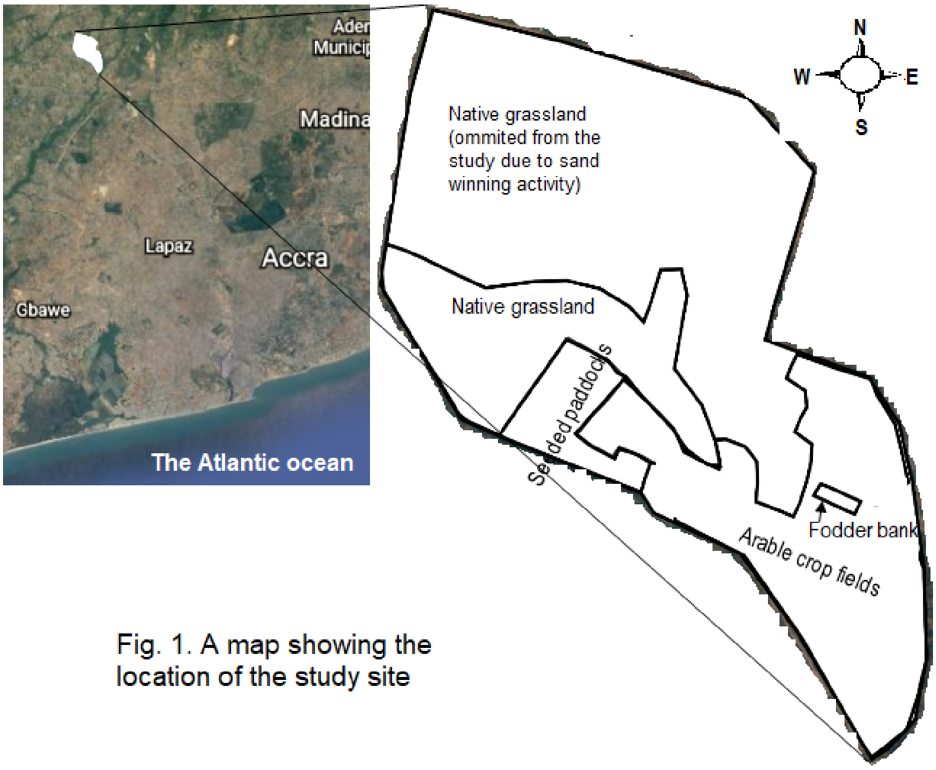

Fig. 1. Location map

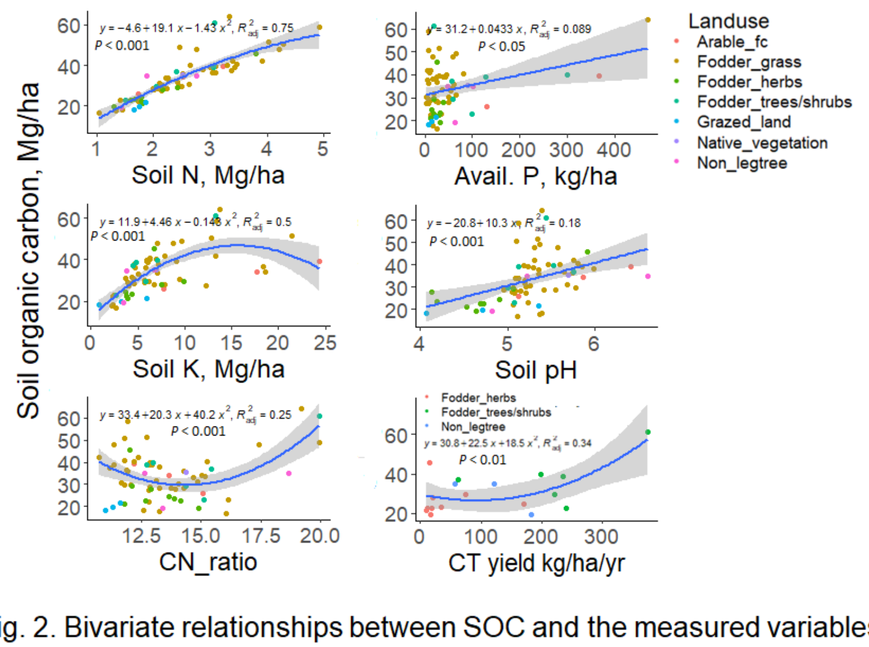

Fig. 2. Best-fit bivariate regression analyses