

Dear Referee,

Thank you very much for your suggestions for improvement.

Following, we would like to share a point-by-point report of the changes made to the manuscript based on your review.

We are happy to answer further questions and include your recommendations to our manuscript.

Sincerely,

Josue De Los Rios

### General comment

The manuscript reports annual SOC stocks over a period of 6 years comparing different management systems. However, bulk density was only determined once at the end of the study in 0-30 cm and estimated for 30-60 and 60-90 cm and, thus, the SOC stock changes are highly speculative. It is unclear whether SOC stocks at the control were in equilibrium or the baseline was drifting. Further, total C is reported as SOC without indicating test for carbonates. More comments and suggestions in an annotated manuscript attached.

(1) Regarding: “bulk density was only determined once at the end of the study in 0-30 cm and estimated for 30-60 and 60-90 cm and, thus, the SOC stock changes are highly speculative.”

#### Response:

The dynamics in SOC stocks we are reporting are only linked to changes in SOC concentrations, not to changes in bulk density.

(2) Regarding: “It is unclear whether SOC stocks at the control were in equilibrium or the baseline was drifting”

#### Response:

The SOC stocks in the control (GC) are not in equilibrium at 30-60 cm depth. Before grassland establishment in 2004, the study site was dominated by an arable cropping system with a 3-year rotation. It is probable that since then, the SOC stocks increase is ongoing. We are addressing that point in the new version.

(3) Regarding: “total C is reported as SOC without indicating test for carbonates”

#### Response:

We did not find carbonate content within the samples after using HCl. Therefore total C is referred as total SOC.

### Abstract

Lines 8-15:

Line 8-9 Comment: The renovation of a grassland ecosystem in a tilled cropland ecosystem does the opposite by sequestering atmospheric CO<sub>2</sub>

Response: We are referring to grassland renovation after a 10-year-old managed (non-native) grassland (also referred as grassland re-seeding), not after a tilled cropland ecosystem. Thus, the grassland system has not changed.

The study reported by Conant et al. 2017 referred in the corrections report improvements for a native grassland that was reseeded. In our case, we are working with a 10-year-old intensively managed grassland, seeded with multispecies mixture of grasses and legumes that was renovated.

Line 10 and 13 comment: Add Soil Depth

Response: Soil depth added

“While plenty studies show no-tillage (NT) as a promising option to increase the (0-30 cm) topsoil SOC stocks of arable lands...”

“Further, the LUC and tillage method effects on the (below 30 cm) subsoil SOC have been often...”

Line 12-13 comment: “(not meaningful without data)”

Response: Data is now introduced in the sentence. See below:

Further, the LUC and tillage method effects on the (30-90 cm) subsoil SOC have been often overlooked by many studies, where about one third of the top meter SOC stocks are stored, and which can be affected by changes in vegetation and residue distribution

Line 14 and 15 comment : list major species including botanical names

Response: Added.

In this study, a 10-year-old multi-species permanent grassland composed mainly of *Lolium perenne*, *Dactylis glomerata*, *Trifolium repens* and *Medicago sativa* was converted to continuous silage maize *Zea Mays L.* (CM) using either NT (NT-CM) or CT (CT-CM), and renovated with *Lolium perenne Trifolium repens* mixture using NT (NT-GR), while some part remained undisturbed as a control (GC).

Line 19-20, 23-24, 25-26 typographic changes:

Responses: Changes accepted

Line 19-20: “Results ~~showed~~ indicated that cropping systems ~~significantly~~ affected SOC dynamics over time...” (Accepted)

Line 19-20: “At 0-30 cm, SOC stocks were ~~significantly~~ reduced after conversion...” (Accepted)

Lines 23-24: “Conversely, SOC stocks remained unchanged ~~after for NT-GR same as~~ compared to the GC. In the subsoil, SOC stocks increased under GC (1.1 Mg C ha<sup>-1</sup> yr<sup>-1</sup>) and remained unchanged in the other systems. (Accepted)

~~“Across the whole profile, In the 0-90 cm, SOC stocks increased in GC...”~~ (Accepted)

Line: 25-26: “The differences in  $\Delta$  SOC between the unploughed ~~not tilled~~ systems (NT-GR, NT-CM and GC) in the 0-30 cm soil layer were strongly related to the annual soil C<sub>i</sub> from plant residues in the topsoil.” (Accepted)

Lines 27-29: Our findings highlight the great potential of NT to slow down the annual SOC losses after grassland conversion or renovation, and that SOC sequestration can occur in the subsoil of permanent grasslands when the topsoil C is already saturated. (Accepted)

Lines 27-29 comment: How 'saturation' determined?

Response: We have made the following changes to the text, as we did not determine SOC saturation:

Our findings highlight the great potential of NT to slow down the annual SOC losses after grassland conversion or renovation, and that SOC sequestration can occur in the subsoil of permanent grasslands when the topsoil SOC is ~~already saturated~~ at equilibrium. This strengthens the need to consider the SOC changes occurring in the top meter of a soil profile after a LUC event.

### Introduction

Line 31-39

Line 31 comment: “Add data and Reference”

Response

Line 31: The “high” SOC stocks in grassland ecosystems is rather general. A specific value cannot be given, when this depends very much on soil texture, climatic region, precipitation, etc.

Line 32 Comment: “In the whole profile”?

Response:

Line 32: We now say “particularly in the topsoil”

This reduction of SOC stocks can be up to two thirds within a few decades after conversion to arable land use, and up to a quarter within the first years after a single renovation event, particularly in the topsoil (Guo and Gifford, 2002; Necpálová et al., 2014; Reinsch et al., 2018a; Kayser et al., 2018).

Line 32-34 Comment: “You miss important meta-analyses, e.g., Don A, Schumacher J, Freibauer A (2011) Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. Glob Change Biol

17:1658-1670. and also this: Conant RT, Cerri CEP, Osborne BB, Paustian K (2017) Grassland management impacts on soil carbon stocks: A new synthesis. *Ecol Appl* 27:662-668”

#### Response

Line 32-34: The Guo and Gifford (2002) study don't provide information about which soil depths is represented in the SOC stock losses occurring in grassland conversion to arable land. However, their study covers down and below 1 m depth. The SOC losses (25 %) after grassland renovation is reported for the topsoil (0-30cm) in Necpalova et al. 2014.

The provided source from Don et al. (2011) is very relevant but limited to tropical regions. The SOC losses due to LUC from grassland to cropland reported there for tropical regions (-12 %) is much smaller than the one reported (50-75%) reported by Guo and Gifford (2002) across tropical, subtropical, and temperate regions. In addition, this value is limited to 0-38 cm soil depth.

The SOC losses reported by Conant et al. 2017 are based on old studies which we had cited already (Guo and Gifford 2002 and Paustian et al. 1997).

Conant et al. 2017 results do not cover grassland renovation of an already managed grassland. Their study refers to the conversion of a native to a managed (seeded) grassland. Our permanent grassland is not native and has been intensively managed since 2004.

Line 34 Comment: “Add data”

#### Response

Line 34: Specific data of how much CO<sub>2</sub> and N<sub>2</sub>O losses is released after tilling grassland is provided in the cited studies.

Line 36 and 39: typographic changes

Response: Changes accepted

Line 36: “... and other greenhouse gases (GHGs) of greater warming potential...” (Accepted)

Line 39: “...contributing to ~~anthropogenic~~ global warming...” (Accepted)

Lines 30-49:

Line 41 comment: “Outdated data, there are many recent meta-analyses on the effects of NT on SOC stocks available”

Response:

Line 41: In our view, the SOC rates from West and Marland 2002 and West and Post 2002 are still valid because they are based on meta-analysis that are also used in future studies. A more recent source is added (The 2019 IPCC report) reporting values in percentages.

Line 46: typographic changes of ~~unploughed~~ → NT

Response: Not changed

Line 46: We avoided changing this term “unploughed” to “NT”, because the grasslands used as control in the reported studies were not converted, nor reseeded using NT or CT. In the current study, NT is referred to the way how soil preparation and seeding is done after conversion or renovation.

Line 47, 48 and 49 typographic changes

Response: Changes accepted

“Few findings from limited locations suggest that SOC can be conserved after conversion and renovation, at the same level as an unploughed adjacent grassland site (Franzluebbers and Stuedemann, 2008; Follett et al., 2009). However, more studies are ~~yet~~ required for different site conditions, like the ones in temperate wet climates, where both high organic C inputs and SOC decomposition occur, and a reduction in crop residue inputs ~~might~~ may follow after conversion or renovation.”

Lines 50-64 typographic changes and add Reference

Response: Changes accepted, and Reference added

Line 50: typographic changes introduced: ~~Not less important~~ → Equally crucial to their effects on the topsoil is

Line 51: Soil depth added to sentence “...subsoil (below 30 cm).”

Lines 52-53: Added “...and increase SOC” and suggested reference (Rasse et al. 2005)

Line 65-69 Comment: “Add Botanical Names”

Response:

In the new manuscript version, we have added the botanical names in Material and Methods earlier in the text (in the lines 90-93).

We prefer to give experimental details in the Material and Methods and not in the Introduction.

## Material and Methods

Lines 72-78

Lines 72-78: typographic changes

Response: Changes accepted

Line 75: ~~organic-C~~ → SOC

Line 77: ~~Historical~~ erosion (accepted)

Line 75: pH information and soil depth information added to sentence???

Response:

We added pH information to Table 1

Line 76 comment: Not meaningful without data

Response: We added the data associated to these differences, as follows:

“Due to this, very high variability in subsoil SOC stocks between the upper and lower half of the site was observed (1.5, 0.8 and 0.3 % in the upper half, opposed to 1.7, 1.1 and 0.6 % in the lower half at 0-30, 30-60 and 60-90 cm depth, respectively), most likely due to erosion, sedimentation processes and downslope transport of DOC.”

Line 78 comment: Summit? Shoulder?

Response: Information not available

Lines 79-87:

Line 80 comment: spatial arrangement? how many plots in total? total experiment area?

Response:

We added details of the number of plots for the permanent grassland (2004-2014) and for the current study Table A1 in Appendix.

Lines 88-95:

Line 86 comment: “Add data”

We added the data from Table S2 and S3 to the main text. We modified text says as follows:

Despite these differences, by the end of 2014 the SOC stocks were not significantly influenced by the pre-cutting frequency and N rates at the different soil depths. However, wider differences at 30-60 cm soil depth compared to 0-30 and 60-90 cm soil depth were observed (0-30 cm: 71.2 – 72.9; 30-60 cm: 34.6 – 41.6, and 60-90 cm: 13.2 – 16.6 Mg C ha<sup>-1</sup>), particularly in the 5 cut system (Table S1, S2, S3).

Lines 87: Tables ~~S1, S2 and S3~~ → S1 – S3 (Changes accepted)

Line 95: ~~Supplementary~~ → Fig. S1 (Change accepted)

Lines 96-104 typographic changes:

Response: Changes accepted

Line 96: “At the ~~experimental setup~~ implementation of the experiment...”, (Changed accepted)

Line 100: “The maize (~~Zea Mays~~) variety Ronaldinio (KWS)...” (Changed accepted)

Lines 106-115:

Line 111 comment: “Add formula to calculate SOC stocks

Response: Formula added

Lines 106-115 Additional changes: We modified the whole “2.1.1 Soil sampling and carbon analysis” section

Soil sampling was performed to 90 cm depth from 2014 (the year prior conversion) to 2020 at the end of each growing season, in fix soil depth increments: 0-30, 30-60, and 60-90 cm. To do so, a sample from each plot was collected using a hydraulic-driven soil auger (inner Ø 2 cm), oven-dried at 30 °C, and sieved to pass a 2 mm mesh size for further analyses. The C and N content (%) were determined using a CN elemental analyzer (Vario Max CN, Elementar) (ISO). No carbonates were detected within the soil samples after, thus estimated total C was equal to SOC.

Soil bulk density measurements were conducted in 2020 in all the treatments at 15 cm soil depth just before tillage in CT-CM. To do so, four intact soil cores from four randomly chosen plots were collected using a steel cylinder (Ø = 5.1 cm; L = 5 cm), according to DIN ISO 11272 (HBU, 1998). No significant differences in soil bulk densities were detected between the treatments. Therefore, a mean soil bulk density of 1.61 g cm<sup>-3</sup> was adopted in all treatments in the 0-30 cm soil depth, regardless the cropping system. For the 30-60 and 60-90 cm soil depths, soil bulk densities of 1.65 and 1.70 g cm<sup>-3</sup> were assumed, respectively, based on the boulder clay features of the subsoil (Struck et al., 2019). Calculations of SOC stocks were done according to Eq. 1:

$$SOC [Mg C ha^{-1}] = C \text{ content } [\%] \times Soil \text{ Bulk Density } [g cm^{-3}] \times Soil \text{ Depth } [cm]$$

Lines 109-110: No inorganic C? Soil pH data?

We have modified Table 1 to include this information. No carbonates were detected within the soil samples after testing with HCl, thus estimated total C was equal to SOC.

#### Statistical analysis

Line 152-154: Requires normal distribution of the data-how was this tested?

Response: Normal distribution and heteroscedasticity was observed and considered in the ANCOVA after graphical residual analysis. We have added the following sentence to the new manuscript version to mention this procedure:

“Normal distribution and heteroscedasticity of the data with respect to the different treatments were assumed in the statistical models after graphical residual analysis.”

#### Tables and Figures

Line 562 (Table A1) comment: “confusing - important is to list the treatments where SOC changes were monitored after 2014 and reported here”

Response: Table A1 summarize the information described in the materials and methods, about the 10-year-old permanent grassland existing between 2004-2014 with the respective study factors and repetitions, and the new cropping systems and N rates with their repetitions introduced from 2015, which are assessed for SOC stocks in the current study.

Table A1 comment: what does this mean, e.g., multiplied?

Response: Multiplication symbol introduced to indicate treatment combinations (Corrected)