### Dear Jennifer Adams Krumins,

We would like to thank you for the critical review, you and the two reviewers have addressed during this first round.

### You will find below our answers in blue colour:

- Regarding your first comment: "I agree with the reviewers that sample size was too small to draw meaningful conclusions. Further, if more than 2 years of data had been collected some of the treatment effects of grazing may have been revealed within the inter-annual climatic effects. If more data had been collected it might be possible to statistically control for the random effects of climate and resolve differences among grazing treatments".

A: One main specificity of grazed grasslands is to exhibit large spatial heterogeneity of their main properties. Many papers have shown this variability due to the herbivores behaviour (defoliation, dejection) creating patches. Considering that 16 samples for each treatment is a low sampling for root mass study in field conditions is a debate. Yes, more samplings are always possible, but our main aim was to study seasonal and annual dynamics of root mass. Thus, we have invested in the frequent sampling: 20 times for 2 years. This has generated 960 samples in total. I don't count here the number of hours necessary to get final data. To conclude working with roots in field conditions and especially in grazed ecosystem, is poorly known and requires controlled protocol for which compromises should be found between sampling size and time to extract data. Having four true replicates of grazing treatments applied for more than 10 years strengthen the data obtained in this study.

#### More specific answer is given to reviewer 1.

"That all said, this is still a nice study demonstrating complex interactions between grazing and environmental variables. I understand and appreciate the use of PCA here to describe the role of the different variables affecting root and shoot responses to grazing. However, I urge the authors to clarify their goals with respect to this statistic so that it is useful to the reader."

## A: We added a sentence in the text (lines 266-268) to explain our objective with such analysis and have given the answer to reviewer 1.

#### Additional requirements of the managing board:

As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:

-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.

## A: The link given in the text was incomplete, and explain why reviewer 1 had no access to data. We have corrected it line 542 (see answer to reviewer 1).

-Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.

## A: R scripts are shown in the S4 supplementary material (line 885).

-Details on experimental procedures are available to readers in the text or as appendices.

-Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

A: this paragraph is now added (line 556).

## **Reviews**

Reviewed by anonymous reviewer, 2020-11-01 17:25

This is an interesting study, which describes the productive response of grass at different levels of grazing intensity comparing a wet year with a dry year. The subject of study is interesting, despite the fact that already there are some related works, more information is still needed to understand the effect of the interaction between grazing and environmental conditions on pasture functioning.

The introduction is very extensive, greater concretion would be desirable. Some expressions are difficult to understand, changing long sentences for several short sentences will make understanding easier, for example, L53-56 or L62-66.

A: we have made some changes to reduce the sentences and improve the language.

Furthermore, in general, the use of language sometimes makes it difficult to understand the content. Perhaps the review of the work by someone specialized can help make the speech more fluid.

A: a professional editing English spoken has previously read the text before submitting it.

Regarding the statistical analysis, I have a doubt. The use of the plot nested in the block as a random factor in the linear mixed-effects model is considered for accounting for temporal pseudo-replication, however, in my opinion at the same time it can control the spatial pseudo-replication and therefore it would not be necessary to calculate the mean values for each plot, being able to use the data of each ingrowth-cores which would increase the degrees of freedom and with it the statistical power of the test.

A: We agree that collecting low number of soil cores can lead to bias linked to the realism of root mass present belowground in ecosystems. That is the reason why, to minimize this effect, we have collected 16 samples per treatment for each date. Averaging them is a mean to integrate the spatial variability in order to detect treatment effect at plot level, the true replicate level.

However, our main purpose is to study the seasonal variation of root growth, which has been highlighted in grasslands (effects of temperature and precipitation on plant growth).

But, we cannot exclude that 16 samples are not enough to detect effect of rotational grazing on root growth. We have added a paragraph in the discussion (lines 454-462).

I am not sure I understand what is the intention of carrying out a PCA, since, in the results, I miss that each of the plots is represented in figure 3. Its representation would allow us to observe if the set of measures of the variables separate the different plots depending on the treatment to which they belong. Otherwise, if the objective is only to observe the correlation between variables, a matrix of correlations between parameters would be more suitable than a PCA, since it would allow us to observe the correlation values and their significance for each pair of variables.

A: This statistic approach allows comparing sets of traits and properties relationships in order to detect response and effect traits, but also to analyse multiple dimensions of traits relationships, not possible with pairs of correlation. By analysing PCA by year, we can detect changes of relationships between traits. Text is added in the materials and methods section (lines 266-268).

In the discussion, the authors state "in our field conditions and after 10 years of treatment application, soil moisture was not affected by the rotational grazing, probably because the temporal scale used (monthly-based) buffer shorter-term response", however, there are other possible explanations for it. Is it possible that the livestock loads used do not increase the apparent density of the soil? Or does the change in plant species between treatments compensate for the lower LAI in the grazed areas? Not all plant species have the same efficiency in the use of water.

### A: we have added sentences in the discussion to incorporate these ideas (lines 409-418).

Authors affirmed "these treatments seem to be better adapted to buffering the negative effect of drought on grassland production than for abandoned grasslands. This is consistent with previous work showing that moderate grazing could be more beneficial than no grazing for drought resistance and recovery of ANPP and BNPP" I'm not sure if the data showed reflects this. Figure 2 shows a marked decrease in the dry year with respect to the wet year in the ANPP for grazing treatments, while the abandoned treatment maintains a similar value in both years. Therefore, the interpretation that I give to these results is that in wet years grazing increases productivity with respect to abandonment, but this increase in productivity disappears in the event of a drought, therefore, the grazing treatment is more sensitive to drought than the abandoned ones. In this context, it would be interesting to know how the years prior to the study have been, at least 2012 and 2013, to know if there is any type of accumulated effect from one year to the next.

# A: the sentence is used for data of root to shoot biomass ratio, but we agree it is confusing. We have rewritten this paragraph (lines 482-485).

On some occasions the authors do not find the expected effect regarding the grazing treatment, giving different explanations for it. However, a possible explanation not considered is the low number of replicates used in this study. When the expected differences between treatments are small, high statistical power is necessary to detect them, which means a sufficient number of replications. Even more so when the effects are heterogeneous in space, as the authors well indicate since the impact of livestock is highly variable in space. Perhaps reanalyze the data without calculating the means per plot can give new results. But regardless of this, I would not disregard the need for a broader sampling to be able to detect the impacts of grazing on the functional characteristics of the roots.

A: We agree that collecting low number of soil cores can lead to bias linked to the realism of root mass present belowground in ecosystems. That is the reason why, to minimize this effect, we have collected 16 samples per treatment for each date. Averaging them is a mean to integrate the spatial variability in order to detect treatment effect at plot level, the true replicate level.

However, our main purpose is to study the seasonal variation of root growth, which has been highlighted in grasslands (effects of temperature and precipitation on plant growth). With four replicates per treatment, we have observed changes of ANPP induced by grazing, as reported in a previous study performed on the same site with a similar protocol (Herfurth et al. 2015).

This is not the first time that response patterns in above and below-ground compartments are not always similar. After defoliation event, shoot growth increases at the expense of root growth (Picon-Cochard et al., 2009); the same response is usually observed with fertilisation. Thus, it is not unexpected that grazing can have an effect on above ground but not on below-ground mass.

The problematic of sampling strategy is now developed in the discussion (lines 454-462).

It seems that there is some confusion among tables S1; S2 and S3 since in the text name S2 to refer to the information shown in S3 and names S1 for the information shown in S2.

A: thanks to have seen these errors. We have now corrected them.

#### References:

There is a lack of some important studies as:

Li, W., Li, X., Zhao, Y., Zheng, S., & Bai, Y. (2018). Ecosystem structure, functioning, and stability under climate change and grazing in grasslands: current status and future prospects. Current Opinion in Environmental Sustainability, 33, 124-135.

Aldezabal, A., Odriozola, I., & García-Baquero, G. (2019). Grazing abandonment delays the effect of temperature on aboveground net primary production in Atlantic grasslands. Rangeland Ecology & Management, 72(5), 822-831.

A: We have added the first paper but not the last one, which is not accessible.

In the manuscript authors indicate that "Data are available online: <u>https://zenodo.org/deposit/4034903#</u>" however, the web page indicates Permission required, so in reality, data are not available. In general, I consider it to be a very interesting article that provides interesting data on certain aspects of pasture operations that are not yet clear, and that will certainly be cited after its publication.

A: Zenodo is a platform freely accessible with an Orcid number. I was not aware that the first link was not working. The good one is given line 542.

Reviewed by anonymous reviewer, 2020-11-03 11:54

General The paper "Intra and inter-annual climatic conditions have stronger effect than grazing intensity on root growth of permanent grasslands" addresses the important question of how

climate and management (herbivory) influences the main drivers of soil C sequestration or soil organic carbon (SOC), i.e. above and belowground plant growth. The manuscript is well written and clear in its objectives to address the importance of root growth, soil fertility, and species composition as drivers of SOC. The approach using an existing long-term trial is appropriate to capture short-term changes. While we know that SOC changes occur over decadal and longer time periods, and thus a longer trial would have been ideal, the scarcity of such trials must be acknowledged.

Neverthless, their findings that climate has a larger influence than management (in this case a gradient of grazing regimes) is in agreement with other studies, and thus supports the overall idea that SOC is driven primarily by climate, and how this affects ANPP (and BNPP). The observations regarding grazing, relatively increased root growth and drought tolerance are not novel (see Klumpp et al 2011 in manuscript) but an important confirmation. An important contribution of the work is that they had access to different grazing intensities, as it is the variety of grazing intensities in various studies that confounds generalizations on its effect on SOC or other components of the biosphere.

As a suggestion, I would have liked to see more than 2 years of data, at least for the ANPP and possibly this is available for an existing 10-year trial? If they have 2 years of BNPP and ANPP as well as longer-term ANPP data available, this would allow some estimation of BNPP over the 10 years. Also once they have data over the longer time period it becomes viable to model time periods applicable to climate models. Without this longer term data I would say the paper still provides valuable data, especially considering the paucity of belowground studies of roots and SOC.

A: We agree with the reviewer that longer-term data are useful to understand dynamics of terrestrial ecosystems like grasslands. However, for this present work, data of ANPP for longer period are out of the scope for this paper. We expect future research to mobilize longer-term ANPP data and simulate BNPP and their links to soil C in grazed grasslands.

Specific comments:

The abstract could be improved with some result details

A: We have added some results.