Response to comments

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Latest version : https://www.biorxiv.org/content/10.1101/2022.07.04.497718v2

by Sébastien Barot, 17 Oct 2022 19:29

I concur with the two reviewers to think that the manuscript is interesting and based on a sound experiment. I particularly like the way two contrasted hypotheses are tested and the fact that the impacts of the treatments are documented through many relevant variables. Working on the impact of herbivore insect on soil functioning is relatively original. Nevertheless, the two reviewers have made numerous valuable comments that should help improving the manuscript. Overall, my general comment is that the theory behind the hypothesis to be tested is relatively complicated, that the data and the combinations of treatments are also relatively difficult to understand. Therefore, any effort to make the text crystal clear and to fully clarify each step of the rationale would be welcomed.

Reviewed by Manuel Blouin, 20 Sep 2022 20:28

Reviewer comments

The study of Ibanez et al. deals with the effect of herbivores on soil functioning in relation to primary production and herbivore diet. It is a complex experiment, given the number of compartments (plants, herbivores, decomposers) and the processes (primary production, diet differences, decomposition of organic matter) that are considered. The experimental design is fine, statistical analyses also, the conceptual background is well exposed and the paper is well written. It misses some coherence between the collected data and the tested conceptual framework.

Response : We hypothesize that the effects of herbivory on soil processes depend on grassland properties and herbivore diet. To test this hypothesis, we introduced herbivores having different diets in contrasted grasslands. From this point of view, we believe that our study is coherent. However, we did not find any effect of the herbivores' diet, contrary to previous results. We did find that the effects of herbivory on microbial processes depended on grassland properties, but in a way that did not match the existing frameworks. Therefore, the lack of coherence is mainly between our initial expectations (based on the existing literature) and the results. We might have introduced our expectations in a more agnostic way in order to avoid this discrepancy. We instead chose to keep the original hypothesis because (1) this corresponds to the initial motivation underlying the experimental design, (2) we believe that the conceptual articulation of the two frameworks (as in the new Table1) can be useful for future research and (3) it is noteworthy that our results are at odds with both frameworks, contrary to previous findings.

Title

I suggest changing “ecosystem productivity”. First, from available information, the authors did not measure productivity, nor production, but aboveground biomass. Second, it is restricted to plant, not ecosystem which include several trophic levels. If the authors can justify that the protocol allows to estimate a Δ of biomass per year, then they could talk about aboveground production.

Response : In our study the net primary productivity of each grassland was inferred as the above-ground biomass collected at the peak of biomass. We considered this one-time estimation to be representative of the yearly production (g dw. yr-1 m-2). We agree that this represents plant productivity, not ecosystem productivity, and changed the title accordingly.

Abstract

L38: This sentence give the feeling that the "productivity model" prediction is validated, which is missleading. The authors could precise "but were influenced by primary production, in a way that differed from the "productivity model", or something equivalent.

Response : We agree with this suggestion, and changed the sentence accordingly.

Introduction

The Introduction is quite long and could be shortened. The authors could stress less on productivity and herbivore diet theories when the same results are expected from both the interpretation, while insist on cases where predictions diverge. They could end the Introduction by diverging predictions from the two conceptual frameworks.

Response : In line with this comment, we have reorganised the introduction. The sentences stressing the cases where both models were aligned were removed. We removed the discussion of Tuomi et al 2019. We did not remove the section presenting the microbial strategies and their importance for ecosystem functioning, since this appears central to our manuscript (and presenting the strategies was a suggestion of reviewer #2). This section was nevertheless shortened, combined in a single paragraph and presented in a different order, which will hopefully make the introduction more understandable. The last section of the introduction was also modified, stressing on the fact that this manuscript addresses 2 major gaps : “We therefore identified two important gaps in previous research on the effects of herbivory on ecosystem functioning : first, the two existing frameworks should be combined (Table 1), and second, the functional characteristics of soil microbial communities should explicitly be included within the frameworks.” (lines 150-153). Please refer to the whole introduction for the details.

From my knowledge, productivity is high in "juvenile" stages, with many exploitative species, and relatively low in "mature" stages, with exploitative species (cf Odum, 1959). The authors could better explain how they will decorrelate productivity and species composition in terms of adaptive strategy.

Response : As suggested by another comment below, we removed all considerations about plant succession, and therefore did not address this comment. Note that in our study sites mature stages having low productivity correspond to forests dominated by *Picea abies*. From this perspective, all the studied grasslands are juvenile stages, the differences between them in terms of productivity and botanical composition being due to management, soil properties, local habitat effects and history.

L80: "recalcitrant litter". The intrinsic recalcitrance of organic matter has been shown to be a concept that poorly explains the degradation of organic matter. From a quick reading, I did not found references to this recalcitrance in the paper of Reich (2014). The authors could be more precise to justify the association between plant types (conservative vs exploitative) and decomposition rates.

Response : In Reich (2014), page 294, it is contended that : "Considerable evidence shows strong impacts of a variety of litter traits associated with the LES on decomposition". The next sentences p. 294 further specify this statement.

The authors could precise, from the Introduction, that they will consider forbs and grasses as exploitative and conservative groups of species. That will help to make a link between the tested conceptual frameworks and the experimental design.

Response : We added in the introduction that : "Since within each site grasses have a higher C/N ratio than forbs (Ibanez et al 2021), grass-feeders were expected to accelerate matter cycling, while an opposite effect was predicted for forb-feeders." (lines 158-160). However, although *within each site* forbs are indeed more exploitative than grasses, *across sites* this is not necessarily so, since in some sites exploitative grasses like *Lolium perenne* and *Dactylis glomerata* have leaf traits comparable to forbs growing in other sites.

Line 179: I was surprised that the authors chose managed and fertilized grasslands among their sites. This could bring an additional complexity through the interaction between fertilizer and soil processes. The impact of this choice on the results could be discussed.

Response : We used those four types of grasslands to cover a wide range of above-ground biomass productivity and plant composition in the study area. We added in the methods that: "Their productivity ranged from 156 to 840 g dw yr-1 m-2, managed grasslands being more productive than natural grasslands (Sup. Fig. 2)." (lines 181-182). We agree that interactions between fertilisers and soil processes might bring additional complexity, which we tried to infer through our data analysis. For instance, the discussion already stated that: "The decoupling between TDN and plant biomass might be explained by management, since extensive and intensive grasslands are fertilized with manure each year; intensive grasslands being the most fertilized and productive ones" (lines 380-382).

Lines 210: the experiment has been repeated in 2016 and 2017. In the Materials and Methods, it is not precised (for example in the Statistical analysis section) how the authors managed the data from the two years. Are there differences? Are the data pooled? Did the authors chose one year and if so, why and based on which criteria? Results and Discussion could be completed accordingly, to make it more clear the nature of presented data.

Response : We did not measure anything twice, except plant species composition (in order to calculate a differential). To make things clear, we specified : "Several ecosystem characteristics were measured, as detailed in the following sections. In short, soil abiotic and microbial characteristics as well as plant biomass were measured at the end of the experiment, in mid-September 2017. Herbivory marks on standing biomass was estimated in 2016, while plant specific abundance was estimated before the experiment in 2016 and after one year in 2017, in order to calculate the effect of herbivory." (lines 202-206)

Line 183-185: The authors say "We hypothesized that the effect of herbivory on these soil microbial characteristics depends on the interaction between herbivore diet and ecosystem productivity.". It is not what I understood from the Introduction, which was more a confrontation of two conceptual frameworks to determine which one fit the best with observations. Since the authors identified situations where the two frameworks provide opposite expectations, they should focus on these different predictions and decide to support one hypothesis rather than the other, based on literature.

Response : We agree with this suggestion. The manuscript now reads: "We hypothesized that the effect of herbivory on these soil microbial characteristics depends on the interaction between herbivore diet and ecosystem productivity. In cases where the two frameworks make opposite predictions, the experiment was designed to compare the relative importance of diet and productivity effects." (lines 163-166).

Materials and Methods

Lines 195-197: "All grasslands were composed of a mixture of forbs and grasses, although the dominant functional group depended on the grassland type (Sup. Fig. 2A).". Sup Fig 2A is about leaf C/N ratio. Supp Fig 1 is about the percentage of eaten leaves. There is no clear information about the relative importance of forbs and grasses in each site. This requires an additional Supp. Figure.

Response : We added the corresponding figure (now Sup. Fig. 2).

Lines 200-201: The authors could explain better what is the rationale to consider three grasshopper species AND their mixture.

Response : We specified that: "If single species treatments have contrasted effects on soil microbes, the mixture treatment should discriminate between additive and non-additive effects." (lines 187-188)

Lines 205-206: The authors should justify why they adjusted the density of grasshopper to the aboveground biomass.

Response : We specified that: “This standardization is necessary to avoid confounding correlations between herbivory load and herbivore diet, or between herbivory load and ecosystem productivity.” (lines 195-197)

Lines 206-207: A reference for the maximal density in this area would be welcome.

Response : We are not aware of previous work on grasshoppers in the study area. We instead added that: “Preliminary field observations revealed that this corresponds to the maximal natural insect densities locally observed in the study area.” (lines 194-195)

Line 276: The authors could better explain the experimental design. There are four types of grasslands, each with three replicates, and five points per replicates (five pseudoreplicates). Does the three replicates come from the same “region”? I mean is there a spatial correlation of the three replicates, which could have an impact on the results and conclusion? Is so, it should be precised. A map as supplementary information could be helpful. Strictly speaking, the five pseudoreplicates should have been averaged since the statistical individual is the site. A comment on this would be appreciated.

Response : Pseudoreplication had already been included in the statistical analysis through the inclusion of random effects at the site level. We included a map (Sup. Fig. 1), and specified in the methods: “The 6 managed grasslands are disseminated in the plateau of Autrans, and the 6 natural grasslands form two distinct clusters containing either 2 forbs-dominated communities and 1 warm grassland, or 1 forbs-dominated community and 2 warm grasslands (Sup. Fig. 1). Since the two clusters contain communities having contrasting plant productivity, botanical composition and soil characteristics, spatial autocorrelation is unlikely to affect the results.” (lines 175-180)

Lines 354-356: Precise RV=0.29 is lower than what, since you are talking about the RV=0.22 just before.

Response : This was indeed unclear. It now reads: “The RV coefficient of the coinertia between five vegetation characteristics and the six microbial characteristics was equal to 0.29 ($p<0.001$). This much lower RV coefficient than that between soil microbial and soil abiotic characteristics (0.59) suggests that the microbial soil characteristics were more related to soil abiotic than to vegetation parameters.” (lines 343-346).

Results

Lines 358-360: Supp. Fig. 3 indicates the variation of the % of forbs in response to grasshopper species, not what you say here.

Response : We apologize for that, the corresponding Sup. Fig. had been forgotten. It has been added (Sup. Fig. 6).

Discussion

Line 405: Given that you refer several times to this new microbial resource acquisition strategies, it could be interesting to present them briefly. This would also help to understand Y-strategists (line 409).

Response : This job was done in the introduction, although we did not explicitly mention "A" and "Y" strategies. This specification has now been included (lines 133-137).

Lines 407-410: As an alternative explanation to root exudates, could it be that the plant-microbes competition is stronger at high SOM levels? If the plant is more efficient than microbes to uptake mineralized nutrients, microbes could be constrained to mineralize more while they can not uptake more because of the plant.

Response : Good point! We added a sentence accordingly : "Furthermore, as found in alpine ecosystems in Cordillera Darwin, Tierra del Fuego (Chile) (Thébault et al. 2014), we suggest that competition between soil microbes and plants for nutrient resources in SOM and TDN-rich soils may contribute to the higher mass-specific microbial investment in resource acquisition in these soils." (lines 399-402).

Lines 447-449: the role of depolymerization is not clear to me.

Response : We clarified this in the manuscript (lines 441-449). The depolymerization of N-containing compounds is a key limiting step in N cycling because polymers are not directly bioavailable to microbes or plants. Extracellular enzymes depolymerize these compounds into monomers that are bioavailable to microbial metabolism for subsequent mineralization or immobilisation in biomass.

Line 462: Please, keep PNM, instead of NMP, as in the whole text.

Response : Done

Line 477: Is it Supp. Fig. 3 instead of Supp.Fig. 2?

Response : We are renumbered and checked all the references to figures.

Lines 477-482: All along the paper, I have not been convinced by reference/discussions about plant succession. The duration of the experiment is clearly not sufficient to get into these considerations. I suggest removing these elements to get a more focused paper.

Response : We deleted the corresponding sentences related to plant succession. We also do not mention succession in Table 2. We nevertheless kept this idea in the conclusion ("The current experiment was conducted over two years and focuses on physiological time scales (root exudation, enzyme production), while the productivity model encompasses plant community dynamics, which occurs on longer time scales." lines 543-544) because it allows us to understand why the results don't match with the productivity model.

Lines 490-494: This intellectual construction works, but it is not very convincing.... Some references to support the link between the TDN and leaf C/N ratio could help.

Response : Our suggestion was originally based on the work of Williams et al. 2022 who found that mean leaf C/N ratio correlates negatively with root exudates. We added Robson et al 2007 and Legay et al 2013, who found a positive correlation between TDN and mean leaf C/N, as in the present study (lines 478-479).

Line 506: “In the present study, high quality plants characterized by low leaf C/N ratio”. This should be stated earlier, otherwise the reader does not understand before the discussion why the Introduction is so developed regarding high versus low quality of plants, and why different herbivore species are considered.

Response : We specified in the introduction that “Since within each site grasses have a higher C/N ratio than forbs (Ibanez et al 2021), grass-feeders were expected to accelerate matter cycling, while an opposite effect was predicted for forb-feeders” (lines 159-161).

Lines 519-523: This paragraph could be shortened.

Response : We deleted the sentences related to the absence of effect of grass feeders on plant community composition.

Conclusion

Lines 540-542: “We did not find any interaction between ecosystem productivity and herbivore diet on soil microbial characteristics, contrary to our expectation.”. Regarding the last comment on the Introduction, I feel that the hypothesis was more than one model was better than the other since it can predict observations in some conditions whereas the other one can not. I did not understand why you expected to find an effect of the interaction.

Response : We agree, and do not mention the “interaction” any more.

Figures

Please, indicate the contribution of each axis in explaining the variance, for the three panels, and the number of observations for Figures 1 and 2, as well as the number of observation in Figure 3.

Response : Done. There are 60 cages in total, with 12 controls, so 48 standardized responses to herbivory.

Reviewed by Tord Ranheim Sveen, 16 Oct 2022 08:53

This is a very interesting study that is generally well written, researched, and presented. On the strong side is the anchoring of the study in the contrasting frameworks of herbivore impact on microbial functional characteristics and plant communities, which is a line related to and followed throughout the paper. The methods are solid and, coming from a soil ecology background, well motivated and sound. Your discussion relates well to your results and your research question and I learnt new interesting things. In particular, the indication of threshold values in SOM and N driving herbivory responses is very interesting and should be investigated further. I have some concerns and questions on details but overall, I am happy to recommend this study for publication. Well done.

Issues

The presentation of the contrasting frameworks (diet vs productivity) could be made easier to follow through a cross-table to explain when the models align and when they contrast. Something like a power analysi table used to explain Type I and Type II errors (example: https://www.scribbr.com/statistics/type-i-and-type-ii-errors/)

Response : We added a cross-table summarizing the predictions of both frameworks (Table 1).

Since time scales is an issue when comparing your results with the productivity model, it would be good to provide an explanation for why you chose to do a 2-year study and not longer.

Response : This issue had already been mentioned in the conclusion of the first version : "The current experiment was conducted over two years and focuses on physiological time scales (root exudation, enzyme production), while the productivity model encompasses plant community dynamics, which occurs on longer time scales." (lines 543-544). Reviewer #1 instead suggested removing discussions of plant succession. We nevertheless kept this sentence in order to explain why the results don't match with the productivity model.

Could there be any effects of mineral fertilizers introduced into the soil that affects the microbial functions you measured? For example, easily accessible mineral N would presumably affect the resource acquisition strategy of the microbial communities by itself.

Response : There were no mineral fertilisers added in these grasslands, "only" organic inputs from the cattle farms. We might indeed expect that organic inputs favor the high yield Y strategy. Since organic inputs are added in intensive grasslands, which are the most productive, this might have had a synergistic effect. We therefore added the following : "This might also be reinforced by organic inputs in the most productive intensive grasslands." (lines 415-416).

l151: I would avoid using the term "infertile" as this is implies there is no or very little productivity. I suggest changing this throughout to "less productive" or similar.

Response : We agree, and changed accordingly.

l388-389: SOM does not equal C sequestration (which is a rate measured over time). Why would you talk about C sequestration at all in this context?

Response : We agree. We changed "C sequestration" for "C content" throughout the paper when required.

L418-420: The adoption of the A vs Y strategies is really interesting and can be fruitful in this context, but it would be good to introduce it more clearly with e.g. a definition of what you consider an A strategist community vs a Y strategist in terms of the functions you measured. What motivates you to say that "the A strategy comes along with plant communities having higher C/N leaf ratios"? Basically, at what EEN is a community A strategists? at l441-442 you then go on to directly say that "A strategists invested even more in extracellular enzymes in response to herbivory". Again, this use of the term is quite careless, since you have not defined any thresholds for your strategies.

Response : A strategists are adapted to low-resource environments through a higher investment in extracellular enzymes allowing the acquisition of assimilable monomers from complex polymers. Y strategists have a lower investment in extracellular enzyme production and thrive in resource-rich environments. Microbial communities are composed of organisms with different resource acquisition strategies but there is actually no threshold available in the literature to define at which level of investment in extracellular enzymes a whole community can be considered as A or Y strategist. Our interpretation is based on a more comparative approach, with increases in extracellular activities being interpreted as an increase in the proportion of A strategists in the community. Several sentences were rewritten or added accordingly to make it clearer in the discussion (please refer to the manuscript for details).

Figure 1 and figure 2A: you should better explain the differences in color of the arrows in the legend.

Response : Done for Figure 1. The colors were already specified in Figure 2A.

The paper could benefit from one last round of language editing, as some of the words and sentences are a bit clunky at times. I made some notes on this with line references below, but please note that this is not exhaustive.

Response : We have edited language throughout the manuscript.

l33: with contrasted productivities

Response : Done

l87: unclear wording ”different axis of variation”. What is the variation implied here?

Response : We simplified this sentence : “...the “diet model” focuses on another aspect of variation of herbivore-plant-soil interactions…” (line 80).

L106: provide instead of provided

Response : We prefer to use the past tense here.

L312: change ”on a large part” to ”to a large extent”

Response : Done

l386: revise sentence, too many ”differences” and ”different”

Response : Done

L389-392: This is trivial and does not need an explanation. Usually, 95% of total N (or TDN) consists of DON.

Response : We deleted the corresponding sentences.

l393: fertilized with what? Presumaly with mineral N? This should be stated.

Response : We specified they are fertilized with manure (line 381).

L402-405: Good explanation and reference, you could also mention the carbon use efficiency concept which talks around this.

Response : we mentioned carbon use efficiency in this explanation of the resource acquisition strategy. The sentence now reads : “However, microbial biomass did not increase with SOM, which implies that high SOM comes along with higher mass-specific extracellular activities for carbon uptake and higher mass-specific nitrogen mineralization, which corresponds to a resource acquisition (A) strategy at the expense of growth yield or carbon use efficiency (sensu Malik et al. 2020)." (lines 390-394)