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RESPONSES TO COMMENTS BY REVIEWER RC1

Comment

We suggest the authors to strictly follow the EGU’s guidelines in editing the text: i.e. abbreviations, % (3% or 3 %?), C (10 C or 10 C?), space in between numbers (10-30 days, or 10 - 30 days?), etc.

Response

We have effected the corrections in accordance with the journal style. See, for example lines 18, 19, 30, 164, etc.

Responses to other comments

- Line 18. ‘(N)’ is added to give ‘nitrogen (N)’.
- ‘nitrogen’ is changed to ‘N’ throughout the paper. Please check lines 19, 20, 149, etc.
- Line 30. ‘(C)’ is added to give ‘carbon (C)’.
- Line 31. ‘(Chabbi et al. 2017)’ is corrected to ‘(Chabbi et al., 2017)’.
- Line 39. ‘carbon’ is changed to ‘C’ and throughout the revised paper.
- Line 44. ‘OM’ is added to give ‘organic matter (OM)’.
- Line 47. ‘organic matter’ is changed to ‘OM’ and throughout the rest of the paper.
- Lines 43-44. ‘physicochemical’ is changed to ‘physico-chemical’.
- Line 81. ‘macronutrients’ is changed to ‘macro-nutrients’.
- Line 95. ‘haplic’ changed to ‘Haplic’.
- Fig. 2. ‘Department 2018’ is corrected to ‘Department, 2019’.
- Lines 109-110 (Old paper). We are grateful to the reviewer for the suggestion. It is well-noted.
- Line 170. ‘14000’ is corrected to ’14,000’.
- Line 179, 183. ‘90oC’ is corrected to ’90 °C’; ‘minutes’ corrected to ‘mins’.
- Lines 178–182. ‘µl’ is changed to ‘µL’.
- Line 338 (old paper). The sentences revised is revised. Please lines 364–367 in the revised paper.
- Lines 44, 368–369. ‘macro and micro aggregates’ is changed to ‘macro- and micro-aggregates’.
- Line 461. ‘interests’ is corrected to ‘interests.’

RESPONSES TO THE COMMENTS BY REVIEWER RC2

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 statement 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 macronutrients or accessions of condensed tannins.

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- We agree that some portions of the manuscript such as **lines 10, 66-68, 180-181, 223-225** need revision to improve readability. We have revised the manuscript accordingly.

- 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 have revised tables 1 and 3 accordingly to show the within and between functional group variability regarding biomass productivity and tannin yield, respectively and have related this information to SOC stocks and soil macronutrients in the revised paper (e.g. lines 290–300, 385–390.)

- Generally, the paper has been shortened and revised to exclude any information that is not justified based on the data. As part of that, the multivariate regressions etc. has been omitted (see later comments) and the paper has focussed more 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.

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.

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 have improved our communication by describing the experimental units better and have shown what the reference points are. Please see lines 102–126 and Table 1.

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 macronutrients 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 subsampled 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.

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- We have realized that the reasoning for selecting these sites was not clear enough and we have improved upon this in the revised 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.
- 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.
- In the revised paper, we have a) improved 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.

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').

- The tendency to make the paper as concise as possible caused as to hold back certain details. Like we have indicated, we have improved the description of the experiment in the revised paper and provide more information. i.e. we have included a map (Fig. 1) to provide information regarding the location of the fields. The other details as provided below have been added to the revised paper as well.
  - 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. The information regarding the management of the fields were obtained by one of the authors who manages the farm from documents available to him.
  - 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.

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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 have improved the description of this in the revised paper (Please see lines 271–278).

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.

- 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 have made this clearer in the revised paper (See lines 115–119).
- 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 heteroscedasticity of the data. We have, however, revisited the analysis again as suggested by the reviewer.

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.

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 has been provided in the revised paper (lines 120–126).

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).

- 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.

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- 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.
- As suggested by the reviewer, we have re-analysed the data to ensure our inferences are factual. To analyse the effect of the different land-use types on soil properties, we performed a one-way ANOVA using generalised linear models. P-values were 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 were checked for normality and homogeneity of variance. In cases of abnormality, the data were log-transformed, and in cases where equality of error variances was not confirmed even after log-transformation, we set 'white.adjust=T' to deal with heteroskedacity using White-adjusted heteroscedasticity corrected standard errors. The statistics section as well as the results and discussions have been modified accordingly to reflect the changes.

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.

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 (see Table 5) 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.

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.

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 15 % decline in SOC. This has been corrected in the revised paper (see line 217).

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.

The reviewer is right to say we cannot attribute cause and effect in a correlation when all we have is an association. We have revised the manuscript and chosen the appropriate vocabularies to describe relationships (lines 240–253).

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

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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?

- 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 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 (Figure 4b).
- 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 0-30cm soil depth considered in this study (Please see lines 330–338).
- 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 (see lines 342–347).
- 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 significances (lines 347–349).
- 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) (Please see lines 328–329).

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?

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 between SOC and the other biophysical factors in the revised paper. This should also clearly indicate the (indeed) strong relation between TN and SOC (Please see Figure 4 and 6).

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

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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.

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

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.

- We have decided to provide a location map and site plan in the revised paper (Fig. 1).
- 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 has been added to the revised paper as suggested by the reviewer (please lines 102–107).

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

Once we did away with the multiple regression models, this equation has completely been omitted from the revised paper.

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.

We can understand the reviewer's suggestion to delete figure 2. However, since the whole paper is more focussed 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 have been made anyway to shorten the paper, especially the discussion, avoiding speculations, etc. as suggested by the reviewer.

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.

Like we indicated earlier, we have added biomass productivity data to the revised tables 1 and 3 in the form of means and standard deviations of each functional group.

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Nitrogen availability determines the long-term impact of land-use change on soil carbon stocks in grasslands of southern Ghana

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Abstract. Enhancing the capacity of agricultural soils to resist soil degradation and to mitigate climate change requires long-term assessments of land-use systems. Such long-term ~~assessments~~evaluations, particularly regarding low-input livestock systems, are limited. ~~This study evaluated~~In the ~~impact~~absence of suitable long-term ~~land-use practices, condensed tannins (CT)~~experiments, this study assessed the outcome of C inputs and ~~soil nutrient status on carbon cycling~~outputs in across an array of plant functional groups in arable and permanent systems of a tropical Savannah~~.~~ after more than 50 years of consistent land use. Soil samples were taken (0~~—~~–30 cm depth) from arable crop fields, grazed-seeded grassland, cut-use permanent crops and native grassland. Soil organic carbon (SOC) stocks ranged from ~~19.9~~17 to ~~36.8~~64 Mg SOC ha^{-1} (mean±sd = 32.9±~~0~~10.2 Mg ha^{-1}). SOC stocks were lower for grazed-seeded grassland relative to cut-use grass, legume trees and shrubs. ~~Within sown systems, nitrogen~~Accordingly, while converting the native grassland to grazed pastures was estimated to have lost 44 % SOC over the period, the conversion to woody legumes resulted in slight (5 %) increments. Within sown systems, nitrogen (N) availability seemed to be the most critical factor that determines the fate of the SOC stocks, with soil ~~nitrogen (~~N~~)~~ concentration and the SOC being highly correlated (r = 0.~~90~~86; p<0.001). ~~Accordingly, while converting the native grassland to grazed pastures resulted in mean annual losses of 0.11 Mg C ha^{-1} (p<0.05), the conversion to woody legumes resulted in slight (0.03 Mg C ha^{-1}, *ns*) increments.~~ In total~~, CT,~~ N, P~~, K~~ and ~~pH (with interaction terms) explained 92% of variations in the long-term changes~~K were significant predictors of SOC ~~stocks.~~density in the soils. Moreover, secondary plant metabolites in legumes, namely tannins, were identified to have an impact on SOC. The ~~regression model showed~~results from this study provide the theoretical basis to test the hypothesis that improved soil fertility management, and the use of tannin-rich plants ~~could~~have the potential to promote SOC storage in the Savannah ecological region in the long-run. Our study ~~suggests~~also shows the ~~cultivation~~potential of legume tree/shrub forage species as an environmentally sustainable land-use option to mitigate agricultural CO_2 emissions from low-input livestock systems in the grasslands of southern Ghana.

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Keywords: grassland, land-use change, legumes, proanthocyanidins, soil carbon sequestration, soil ~~chemical~~ properties, sub-Saharan Africa

1 Introduction

~~Sequestration of~~Increments in s carbon (C) sequestration rates in soils ~~at a rate~~ of 0.4 % per year ~~was~~were suggested to compensate for the global emissions of greenhouse gases by anthropogenic sources (Chabbi et al~~.~~., 2017). Grasslands, on a global scale sequester around 0.14 Mg C ha^{-1} year^{-1}, thus storing 685 Gt C in the upper soil (to 1 m depth). This pool size is nearly 50 % more C than that of forests (346 Gt C), and 70 % more than wetlands (202 Gt C) (Grace et al., 2006; Gobin et al., 2011; Conant et al., 2017). About 60 % of all grasslands occur in the tropics, and these contain 10~~—~~–30 % of the global soil C stocks (Caquet et al., 2012). Besides mitigating climate change, increased soil ~~carbon~~C sequestration ~~will~~could enhance ecological efficiency and delivery of other related ecosystem services, especially food, water and biodiversity (Stringer et al. 2012; Conant et al. 2017). However, large areas of native grasslands worldwide have undergone a substantial use-intensification or have been converted into pasture and croplands (Sterling and Ducharne, 2008; Taube et al., 2014~~) leading~~). This action has led either to reduced C sequestration rates or net losses of soil organic ~~carbon~~C (SOC) (Johnston et al., 2009; Crews and Rumsey, 2017; Reinsch et al., 2018). This change is also visible in sub-Saharan Africa, where overgrazing and other land uses are factors that affect the C cycle (Grieco et al., ~~2012).~~2012) and might constrain the attainment of the "4 per mille" agenda (Minasny et al., 2017) in the sub-region.

Generally, conversion of natural to managed ecosystems depletes soil C stocks, and the conversion of native grasslands to crop production results in approximately 50 % loss of SOC in global grassland ecosystems (Lal, 2018) as a consequence of destabilizing stored SOC. Adequate organic matter (OM) input into soils is necessary for increased ~~carbon~~C sequestration. However, ~~the~~ sequestered ~~carbon~~C is sensitive to management and land-use changes, and particularly to grazing, ~~as well as~~ changes in species composition ~~and~~as well as mineral nutrient availability (Conant et al., 2017). Nevertheless, management practices that increase the supply of quality ~~organic matter~~OM could promote ~~carbon~~C storage in soils. Accordingly, soil nutrients have implications for plant primary productivity and ecosystem functioning (Post et al., 2012; Marques et al., 2016~~).~~); however, their exact effects on C sequestration, particularly in grasslands dominated by C4 species, are not well understood (Milne et al., 2016). For example, the impact of N-fertilization on SOC dynamics remains controversial because of its dependence on other parameters such soil pH, available phosphorus (P) and potassium (K) as well as the frequency of tillage management (Khan et al., 2007; Lal, 2008, Reinsch et al., 2018).

~~As much as increased~~ Both the C input into soils ~~is~~derived from plant residues and organic manures, as well as the carbons stabilization in the soil are essential for an increased soil C sequestration~~, its stabilization in the soil has come into sharp focus.~~. Some SOC stabilization mechanisms that have been proposed include ~~physicochemical~~physico-chemical protection of SOC by micro- and macro-aggregates, spatial separation of SOC from decomposers by encapsulation, occlusion and hydrophobicity and mineral-organic associations (Kleber et al., 2014; Song et al., 2018; Alberto Quesada et al., 2020). Moreover, plant secondary metabolites in both above- and belowground biomass have recently been discussed to have an impact on both C and N cycles, with the potential to either increase or reduce C immobilization rates in soils (Kraus et al., 2003; Halvorson et al., 2011; Tamura and Tharayil, 2014; Chomel et al., 2016; Adamczyk et al., 2016, 2017; Kagiya et al., 2019). Condensed tannins (CT), for ~~examples~~example, enter the soil as leachates and via decomposed litter from plant leaves and roots (Hättenschwiler and Vitousek, 2000). Although forages naturally contain lower levels of CTs to remain suitable for livestock feeding (Mueller-Harvey et al., 2018), the concentration of CTs may reach potent levels in the long-term, because CTs are recalcitrant and could remain in the soil for decades (Tamura and Tharayil, 2011). However, the role of CTs in mitigating CO_2 emissions, particularly from tropical soils, has received minimal attention.

In livestock systems, where a large part of the net primary production is exported from the soil as hay or silage, belowground biomass is a significant source of C input. Mechanisms driving SOC sequestration in livestock systems, particularly low-input systems in the tropics, are not well understood, due to lack of research data, and sometimes with conflicting results even for such important factors as grazing (McSherry and Ritchie, 2013). Previous studies have shown mixed effects of grazing on soil ~~carbon~~C, including positive (Reeder and Schuman, 2002), neutral (Shrestha and Stahl, 2008) or ~~negative~~adverse effects (Pei et al., 2008). Due to the complexity of the different above described controlling factors on SOC stocks, long-term experiments are ultimately necessary to validate the assumptions that have been made. ~~Therefore,~~In the ~~location~~absence of ~~the current~~

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~~study, which is~~ long-term experiments in Sub-Saharan Africa that were designed to answer this research question, we selected a research ~~exhibition~~ farm that has cultivated and managed ~~(harvested and weeded to maintain plot purity)~~plots of different plant species according to the same principles since 50 years~~, with surrounding native and grazed natural grasslands under identical climatic and environmental conditions,~~. The research farm is located in a large nature reserve of predominantly undisturbed grassland, of which around 50 years ago parts were simultaneously converted to farmland and other parts being used for the establishment of the research plots. This offers the unique ~~opportunities~~opportunity to test the long-term effects of converting native grassland to agricultural land on SOC storage~~. These resources are particularly valuable as long-term experiments designed for this purpose are absent in the sub-Saharan Africa region. The~~, using the undisturbed grassland that has not been changed within these 50 years as reference baseline and comparing how the different land-use changes introduced back then have affected the SOC stocks until today. Thus, the main aim of this study was to assess the impact of long-term land-use practices on soil ~~carbon~~C storage as well as the potential role of tannin-rich forages and soil nutrient status.

In detail, the current case study was conducted to test the following hypotheses:

i. Soil C stocks in sub-Saharan Africa are profoundly affected by the type of land-use management
ii. Soil ~~macronutrients~~macro-nutrients and CT can predict long-term changes in SOC stocks of tropical grasslands
iii. Plant functional groups influence the relationship between SOC and soil N.

2- Materials and ~~methods~~Methods

2.1 Site characteristics

The study was conducted at ~~and around~~ a research ~~exhibition~~ farm of the Council for Scientific and Industrial Research (CSIR), Ghana, located at 5°~~70´N~~ 70'N, 0°~~29´W~~ 29'W and 49 m a.s.l~~. The surface lithology is of non-carbonate sedimentary with coarse sandy loam soils belonging to the haplic acrisol group (IUSS, 2014).~~, located some few km from Accra (Fig. 1). The mean monthly temperature ranged from 21 to 31 °C and a monthly rainfall of 13–205 mm (annual rainfall is approximately 800 mm). The major rainy season is from April to mid-July with the minor rainy season in October (Fig. ~~1~~2). Thus, the climate is moist semi-arid with a growing period lasting between 120~~—~~–180 days (Ghana Meteorological Services Department, 2018). ~~The~~ The surface lithology is of non-carbonate sedimentary with coarse sandy loam soils belonging to the Haplic acrisol group (IUSS, 2014). The soil at the site had the following features at the 0-10 cm soil depth: sand 80.34 %, silt, 12.64 %, clay, 7.02 %, pH (water) 5.86; N, 0.133 %, available P, 2.10 ppm; available K, 55 ppm and O.M., 2.61 % (Barnes, 1999). The native land cover of the study location is a ~~native~~ tropical grassland Savannah~~ with very~~.

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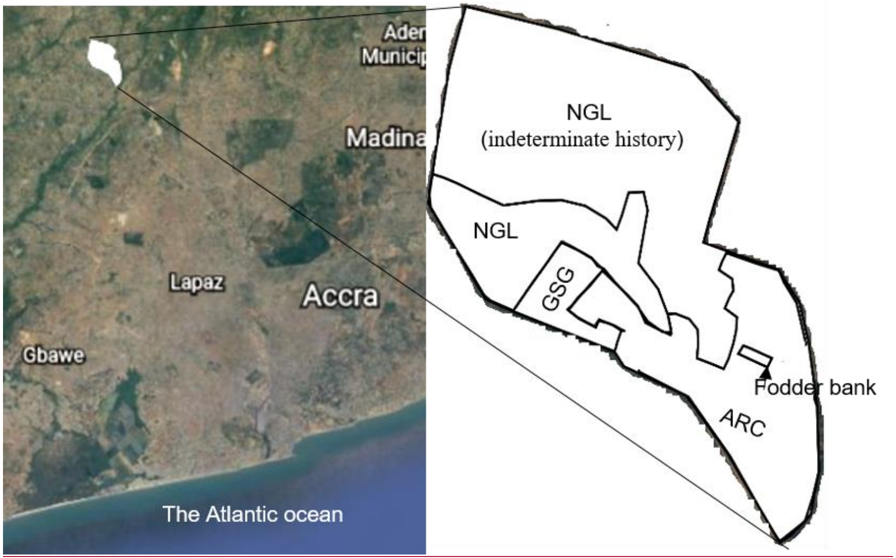

Figure 1: Location and plan of the study site. The native grassland (NGL) remains uncultivated since the farm was established in 1966. The portion of NGL labelled "indeterminate history" was excluded from the study due to high uncertainty associated with its history. The fodder bank consisted of cut-use grasses (CG), legume herbs (CLH), legume tree/shrubs (CLTS) and non-legume tree/shrubs (CNLTS); Map from Google Earth (2020).

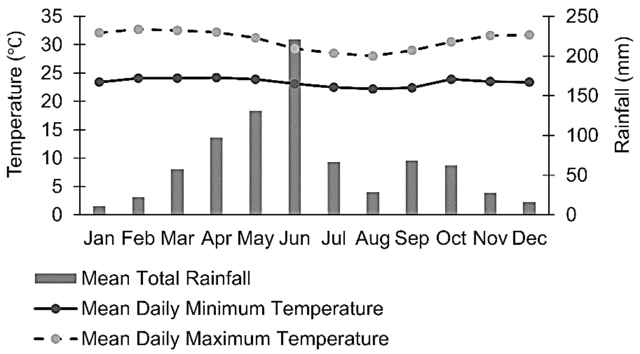

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Figure 2: Climate (30-year average) of the study area (Data source: Ghana Meteorological Services Department, 2019)

The research farm, located on the grazed-seeded grasslands (GSG) keeps Sanga cattle, a cross between the humped Zebu type cattle and the local West African Shorthorn, known for their resistance against trypanosomiasis (adult weight ranging 300-330 kg), and Djallonké sheep (adult weight ranging 25-37 kg), managed semi-intensively. 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. While the exact stocking density is not recorded, typical stocking capacity around that region has been reported to be in the range of 3.5-5 LU ha^{-1} (Timpong-Jones et al., 2013). Compared to that, the Native grasslands (NGL) is infrequently grazed in a nomadic fashion. Also here, no records about stocking densities are recorded, yet grazing is limited ~~disturbance. Parts of this native grassland have been~~ to the dry season when feed is scarce at the GSG-plots.

The site was selected 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 land usage until parts were converted to different ~~agricultural~~ uses ~~since 1966. The different uses are summarized in Table 1.~~in 1966 and beyond (Fig. 1). Briefly, ~~they comprise~~the land uses we encountered comprised arable field crops and grazed-seeded grasslands (both at field scales with at least 1 ha), as well as a fodder bank that also served as a forage museum, housing live plant species brought from all over the ~~above mentioned experimental research exhibition farm. The research exhibition farm comprised~~world. Our study considered 59 species with relevance for forage production~~,~~ (Table S1), representing four plant functional groups: cut-use grasses (38 species), cut-use legume herbs (11 species), cut-use legume trees and shrubs (7 species), and cut-use non-legume trees and shrubs (3 species) at plot scales (25~~–30 m^2). Each plot was harvested once every year and weeded to maintain species purity, with no other management being conducted. Individual species of each functional group constituted a replicate. Similarly, the different fields under each land-use type constituted a replicate.~~–30 m^2). As for a subset of species, several accessions of the same species were present, and we sampled 72 plots from the 59 species. The management practices associated with the land-use types are summarized in Table 1.

2.2 Soil sampling~~, preparation~~

Field/plot selection was based on two main criteria- (i) that the field was established in 1966 and ~~analyses~~(ii) the management remained relatively stable over the 50-year time-frame. Information regarding the management of the fields and biomass productivity of the species were obtained from documentations available at the farm. Plots/fields that underwent some changes in management or re-established for any reason were omitted from the study. Consequently, there were no replicated plots, as in a traditional agricultural experiment, in the case of the cut-use forage species, to control for variation. Hence, we categorized the forage species into functional groups, with the individual species of each functional group constituting a pseudo-replicate. ~~The~~

Table 1. Brief description of land-use types at the study site

Land-use type	Description
Native grassland (field) (NGL)	Dominant plant species included *Panicum maximum*, *Cyperus spp*, *Talinum triangulare* and *Panicum decumbens* and intermittent occurrence of shrubs, including *Acacia sp.* and *Azadirachta indica*, lightly grazed by sheep and cattle; aboveground biomass yield is 5.7 – 7.2 t ha^{-1} (Timpong-Jones et al., 2013); uncultivated since the last 50 years
Arable crop production (ARC) (field)	*Three fields; sown crops: Manihot esculenta*, *Arachis hypogea*, *Zea mays* and *Vigna unguiculata*, as well as fodder grasses and legume herbs; two fields utilized zero tillage, while one was fully tilled; only one of the fields received fertilizer

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Grazed-seeded grasslands (GSG) (field)	Single-species pastures -*Brachiaria brizantha*, *B. ruziziensis* and *Digitaria decumbens*; grazed rotationally by Djallonke sheep (25-37 kg adult weight) and Sanga cattle (300-330 kg adult weight).
Cut–use forage plots	Monoculture (25-30 m^2); 38 species of grasses (CG), 11 species of legume herbs (CLH), 7 species of legume trees/shrubs (CLTS) and 3 species of non-legume trees/shrubs (CNLTS), respectively, no soil amendments. Plots were weeded to maintain plot purity and harvested twice in the wet season and once in the dry season. Prostate and erect grasses and herbaceous legumes were harvested at 6-10 cm and 10-20 cm high, respectively while shrubs were defoliated at 30 cm high. Annual dry matter yields (mean±sd) from the forages were 8.63±3.57, 4.08±1.41 and 6.53±2.17 Mg ha^{-1} for shrub/trees, legume herbs and grasses, respectively.

Soil samples were taken from the native grasslands and arable crop fields by zone-based composite sampling, where we divided the fields into three zones (northern, central, and southern). For the seeded-grazed paddocks, we randomly selected three paddocks out of the nine for soil sampling. We collected 10 – 20 subsamples (depending on the size of the zone) spread evenly across each zone in a zig-zag pattern, to constitute a composite sample, each composite soil constituting a replicate. In the case of the plot-scale samples, the sampling procedure ~~adopted in this study ~~followed recommendations by Saiz and Albrechts (2016). Sampling locations were determined by roughly locating the first at the centre of the field, with three replicates laid out according to a pattern of three axes separated 120° to a primary axis pointing north. Replicates were selected along these axes at approximately mid-distance between the centre of the field and its boundaries.

Before any sampling, surface litter was removed by hand. For each sampling plot or field, ~~four replicates of ~~soil samples were taken (0–30 cm soil depth~~).~~) using a soil probe of 1-inch diameter. Replicate samples obtained were bulked, thoroughly mixed, and a representative sample taken into a zipped polythene bag for analyses. Due to the higher number of accessions, the total number of soil samples obtained from the cut-use forages was 72, derived from 59 species. For the estimation of soil bulk density, ~~four~~3-8 sets of soil samples were taken from each experimental unit (replicate plots/fields~~)~~), depending on size, using a stainless-steel core sampler at depths of 0~~—~~–5~~ cm~~, 5~~—~~–10~~ cm~~, 10~~—~~–15~~ cm~~, 15~~—~~–20 ~~cm ~~and 20–25 cm. Soil bulk density and C stock were estimated according to methods by Guo and Gifford (2002).

2.3 Soil analyses

Soil samples meant for C and nutrient analysis were initially oven-dried at 30ºC for 48 hours. Dried samples were sieved with a 2 mm sieve to remove coarse particles and plant roots. Sieved samples were milled and stored in a desiccator before analyses.

Soil samples were analyzed for C and total soil N (~~TN~~*t*N) using the C~~/~~-/N analyser (Vario Max CN, Germany), using aspirin (50 mg; N = 9.7 %; C = 34.0 %) and a standard soil sample (1 g; N = 1.2 %; C = 1.4 %) after every 10 test soil samples to aid calibration of the equipment. Bulk density was estimated after oven drying at 105ºC. It was assumed that soil samples did not contain inorganic C because pH values were less than seven and because no liming or any other amendment was carried out during the past 50 years; therefore, total C was considered as SOC. Additional randomly performed HCl tests confirmed this assumption.

Soil pH was determined according to methods by Wiesmeier et al. (2012). Soil pH was measured directly with a pH meter (Microprocessor pH/ION Meter, PMX 3000, WTW) after adding 0.0125 M $CaCl_2$ solution to each sample in the ratio of 1:2.5 (soil: $CaCl_2$ solution). Plant available ~~phosphorus (~~P~~)~~ and exchangeable ~~potassium (~~K~~)~~ were extracted from 1 g air-dried fine soil (<2 mm) using Bray 2 solution, the reagents being 0.1 M HCl and 0.03 M NH_4F (Bray and Kurtz, 1945). K was determined using flame-photometry, and P measured calorimetrically at 882 nm (Miller and Arai, 2016) after reaction with ammonium molybdate and development of the ~~‘Molybdenum~~'Molybdenum' (Mo) ~~Blue’~~Blue' colour (within 30 min.).

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2.~~3~~ 4 Plant sampling and proanthocyanidin analyses

Plant samples for CT determination ~~were taken~~harvested from forage species at the time of soil sampling ~~sites in January 2018,~~ which was during the late annual growth stage. Sampling included leaves and leaf stalks from dicots, which consisted of legume herbs, legume and non-legume tree and shrub species (Supplementary table S-1). After cutting, samples were immediately cooled on ice before being freeze-dried, milled with a ball mill and stored in a freezer at -28 °C until further analyses. Condensed tannins (CT, syn. proanthocyanidins), which consisted of extractable CTs (ECT), protein-bound tannins (PCT) and fibre-bound tannins (FCT), were determined according to methods prescribed by Terrill et al. (1992). ~~The CTs were extracted from 20 mg plant samples using an acetone/water mixture (80/20 v/v), and their concentrations determined using a spectrophotometer (Libra S22, Biochrom) at 550 nm. Total CT (TCT) was calculated as the sum of ECT, PCT and FCT for each candidate species.~~Despite newer analytical techniques being available, Terrill et al. (1992) provide the benefit of separating again between protein- and fibre-bound tannins and hence was considered to be most suitable. Extractable condensed tannins (ECT) were extracted from 20 mg plant samples using an acetone/water mixture (80/20 v/v), vortexed for 5 minutes and shaken on a plenary shaker (280 min^{-1}) at 4 °C overnight. The samples were then centrifuged for 10 minutes (14,000 rpm) and decanted into 2 mL Eppendorf tubes. Residues were extracted again using the same setup with the plenary shaker now shaking for 3 hours, centrifuged and the supernatant decanted on top of the first extract. Acetone was evaporated in each case in an Eppendorf concentrator plus (Eppendorf, Hamburg, Germany) at room temperature for 90 min. Extracts were frozen overnight and after that freeze-dried for 24 hrs. 1 mL of UPLC-grade water was added to the freeze-dried extracts, vortexed and filtered with a PTFE filter (0.2 µm).

~~2.4 Calculations and statistics~~

Protein-bound condensed tannins (PCT) were extracted twice from the residues by adding 10 g L^{-1} sodium dodecyl sulphate (SDS) and 50 g L^{-1} 2-mercaptoethanol in 10 mM Tris/chloride adjusted to pH of 8, vortexed for 5 min and then placed in a continually boiling water bath for 60 min and cooled on ice to room temperature. The mixture was then centrifuged for 10 min and the supernatant decanted into 2 mL Eppendorf tubes in triplicates. For analysis, 960 µL of n-Butanol/HCl (95:5 v/v) solution was added to 240 µL of the extract of either ECT or PCT, vortexed for 5 min and heated in an oven at 90 °C for 90 mins, cooled on ice and transferred to a spectrophotometer (Libra S22, Biochrom) and analyzed at 550 nm to determine the CT concentrations. For the fibre-bound condensed tannin (FBCT) concentration in the samples, 1200 µL of BuOH/HCl (95/5, v/v) and 120 µL SDS was added to the residues from the extracts, vortexed for 5 mins, centrifuged for 10 mins and heated in an oven for 90 mins at 90 ºC, before being cooled on ice to room temperature, centrifuged for 1 min and then measured in the spectrophotometer at 550 nm. Total CT (*t*CT) was calculated as the sum of ECT, PCT and FCT for each candidate species. Annual *t*CT was estimated by multiplying *t*CT with the mean biomass yield of each functional group. The dataset, as well as a brief description of the site, materials and methods adopted to generate data, is available in Nyameasem et al. (2020).

2.5 Calculations and statistics

Due to lack of data regarding the initial SOC stocks of the farm, we ~~Soil bulk density and C stock were estimated according to methods by Guo and Gifford (2002). Change in soil C stock as a result of the land-use change was~~ estimated ~~as the difference between~~per cent changes in soil C stock ~~of~~ using a pseudo baseline (native vegetation ~~(~~as a natural baseline~~) and the land use type under consideration (;~~ FAO, 2019). ~~Annual changes in soil C stock were estimated by dividing each difference by the number of years (50 years) since the native grassland was~~ Since the plots/fields were converted~~.~~ during the same time frame, the status of the converted fields would have been assumed to be similar to the native vegetation if they had remained unconverted. To ~~analyse~~analyze the effect of the different land~~-~~ use types on SOC and soil properties, we performed a one-way ANOVA~~, followed by Tukey's~~ using generalized linear models. Also, we tested the effects of plant functional group (legume trees/shrubs, legume herbs, non-legume trees/shrubs) and plant part (leaves, leaf stalks) on the polyphenol content of the forages in a two-way ANOVA using linear mixed-effect modelling, with species as a "random factor". *P*-values were estimated based on type II sum of squares (SS) in the case of the one-way ANOVA and type III SS in the case of the two-way ANOVA due to the dissimilarity of the sample sizes (Fox and Weisberg, 2019). In cases where *P*-values were significant,

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Tukey's post hoc tests, using the 'lsmeans' function of the 'multcomp' package (Bretz et al., 2011) was performed to permit pairwise comparisons of means ~~(p<0.05). In cases where~~.

Before the ANOVA, data were checked for normality ~~(Shapiro-Wilks)~~and homogeneity of variance. In cases of abnormality or heteroscedasticity, data were log-transformed or corrected using 'White-adjusted heteroscedasticity corrected standard errors' from 'car package of R. Where data normality or the equality of ~~error~~ variances ~~(Levene's test) required for ANOVA~~ were not confirmed ~~per data set~~even after log-transformation, a non-parametric test (Kruskal-Wallis) was used, followed by ~~Dunnett T3's~~ Dunnett's test post hoc tests to permit pairwise comparisons of means~~. We tested the effects of plant functional group (legume trees/shrubs, legume herbs, non-legume trees/shrubs) and plant part (leaves, leaf stalks) on the polyphenol content of the forages using linear mixed-effect modeling, with species as a "random factor". Non-normal data were transformed before analyses of variance. Means with significant differences were separated using Tukey.~~

of the unequal sample sizes (Zar, 2010). Full correlations ~~and multiple~~ , bivariate (linear ~~regressions~~and exponential) and a full hierarchical model were ~~conducted~~fitted to the datasets to establish relationships between the measured variables and ~~changes in SOC stock (ΔSOC). A full hierarchical model was fitted to each of the two datasets (Table 5) to assess the extent to which the measured variables could predict the observed changes in SOC stocks. The backward elimination procedure (based on the lowest contributing variable) was used to generate a total of 17 candidate models. Due to differences in sample size, Akaike's~~SOC as well as to identify main effects and interaction terms as predictors of SOC. Akaike's Information Criterion with small-sample bias adjustment (AIC_C~~) and respective AIC weights (~~, Akaike, 1985; Burnham et al., 2011) was estimated and used to ~~narrow the models down to~~identify the best-fit model ~~(model with the highest AIC weight) per each dataset. Finally, the model with the highest coefficient of determination (R^2) and lowest error term was selected as the best equation (Eq. 1):~~. All the statistics were performed using in R (R Core Team, 2019).

~~$\Delta SOC(Mg\ SOC\ ha^{-1}yr^{-1}) = -1.74 - 0.073TCT + 0.967N + 7.57P - 2.31K + 0.174pH - (0.12N * pH) + (0.433K * pH) - (2.87N * P) + (0.006TCT * N) - (0.054TCT * P)$, (1)~~

~~where TCT is total condensed tannins (mg kg^{-1} DM), N is total soil nitrogen (Mg ha^{-1}), P is plant-available phosphorus (Mg ha^{-1}), K is exchangeable potassium (Mg ha^{-1}) and pH is soil acidity or alkalinity.~~

3 Result

3.1 Land-use change ~~and~~, soil C stocks and soil chemical properties

~~Land~~ The land-use types impacted soil C stocks in the upper soil layer (0–30 cm), with ~~the~~ group means ranging from 19.9 to 36.8 Mg C ha^{-1} and with a mean and standard deviation of 32.9±10.2 Mg ha^{-1}. Whereas SOC stocks ~~were 85% and 78% lower (p<0.01) for~~did not differ between the agricultural soils and native grassland soils (P>0.05), grazed-seeded grassland soils had 86 % and 77 % less (P<0.01) SOC density relative to cut-use trees/shrubs and grasses, respectively (~~Figure. 2A~~Fig. 3A). Among the cut-use forage systems, SOC ~~stocks were 77~~was 36 % higher for cut-use grasses ~~compared~~relative to legume herbs (~~p~~P<0.0~~1). Converting~~5). Using the native grassland ~~to agricultural land-uses resulted in~~as a pseudo baseline, we observed SOC stock changes ~~in SOC,~~ ranging from ~~-309 – 43 kg ha^{-1} yr^{-1}~~;44–5 %, with a mean ~~annual~~ loss of ~~-16% (Figure 2B). The~~ 15 % and the most considerable negative change ~~of 44% occurred~~occurring in grazed-seeded ~~grasslands, being significantly higher (p<0.05) than the losses observed in soils of cut-use grasses in~~ grassland soils (Fig. 3B). However, there appeared to be near zero to positive changes in cut-use grass and legume trees/shrub ~~fields. Also~~soils. Among the cut-use fodder groups, SOC losses in soils of cut-use legume herbs were 26 % greater ~~than in soils of~~relative to cut-use ~~grasses (p<0.05).~~grass soils.

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~~3.2 Land use change and soil chemical attributes~~

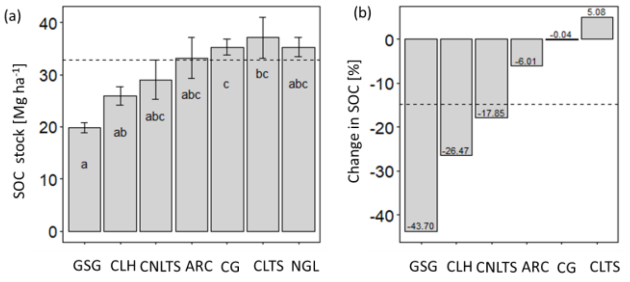

Figure 3. Barplots (with error bars) of SOC stocks (A) and per cent changes in SOC stocks (B) due to native grassland conversion under different land-use scenarios. [Different lowercase letters indicate significant differences between the land-use types ($p<0.05$; se = 1.13); CLTS= cut-use legume trees/shrubs; NGL= native grassland; CG= cut-use grasses; ARC= arable crops; CNLTS=Cut-use non-legume trees/shrubs; CLH= cut-use legume herbs; GSG = grazed-seeded grasslands; broken horizontal line indicate the means].

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Table 2. Soil organic carbon, soil macro-nutrients and other chemical properties of soils under different long-term management

Soil chemical trait	Native grassland	Arable crops	Grazed-seeded grassland	Cut-use forage production				*P*-value	SEM
				Grass	Legume herb	Legume trees/shrub	Non-legume tree/shrubs		
tN, Mg ha^{-1}	2.52^{ab}	2.48^{ab}	1.75^{ab}	2.66^{b}	1.94^{a}	2.55^{ab}	1.93^{ab}	0.0196	0.09
AvP, kg ha^{-1}	89.6^{ab}	185^{b}	15.0^{a}	37.8^{a}	28.1^{a}	112^{b}	73.6^{ab}	< 0.0001	8.23
K, Mg ha^{-1}	6.96^{ab}	16.6^{b}	3.34^{a}	7.42^{ab}	4.45^{a}	7.43^{ab}	4.80^{ab}	0.0028	0.52
C: N ratio	14.4^{b}	13.6^{ab}	11.3^{a}	13.5^{ab}	13.6^{ab}	14.6^{ab}	15.2^{b}	0.0489	0.22
Soil pH	5.69^{bc}	5.80^{c}	4.71^{ab}	5.35^{bc}	4.79^{a}	5.27^{abc}	5.42^{abc}	0.0037	0.05

tN= soil total nitrogen; Av. P= plant-available phosphorus; K= soil potassium. ^{abc}Mean values in the same horizontal row with different letters are significantly different ($p< 0.05$); SEM, standard error of the mean

Table 3. Condensed tannin (CT) profile and CT yield from three plant functional groups growing at the experimental

Functional group	Condensed tannins (g kg^{-1} DM)				*t*CT yield* (kg ha^{-1} yr-1)
	ECT	PCT	FCT	*t*CT	
Legume herbs	2.63^{a}	2.60^{a}	2.78^{a}	8.01^{a}	32.7^{a}
Legume trees/shrubs	10.6^{b}	8.52^{b}	6.07^{b}	25.1^{b}	217^{b}
Non-legume trees/ shrubs	5.83^{ab}	3.24^{ab}	4.79^{ab}	13.9^{b}	120^{b}
P-value	<0.001	<0.0001	<0.0001	<0.0001	<0.0001
SEM	1.06	0.72	0.65	1.99	16.9

ECT= extractable condensed tannins; PCT= protein-bound condensed tannins; FCT= fibre-bound condensed tannins; *t*CT= total condensed tannins (ECT+PCT+FCT); By plant functional groups,

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values in a same column with different letters are significantly different. *Annual *t*CT yield was estimated using the mean annual dry matter yields

The ~~land-use types caused variations in the soil properties, with mean~~mean (±sd) ~~soil organic matter, total nitrogen, available P~~OM, *t*N, *av*P and exchangeable K ~~of~~ concentrations observed for the site were 1.32±0.41 %, 0.057±0.02 %, 10.4±11.9 mg kg^{-1} soil and 68.1±36.7 mg kg^{-1} soil, respectively~~, varying most within each land-use type (coefficient of variation > 30%). Soil total N (TN) was generally low, yet lowest for grazed-seeded grasslands and non-legume tree/shrub,~~. Whereas *t*N, *av*P and K concentrations among the native grassland and agricultural soils ($P>0.05$), cut-use grasses soils contained 37 % more N compared with legume herb soils (Table 2). Arable crop soils contained 5, 7 and 12 times more *Av*P density compared ~~to cut-use grasses and legume tree/shrubs (Table 2). For the tree/shrub species (deep rooted), the legumes had 56~~with cut-use grass, herbs and seeded-grazed soils ($P<0.001$), respectively (Table 2), while exchangeable K density in arable crop soils was five and four times higher ($P<0.05$) compared with seeded-grazed and legume herb soils, respectively (Table 2). CN ratio was 27 % higher ~~($p<0.01$) TN relative to the non-legumes. For the shallow-rooted species, cut-use grasses had 52% more N compared legume herbs (Table 2). Plant available P only differed between grazed-seeded grasslands and non-legume tree/shrubs ($p<0.05$), which was five-fold higher for non-legume tree/shrubs (Table 2). Exchangeable K differed only between cut-use grasses and legume herbs ($p<0.05$), although it was almost three times higher in food crop soils~~ in native grassland soils ($P<0.05$) compared ~~to the soils of other land-use types (Table 2). CN-ratio~~with seeded-grazed and was lower (~~p~~$P<0.05$) for grazed-seeded grasslands compared ~~to cut-use grasses, legume herbs and~~ with non-legume trees/shrubs. ~~Meanwhile,~~It appears CN-ratio was not ~~affected by plant functional grouping.~~different among the forage species ($P>0.05$). Soil pH ranged from moderately acidic to neutral and was affected by the land-use types~~. The cut-use legume herb soils were~~, and was more acidic ~~compared~~ in the cut-use herb soils relative to the grasses, arable crop and native grassland soils and was more acid for seeded-grazed fields relative to ~~cut-use grasses ($p<0.05$), but not the others (Table 2).~~ arable crop soils ($P<0.05$).

3.~~3~~2 Plant secondary metabolites

~~Total condensed tannins (CTs)~~ **in** ~~the~~**cut-use forages** ~~ranged from 2 – 67 mg kg^{-1} DM of plant. Extractable CT (ECT) ranged between 0.1 – 3.0%; protein-bound CT (PCT) ranged between 0.1 – 1.7% fiber-bound CT (FCT) between 0.04 – 2.0% and total CT (TCT) between 0.2 – 5.7% of dry matter (DM). The distribution of the tannins were affected by plant functionality ($p<0.001$). In both leaves and leaf stalks, ECT, PCT, FCT and total CT were lower in cut-use legume herbs compared with legume trees/shrubs ($p<0.001$) but not non-legume trees/shrubs (Table 3). Total CT was about four-fold in legume trees/shrubs compared with levels in the legume herbs (Table 3). However, the condensed tannins were not affected by the part of plant analyzed (Table 3). Both leaves and leaf stalks contained similar levels of ECT, PCT, FCT and TCT ($p>0.05$). 3.4~~The CT distribution in the forages was affected by plant functional groupings ($P<0.001$) but not by the part of plant analyzed or by plant group - plant-part interaction terms ($P>0.05$). Accordingly, the functional group effect on CT distribution in the forages is presented in Table 3. ECT, PBCT and FCT in the legume herbs were 4, 3 and 2 times, respectively, lower ($P<0.001$) compared with legume trees/shrubs but not with non-legume trees/shrubs ($P>0.05$). However, *t*CT per DM and total annual *t*CT yield were 2-3 times and 4–6 times, respectively, higher ($P<0.001$) in both tree/shrubs compared with the herbs (Table 3).

3.3 Relationships between soil organic carbon and soil/plant chemical parameters

~~Bivariate regressions showed positive correlations between changes in SOC and all tested soil properties (r= 0.13 – 0.9; $p<0.05$), except CN, with correlations of SOC and TN, K and pH being significant (Table 4). The impact of N on SOC storage or loss was highest among the soil properties. The response size of changes in SOC to changes in N content was larger in legume trees/shrubs (slope = 15) compared to response size in the grasses (with a slope = 11) and the legume herbs (slope = 10; Fig. 3). All the CTs (ECT, PCT, FCT and total CT) correlated positively and significantly ($p<0.05$) with changes in SOC stock, with the highest impact from PCT (Table 3). Full multiple regression analyses showed that the soil chemical variables~~

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Commented [CM1]: 67 Mg kg DM is 6.7 percent, so this range is 0.2-6.7%. Below you write the range Is 0.2-5.7%.. It is fine if the information is provided once, so please check which number is correct and then only provide the information once...

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~~(N, P, K and pH), including interaction terms, explained 82% of the variations in SOC stocks when the native grassland was converted to agriculture. However, including TCT (and interactions with soil chemical traits) in the models explained an additional 10% of the variations in SOC stocks as affected by land-use types (Supplementary Table S-2), hence improving the predictability of SOC changes, and raising the adjusted coefficient of determination to 0.92 (*Eq.* 1). Table 5 shows details (ANOVA) of the best model explaining changes in SOC stock due to native grassland conversion. Soil N, exchangeable K and plant-available P concentration in soils had a significant impact (*p<0.05*) on the observed changes in SOC stock at the site.~~

~~4~~ Pairwise correlation between the measured variables suggested significant (P<0.01) positive associations between SOC and all the soil chemical properties (Table 4), with the strongest association between SOC and *t*N (r = 0.86). Although the CT variables correlated positively with SOC and *t*N, the associations were weak and insignificant except for annual *t*CT yield (Table 4). Whereas *t*N, *ExK* and CN ratio related exponentially with SOC, *AvP* and soil pH related linearly with SOC (Figure 4a–e). Generally, higher *tN*, avP and soil pH appeared to be associated with higher SOC stocks; however, there appears to be a weaker association between SOC and *t*N at higher soil N concentrations. Whereas the response of SOC to *t*N was similar for non-legume trees/shrubs, grasses and legume-herbs (mean slope=10.5), it was about 36% higher compared with legume tree/shrubs (slope = 14); Figure 5). Higher soil K density was associated with higher SOC stocks, but amounts beyond 16 Mg ha^{-1} appeared to depress SOC accumulation whilst the relationship between SOC and CN ratio appeared positive only beyond a ratio of 15 (Figure 4c-d). SOC related exponentially with *t*CT yield with generally higher biomass CT associating positively with SOC at increased rates beyond 200 kg ha^{-1} (Figure 6). A full regression model predicting SOC density using the measured soil and plant chemical variables as predictors showed significant effects of *t*N, *Av*P, K and *t*CT as well as *Av*P x pH and *t*N x avP x K interaction terms (*P*<0.05) in the model, with *t*N having the greatest effect (Table 5).

Table 4. Pearson correlation coefficients showing the relationships between soil and plant chemical parameter

	Soil pH	*ECT*	*PBCT*	*FBCT*	*tCT*	*Ann. tCT*	*AvP*	*ExK*	*SOC*	*tN*
CN ratio	0.01	0.24^{*}	0.15	0.21^{*}	0.24^{*}	0.33^{*}	0.18	0.14	0.29^{**}	-0.22^{*}
Soil pH		0.02	-0.05	-0.11	-0.04	0.28^{*}	0.24^{*}	0.30^{**}	0.53^{***}	0.55^{***}
ECT			0.59^{***}	0.66^{***}	0.90^{***}	0.84^{***}	0.22^{*}	-0.04	0.14	-0.01
PBCT				0.66^{***}	0.84^{***}	0.78^{***}	0.22^{*}	-0.04	0.12	0.02
FBCT					0.87^{***}	0.63^{***}	0.05	-0.08	0.01	-0.13
tCT						0.93^{***}	0.20^{*}	-0.06	0.11	-0.04
Ann. tCT							0.31^{*}	0.39^{**}	0.47^{***}	0.29^{*}
AvP								0.27^{**}	0.29^{**}	0.19^{*}
ExK									0.62^{***}	0.55^{***}
SOC										0.86^{***}

ECT= extractable condensed tannins; PCT= protein-bound condensed tannins; FCT= fibre-bound condensed tannins; *t*CT= total condensed tannins (ECT+PCT+FCT); Ann. *t*CT = annual *t*CT yield; Significance levels *<0.05, **<0.01, ***<0.001.

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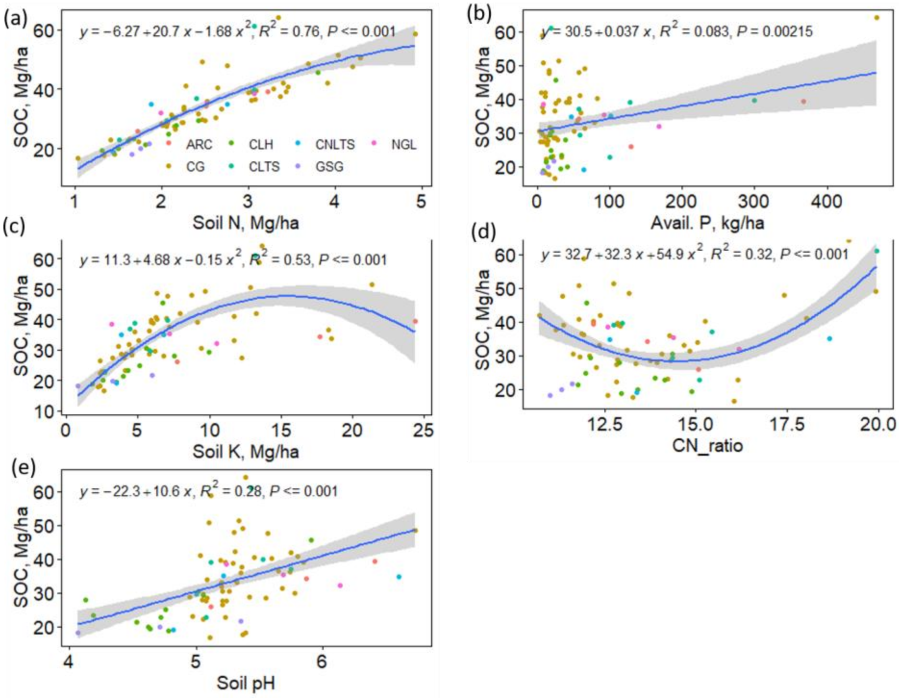

Figure 4: Bivariate regression curves showing the relationship between SOC and the measured soil variables. [The grey band shows the 95 % confidence interval; CLTS= cut-use legume trees/shrubs; NGL= native grassland; CG= cut-use grasses; ARC= arable crops; CNLTS=Cut-use non-legume trees/shrubs; CLH= cut-use legume herbs; GSG = grazed-seeded grasslands]

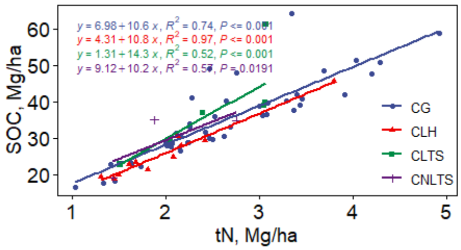

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Figure 5: Relationships between SOC and soil total N as influenced by plant functional groups. [CLTS= cut-use legume trees/shrubs; CG= cut-use grasses; CNLTS=Cut-use non-legume trees/shrubs; CLH= cut-use legume herbs]

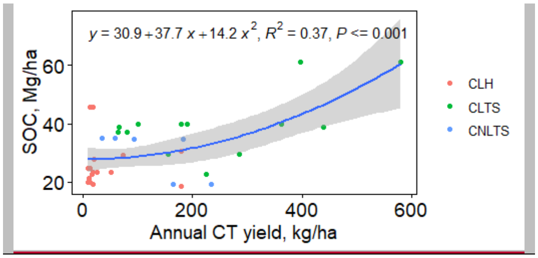

Figure 6: A graph showing the stoichiometric relationship between SOC and leaf biomass CT [The grey band shows the 95 % confidence interval; CLTS= cut-use legume trees/shrubs; CNLTS=Cut-use non-legume trees/shrubs; CLH= cut-use legume herbs]

4.0 Discussion

4.1 Land use change and carbon stocks

~~Our study reported differential distribution of SOC~~We observed a wide range of SOC stocks (17–64 Mg SOC ha^{-1}) across the land-use types~~. This observation affirms~~ at the ~~claim by previous authors~~study site, which~~ (Amézquita et al., 2008; Stahl et al., 2016) that the type of land use affects soil C cycling and determines the ultimate C storage potential of soils. The range of SOC stocks observed in the current study~~ largely agrees with ~~previous~~previously reported ranges of 10~~—~~–50 Mg ~~C~~SOC ha^{-1} for vegetation gradients varying from Sudanese-Sahelian Savannah to a sub-tropical forest (Saiz et al., 2012; Bessah et al., 2016). Similar to previous reports (Olson, 2013; Bessah et al., 2016), ~~we observed that converting native~~ the use of grassland ~~to seeded grassland or arable land~~for agriculture resulted in both positive and negative responses by soil C dynamics~~ with a mean net loss of 16% within 50 years.~~

~~Under different climate change scenarios,~~, Tan et al. (2009) projected changes of -4 to -23~~% (2000 to 2100) change~~ % in soil C stocks of cultivated savannahs of Ghana~~. Thus, the magnitude of change in~~ on a hundred-year scale, depending on the climate change scenarios. Hence the 15 % loss of SOC ~~stocks (-16%) due to land-use change~~ in 50 years ~~observed in our current study could~~ appears to be ~~considered high. However, we observed~~on the higher side. Nevertheless, there was an indication of C sequestration~~ (31 kg C ha^{-1} year^{-1})~~ in the case of cut-use legume trees/shrub production. Similarly, a previous study (Shanmugam et al., 2018) reported a mean annual change of 0.67±0.95 Mg C ha^{-1} for tropical mineral soils of secondary

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Commented [CM3]: Well, his predictions do not include land use change but just climate change if I read this correctly… SO those percent would be on top of our land use change percentages, making it even more drastic…

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woodland or Savannah converted to cultivated pasture or cropland. ~~Thus, the type of land-use system adopted after converting native grasslands may have a significant influence on soil C dynamics. At the farm level, where climatic and soil properties are relatively uniform, differences in land use might influence the quantity and quality of organic matter added to the soil. These variables might have induced plant-soil feedbacks to regulate soil C cycling at the micro level to cause local variations in C stocks (Post et al., 1982; Saiz et al., 2012; Chen et al., 2018).~~Therefore our observations are not surprising as the type of land management adopted after converting native vegetation affects soil C cycling and determines the ultimate C storage potential of soils (Amézquita et al., 2008; Stahl et al., 2016 ~~These causative factors are discussed in the subsequent paragraphs.~~

~~4.2 Grazing intensity and soil carbon storage~~

). From our study, SOC stocks of arable crop farming did not differ from the native grassland. Arable crop production is known to deplete SOC due to tillage effect, which tends to facilitate SOC cycling. 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 and sometimes weedicides. Thus, this is, of course, a much less invasive technique compared to regular ploughing (but a 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.

The low SOC stock in grazed-seeded grasslands was half (20 vs 35 t SOC ha^{-1}) that of ~~native grassland~~cut-use grasses and legume trees/shrubs, suggesting a utilization effect. ~~This observation is consistent with earlier studies (McSherry and Ritchie, 2013) which reported that the~~The effect size of grazing on SOC stocks might be dependent on the interactions of several factors, including grazing intensity~~. Although this study could not obtain adequate information about the stocking rate of the grazing fields, the low nutrient profile of the soils (Table 2) suggests a degradation and overuse of soils.~~ (McSherry and Ritchie, 2013). Overgrazing has been widely reported as responsible for the loss of SOC and a decrease in soil fertility in West Africa (Schönbach et al., 2011; Saiz et al., 2012). Generally, higher grazing intensities decrease soil C and N by direct removal of aboveground herbaceous biomass, thus reducing the potential of CO_2 fixation in photosynthetic tissue and reduction in belowground C inputs through lower root production and higher root litter turnover (Semmartin et al., 2010). On the other hand, moderate grazing may increase tiller density and aboveground productivity, particularly in C4 dominated grasslands (McSherry and Ritchie, 2013), compared to cut-use systems, thus leading to higher C inputs because of additions from crop residues, when abiotic factors such as irrigation and nutrient supply are appropriate. Thus, reducing grazing intensity can not only protect the ~~above-ground~~aboveground biomass of grasslands but also improve soil texture to enhance the accumulation of organic C (Xu et al., ~~2018). In this instance, the relatively low nutrient concentration observed in the grazed-seeded grasslands matches the low C stocks.~~2018).

~~4.3 Species composition and carbon stocks~~

Table 5. Results of full univariate ANCOVA model to explain the status of soil organic carbon density at the study site ($R^2 = 0.848$, Adjusted $R^2 = 0.853$, Adj. $R^2 = 0.830$, $AIC_C = 698.60$*)

Source	df	Type III sum of squares	F value	Pr(>F)
*t*N	1	2694	144.9	0.000
*av*P	1	73.53	3.955	0.049
K	1	414.1	22.27	0.000
pH	1	34.99	1.882	0.173

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*t*CT	1	230.6	12.40	0.001
*t*N x *av*P	1	28.62	1.539	0.218
*t*N x K	1	3.316	0.178	0.674
*av*P x K	1	202.1	10.87	0.001
*t*N x pH	1	56.44	3.036	0.085
*av*P x pH	1	116.7	6.277	0.014
K x pH	1	9.120	0.491	0.485
*t*N x *av*P x K	1	73.85	3.972	0.049
*t*N x *av*P x pH	1	13.26	0.713	0.400
N x K x pH	1	11.33	0.609	0.437
*av*P x K x pH	1	11.76	0.632	0.428
*t*N x *av*P x K x pH	1	33.69	1.812	0.181
Residuals	100	1859		

Akaike's Information Criterion (AIC) (Akaike, 1985; Burnham & Anderson, 1992) with the small-sample bias adjustment (AICC = n[ln (SSE/n)] + 2K + [(2K*(K + 1))/(n-K-1)]) (Hurvich & Tsai, 1995; Burnham & Anderson, 2002); *t*N = total N, *t*CT= total condensed tannins, *av*P = available P.

The effect of plant functional differences on SOC stock was evident in this study. Under similar growing conditions, we observed higher C stocks in fodder grass fields (36 % higher; p<0.01) compared to legume herb fields. Earlier reports (Alonso et al., 2012; Shanmugam et al., 2018) suggested that species composition of grasslands influences the quantity and quality of ~~organic matter~~OM input, and for that matter, C sequestration in the soil. Dry matter productivity is an essential factor that influences the accumulation of soil C. ~~Although we could not obtain information on above~~Above-ground biomass ~~production of the various plants encountered, earlier reports indicated~~productivity is relatively higher ~~above-ground biomass production~~ for fodder trees/shrubs, followed by grasses ~~compared~~and lowest in herbaceous legumes (Table 1). SOC stocks under the cut-use forages appear to follow a similar trend (Table 2, Figure 3). Thus, the higher SOC stocks observed under legume trees/shrubs relative to legume herbs ~~under extensive system in West Africa~~ could be partly attributed to the higher ability of legume trees/shrubs to produce relatively high biomass (Barnes and Addo-Kwarfo, ~~1996~~1999; Adjolohoun et al., 2008~~).~~), maintain soil fertility (as shown in Table 2), control runoff soil erosion (Franzel et al., 2014) and to maintain SOM.

~~Moreover, grasslands~~Grasslands containing more C4 grass species were reported to store more SOC compared ~~to~~with grasslands with more C3 and legume species (Yang et al., 2019). The grass species considered in this study consisted mainly of C4 grasses, whereas the legume herbs were mainly C3 shallow-rooted plants. C4 plants possess morphogenic and architectural traits that enhance their ability to out-perform C3 plants, particularly under harsh environmental conditions (Lanttanzi, 2010). They tend to develop high leaf area index, which enhances their ability to capture light, ~~nitrogen~~N and water compared to their C3 counterparts. Besides, C4 plants have higher photosynthetic efficiency in the use of water and N and yield higher quantum productivity (Taylor et al., 2010) compared to C3 plants. The inherently lower Rubisco concentration and the more lignified tissue in C4 plants limit organic matter decomposition, thus producing more recalcitrant organic ~~carbon~~C (Yang et al., 2019).

Moreover, C4 plants are reported to partition C towards roots in N limited situations; thus, they have a higher ability to fix soil C under N-stress situations (Sage and Pearcy, 1987; Long, 1999). ~~Moreover,~~Another reason could be that C4 plants have a higher concentration of amino acids and organic acids in their root exudates compared to C3 plants (Nabais et al., 2011~~); the organic acids~~) which could protect SOC and N from microbial decomposition. Therefore, the relative differences in aboveground biomass characteristics could be responsible for the observed differences in SOC stocks under fodder grass and legume herbs fields.

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~~The higher SOC stocks observed under legume trees/shrubs compared with legume herbs could be partly attributed to the higher ability of legume trees/shrubs to produce relatively high biomass (Barnes and Addo-Kwarfo, 1999), maintain soil fertility (as shown in Table 2), control runoff soil erosion (Franzel et al., 2014) and to maintain SOM. Moreover, higher CT content (Table 3) in legume trees/shrubs might slow decomposition rate of soil organic matter (Dong et al., 2016) to promote SOC accumulation. The rate of change of SOC to changes in total soil N was higher in legume trees/shrubs, relative to grasses and herbs (Fig. 3).~~

~~Our findings further extend the benefits of cut-use trees/shrubs as they already contribute to food security, incomes and livelihoods in Africa. These plants are deep-rooted, resistant to drought and maintain high protein levels during the dry season when high-quality feed is scarce (Wambugu et al., 2011). Additionally, fodder trees /shrubs provide by-products such as stakes, firewood, bees and seeds. The high CT content of cut-use legume trees/shrubs helps to reduce methane emissions per unit of output and reduce carbon emissions by substituting for commercially manufactured concentrates (Franzel et al., 2014). The positive effects of tannins on ruminant nutrition, particularly on enteric methane emission and reduced urine N excretion, have been well reported (Hristov et al., 2013; Nyameasem et al., 2017).~~

4.2 Impact of differential land uses/plant types on soil quality

We~~CTs may have both positive and negative effects on livestock, depending on the concentration of CT in the plants. This nutritional implication is particularly vital with tropical shrubs and tree species where high CTs are known to impair protein availability (Jayanegara et al., 2018). However, the proportion of total CT allocated to ECT (33–44%) observed in this study (Table 3) suggests the forages are suitable for livestock feeding (Mupangwa et al., 2000; Jackson et al., 1996).~~

~~4.4 Drivers of carbon storage at the micro level~~

~~4.4.1 Soil properties~~

~~The mean nutrient concentrations in the soils suggest the soils are highly depleted of N and organic C. Although legumes are known to fix atmospheric N in soils, we~~ observed higher N concentration in cut-use grass fields compared to legume herb fields~~. In~~ although legumes are known to fix atmospheric N in soils. Indeed, in semi-arid and Savannah ecosystems of sub-Saharan Africa, legume herbs and legume browse species could fix about 8–217 and 61–643 kg ha-1 ~~year~~yr-1 atmospheric N, respectively (Hassen et al., 2017). ~~Legume~~Accordingly, legume-based systems can produce high biomass yield even in the absence of fertilizer application, showing a high N-cycling efficiency (Schmeer et al., 2014), generally increasing soil N concentration. ~~Several~~However, several factors might affect the ability of the legumes to synthesize N, including environmental conditions, N uptake by plants and soil pH (Nutman, 1977; Sage and Pearcy, 1987). Pure grass stands may show a high fraction of C and N allocation belowground (Loges et al., 2018), particularly when the root-functional-traits equilibrium theory is considered (Brouwer, 1983), and have amino acid-rich roots (Nabais et al., 2011), leading potentially to a more excessive N and C accumulation in the soil. These attributes of grasses might explain why soils in grass plots had higher ~~TN compared to the legume herbs. The higher TN in legume trees/shrubs compared to non-legume trees/shrubs is attributed to biological nitrogen fixation, which seems to have been more efficient in the woody legumes than in the legume herbs, potentially due to better compatibility with prevalent rhizobia strains.~~tN compared to the legume herbs. The fairly tight C: N ratio (14:1) observed for the agricultural systems reflects the stoichiometry of stable soil OM at the study site.

The site has seen a general decline in soil fertility (tN and SOM) although there were marginal increases in available P and K when compared to values reported for the site by Barnes (1999). While P and K ~~levels~~densities were moderate in arable ~~food~~ crop soils, they were low in the other land-use types (Bationo et al., 2018; Apal Agricultural Laboratory, 2019). The relatively high P and K levels observed in the food crop fields may probably be as a result of annual supply through fertilizer application

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(Table 1). Cut-use grasses and legume herbs appeared to deplete soil P, but not legume of non-legume trees and shrubs (numerically if not statistically). This trend might be partly related to differences in the root architecture. The grasses and herbs are shallow-rooted and therefore exploit nutrients from within the upper soil depth, while the trees/shrubs have roots below the 30 cm soil depth considered in this study, and therefore might not be able to exploit nutrients extensively from the upper depth of the soil. The relatively low nutrient concentration observed in the grazed-seeded grasslands matches the low C stocks (Table 2). It is, particularly, striking that P and K are much lower in grazed grassland despite the high excreta deposition associated with grazing. Although we could not ascertain the stocking rate on the grazed-seeded pastures, it appears there was frequent overgrazing of the pastures, which is a regular feature in this region, exposing the soils to nutrient loss via erosion (Bationo et al., 2018).

The soils were generally acidic, a common ~~feature~~characteristic of tropical soils (Jayne et al., 2015), however, the < 5.5 pH observed in grazed-seeded grasslands, cut-use legume herbs and non-legume trees/shrubs soils might cause aluminium and manganese toxicities. Low pH could also cause molybdenum, calcium, magnesium or potassium deficiencies and perhaps reduced microbial activity (Apal Agricultural Laboratory, 2019). ~~Apart from the arable crop fields, the pasture fields/plots received no nutrient replenishment during the period under consideration.~~ Among the land use systems, cut-use legume herbs and seeded-grazed soils appeared more acidic relative to the others. Legume plants commonly form symbiotic associations with rhizobia and accumulate most of their N through symbiotic N fixation. During this process, legume plants take up more cations than anions and release more H^+ ions from roots to the soil, leading to low pH values in both the rhizosphere and bulk soil (Zhao et al. 2009; Yang et al. 2016). This effect might differ between legume herbs and legume trees/shrubs due to differences in their root architecture. Grazing fields are associated with high N and C returns from animal excreta, but C and N cycles might cause acidification in grazed fields, for example, nitrate leaching might increase the concentration of H ions, which might lead to decreased soil pH (Ridley et al., 1990).

~~Soil nutrients have implications for plant primary productivity and ecosystem functioning (Post et al., 2012; Marques et al. (2016). However, most Ghanaian soils are inherently infertile (Bationo et al., 2018) as result of leaching, soil erosion by rainfall (0.6–0.9 t ha-1) and the fact that nutrients removed by crop harvest are not replaced by the corresponding amount of plant nutrients (Bonsu, 1979; Bationo et al., 2018). Consequently, the low concentration of soil nutrients across the fields explains the low potential of the soils to store SOC. These fragile soils, therefore, require sustainable forms of agricultural land-use systems to ensure higher below-ground NPP for increased C stocks.~~

4.~~4.2~~ 3 Condensed ~~tannins~~tannin concentration in ~~aboveground biomass~~ cut-use forages as affected by functional group differences

The total CT of below 67 g kg^{-1} DM observed in this study is similar to values reported for tropical forages, with lower concentrations in legume herbs compared to browses (Mupangwa et al., 2000; Sottie et al., 2016). CTs may have both positive and negative effects on livestock, depending on the concentration of CT in the plants. ~~2016).~~The nutritional implication of CTs is particularly vital with tropical shrubs and tree species where high CTs are known to impair protein availability (Jayanegara et al., 2018). Previous authors have reported higher values of more than 70 g CT kg^{-1} DM for some tropical shrubs and trees (Jackson et al., 1996; Rosales, 1999; Pereira et al., 2018), which invariably limits their use as fodder for ruminant livestock. ~~The proportion of total CT allocated to ECT was similar to the range of 12 – 44% reported by Mupangwa et al. (2000) but lower than 70 – 95% reported for some tropical browse species (Jackson et al., 1996).~~

~~A previous study by Mudau et al. (2007) demonstrated that regardless of season, application of nitrogenous, P and K fertilizers increased the total polyphenols in a quadratic manner in bush tea, suggesting that N, P and K limitation might affect the synthesis of CTs by the plants and for that matter SOC storage. The low nutrients in soils could partly explain the lower concentration of CTs in the forages encountered~~~~ compared to earlier reports on similar species (Jackson et al., 1996; Rosales, 1999; Pereira et al.,~~ The proportion of total CT allocated to ECT was similar to the range of 12–44 % reported by Mupangwa

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et al. (2000) but lower than 70–95 % reported for some tropical browse species (Jackson et al., 1996). Compared with other tropical forage species, the *t*CT shares of below 7 % indicate comparably low tannin concentrations, which indicates their suitability for livestock feeding (Mupangwa et al., 2000; Jackson et al., 1996).~~2018). Thus, increased soil fertility might have some implications for CT concentration in forages. The proportion of extractable CTs and bound CTs, even within species, may change with plant maturity and environmental conditions (Jackson et al., 1996) and may influence the feeding value of the forage.~~

~~4.4.3 Regression analyses~~

~~Soil macronutrient concentration and polyphenol content were~~ However, further analyses on forage quality parameters and tannin composition, as well as on other plant secondary metabolites might be required to give reliable estimates of digestibility.

4.4 Relationships between SOC and soil and plant chemical properties

Our correlation and regression analyses (Figure 4, Tables 4 and 5) indicated significant ~~factors that drove organic C dynamics (Table 5), confirming our hypothesis that the chemical status of soils might be influential in C storage or loss in extensive land-use systems. We observed~~relationships between the soil chemical properties and SOC. Similar to reports of Stahl et al. (2016), we found a close association between C and N. Nevertheless, the relationship between ~~soil C~~N and ~~N, suggesting N as an essential factor in the soil C cycle. However, the relationship~~SOC is ambiguous in the literature, ~~and~~with evidence of different responses of soil CO$_2$ fluxes to N levels in soils ~~exists~~ (Yan et al., 2016), including increases (Wang et al., 2015), decreases (Jiang et al., 2010), and no significant differences (Li et al., 2012). N cycle is strongly interconnected with the C cycle, as N is required to build up biomass (and hence C). Whereas Six et al. (2002) attributed the positive relationship between soil C and N to protection by both macro~~-~~ and micro~~-~~-aggregates against mineralization, Waldrop et al. (2004) explained that greater N availability ~~also decreases~~reduces decomposition rate of SOC by regulating production and activity of microbial extracellular enzymes. Indeed, when N becomes available in N-limited soils, photosynthetic reaction increases, thus enhancing SOC storage, but ~~inadequate~~small N may limit CO$_2$ fertilization (Lal, 2018). Accordingly, Tan et al. (2009) observed that increasing N application to about 30~~–~~–60 kg ha^{-1} year^{-1} could result in positive changes in soil C stocks of cultivated grasslands of Ghana. Indeed, the multiple linear regression model (Table 5) showed that *t*N had the most significant effect on SOC density, and with a greater effect size in legume tree/shrub species compared with the legume hers, non-legume trees/shrubs and grasses (Figure 5).

Interaction effects ~~between~~involving N~~ and~~, P~~, N~~ and K as well as pH and K ~~and pH had significant~~were significantly associated with SOC stocks (p<0.05~~) impact on the models,~~), suggesting that the effect of N on SOC sequestration might be dependent on the ~~other variables.~~availability of P, K and soil pH. The associative effect of N and P on SOC density was also reported by an earlier study (Bradford et al., 2008) where P and N additions led to a more significant C sequestration in soils. In a simulated study (Li et al., 2014), a combination of N and P fertilizers increased SOC storage but reduced microbial ~~biomass and~~ activity, as well as C mineralization, compared to adding N or P fertilizer alone or no fertilization. In contrast, Graham et al. (2014) reported a significant release of CO$_2$ when soil N increased. ~~It appears that~~In the current study, it the relative proportions of soil nutrients impact SOC storage~~. Thus,~~, and soils deficient in P ~~and cations (Ca^{2+}, Mg^{2+}) may~~might not be able to use N efficiently, ~~resulting in increased~~which might increase N losses by gaseous emissions and leaching~~,~~ and ~~depletion of~~deplete SOC pools~~. The theoretical basis, according to linear mixed-effects regression models (Table 5), shows that associative effects of CT and soil chemical properties may predict changes in SOC stocks in tropical sand soils~~.

The theoretical basis, according to linear mixed-effects regression models (Table 5), suggested *t*CT concentration in aboveground biomass as one of the potential factors that might affect SOC storage at the site (Table 5). This observation partly confirms our hypothesis that aboveground CT concentration might be influential in C storage. Although CT concentrations in the soils were not considered in this study, we speculate that the higher CT content in legume trees/shrubs (Table 3) might have slowed the decomposition rate of soil OM (Dong et al., 2016) to promote SOC accumulation (Kraus et al., 2003; Halvorson et al., 2011; Tamura and Tharayil, 2014; Chomel et al., 2016; Adamczyk et al., 2016, 2017; Kagiya et al., 2019).

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By extension, the higher potential of the tree/shrubs to produce more mass and more CTs per unit area might explain the higher SOC stocks under trees/shrubs relative to the herbs. However, the bivariate regression analysis showed that positive associations between *t*CT and SOC only occurred at concentrations above 200 kg ha^{-1} yr^{-1} (45 mg kg^{-1} DM). Although tropical herbs contained relatively higher CTs than their grass counterparts, the CT concentration might not be potent enough to impact SOC storage as shown in this study (*t*CT = 10±8; < 45 mg kg^{-1} DM). Although tropical herbs contained higher CTs than their grass counterparts, SOC density was lower in soils under the herbs compared with grasses. Even when the CT concentration in the biomass is low, it might be that in the rhizosphere it is higher due to accumulation. Below-ground biotic and abiotic conditions, including the presence of some fungi (e.g., *Basidiomycetes* and *Ascomycetes*) and bacteria species (e.g., *Pseudomonas*), temperature, pH, oxygen and substrate availability are factors that affect the degradation of phenolic compounds in soils (Min et al., 2015). Although the effect of pH on polyphenol degradation is not clear, under laboratory conditions low soil pH (< 5) was shown to be optimal for extracellular enzymes involved in degrading phenolic compounds in soils (Min et al., 2015). In our study, soil pH was lower in soils under the legume herbs compared with the grasses (Table 2). This low soil pH might have facilitated a faster degradation of potential accumulated CTs and mitigated the assumed effect of CTs on SOC.

We observed significant positive associations between annual CT yield and all the measured soil parameters. A previous study (Mudau et al., 2007) demonstrated that regardless of season, application of N, P and K fertilizers increased the total polyphenols in bush tea, suggesting that N, P and K limitation might affect the synthesis of CTs by plants and for that matter SOC storage. The low nutrient density in the soils might partly explain the lower concentration of CTs in the forages, compared to earlier reports on similar species (Jackson et al., 1996; Rosales, 1999; Pereira et al., 2018). Thus, the proportion of extractable CTs and bound CTs, even within species, might change with plant maturity and environmental conditions (Jackson et al., 1996) and might influence the feeding value of the forage. In consequence, increased soil fertility might have some implications for CT concentration in forages for that matter, livestock feeding and SOC storage.

We speculate that at the farm level, where climatic and soil properties are relatively uniform, differences in land use might influence the quantity and quality of organic matter added to the soil. These variables might have induced plant-soil feedbacks to regulate soil C cycling at the micro level to cause local variations in C stocks (Post et al., 1982; Saiz et al., 2012; Chen et al., 2018).

4.5 Implications of the study

This study shows that soil nutrients have implications for SOC input and removal, and by extension, for primary plant productivity and ecosystem functioning (Post et al., 2012; Marques et al., 2016). However, most Ghanaian soils are inherently infertile (Bationo et al., 2018) as result of leaching, soil erosion by rainfall (0.6–0.9 t ha-1) and the fact that nutrients removed by crop harvest are not replaced by the corresponding amount of plant nutrients (Bonsu, 1979; Bationo et al., 2018). Consequently, the low concentration of soil nutrients across the fields may partly explain the low SOC stocks observed for soils in the study site. These fragile soils would require sustainable forms of agricultural land-use systems to ensure higher belowground NPP for increased C stocks.

The forage legume herbs showed lowest yields (Table 1), indicating low bioactivity in comparison to grasses while the legume shrubs had the highest yields and thus captured a lot of N from the biomass. In addition to that soils under the shrubs contained similar *t*N compared with grasses, suggesting that most of the tested forage legume herbs were not suitable with regards to biomass yield for forage and soil carbon sequestration. Legume shrubs should be preferred as they provide more forage and ecosystem services. Invariably, our findings further extend the benefits of cut-use trees/shrubs as they already contribute to food security, incomes and livelihoods in Africa. These plants are deep-rooted, resistant to drought and maintain high protein levels during the dry season when high-quality feed is scarce (Wambugu et al., 2011).

5.0 Conclusion

~~Our study~~We tested the hypothesis that the conversion of native grasslands can cause considerable losses in soil C stocks of sub-Sahara Africa. ~~Most~~Although SOC stocks under in the agricultural soils did not differ statistically from the native grassland soils, most of the land transformations resulted in declining SOC stocks~~; however,~~, questioning the ~~degree~~potential of ~~loss varied according~~such farms to ~~land-use types and the management system adopted after the native grassland was converted.~~

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~~The~~achieve the 4-permille target. However, the observed wide range of SOC stocks~~, however,~~ suggests an enormous potential for SOC storage in the area. Our findings further extend the benefits of cut-use trees/shrubs as they already contribute to food security, incomes and livelihoods in Africa. The inclusion of cut-use shrubs/trees ~~in pastoral systems of the area is recommended due to~~ or their integration with grass fodder production could be researched further as they show a potential to store more C ~~as well as their ability to supply feed even during dry periods~~. All the measured soil chemical properties correlated positively and significantly with SOC, endorsing the fact that soil chemicals are essential drivers of NPP in ~~ecosystems.~~ecosystem. Hence a boost in productivity is also most likely to result in a boost in SOC. Condensed tannins were positively associated with SOC and could be explored further to harness their potential to extend the residence time of SOC in soils. ~~However, discrepant~~Discrepant pasture management, such as improper livestock stocking rate, grazing or forage harvest, species selection and nutrient management, could affect the transformation efficiency of plant CT to soil CT. We ~~suggest~~hypothesize that sustainable soil management practices, coupled with the adoption of CT-rich forages, ~~could~~might improve ~~the~~ SOC storage capacity of livestock production systems in this ecological region of Ghana.

Data availability

All data used in the analysis, tables and figures are made available at https://doi.org/10.6084/m9.figshare.12016158.v1.

Author contribution

J. K. Nyameasem acquired funding to carry out the study, J. K. Nyameasem, C. S. Malisch and T. Reinsch ~~conceived~~conceptualized the research ~~idea~~experiment and the hypotheses; J. K. Nyameasem, C. Y. F. Domozoro and E. Marfo-Ahenkora ~~collected~~carried out the data~~, J. K. Nyameasem and C. Malisch I. analysed samples chemically as well as the data statistically;~~ collection, J. K. Nyameasem~~, C. Malisch, T.~~ performed the soil and plant analyses, as well as developed the model code and performed the data analysis; J. K. Nyameasem, C. Malisch, and T. Reinsch and I. Emadodin prepared the manuscript with contributions from all co-authors; F. Taube ~~supervised the whole study~~provided oversight and took leadership responsibility for the research activity planning and execution.

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Competing interests

The authors declare that they have no competing interests.

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