12/24/2023

Dear Dr. Caldarau and all other special issue editors,

Please accept the revision of our manuscript entitled “*Long-term fertilization increases soil but not plant nor microbial N in a Chihuahuan Desert Grassland*” for re-review for potential publication as an article in the special issue of *Biogeosciences:* Ecosystem experiments as a window to future carbon, water and nutrient cycling in terrestrial ecosystems. This novel research is being submitted here because it furthers to our understanding of how long-term N deposition may affect dryland ecosystems, including the potential for N-release to other.

In our manuscript, we examine the impacts of *multi-decadal nitrogen additions* on the ecosystem N budget, including the *soil, microbial and plant N pools.* We justify this experiment because of the increasing recognition of possible secondary limitation of the drylands by nutrients, during years or seasons when the system is receiving sufficient water. Despite this recognition, the high rates of N deposition in arid lands near urban development, and the large extent of arid land ecosystems globally, we have limited knowledge of the impacts of increased N inputs, particularly on long time scales.

After 25 years of N addition, there appears to be little impact on the soil microbial community or the plant community and only minimal increases in N pools within the surface soil. While perhaps encouraging from a conservation standpoint, we calculate that greater than 95% of the nitrogen added to the system is not retained and is instead either lost deeper in the soil profile or emitted as gas, leading to environmental concerns of contaminated water or air pollution. This is one of the longest running fertilization experiments in an arid ecosystem and provides important context to long-term N-deposition experiments in other ecosystems.

Our revisions are described below in our response to reviewers. The text in red is our response submitted after first review, and the text in blue is our response once our proposed revisions were encouraged. We have included in this submission a marked-up version using track changes in Word, and also a new manuscript with all the changes accepted.

Sincerely,

Violeta Mendoza- Martinez (Colorado State University)
Scott L. Collins (University of New Mexico)
Jennie R. McLaren (University of Texas at El Paso)

Current contact information for corresponding author, Jennie R. McLaren

The University of Texas at El Paso
Department of Biological Sciences
500 W University
El Paso, Texas 79902
915-747-8903 (Office)
jrmclaren@utep.edu

Short Summary:

We examine the impacts of *multi-decadal nitrogen additions* on a dryland ecosystem N budget, including the *soil, microbial and plant N pools.* After 25 years, there appears to be little impact on the soil microbial or plant community and only minimal increases in N pools within the soil. While perhaps encouraging from a conservation standpoint, we calculate that greater than 95% of the nitrogen added to the system is not retained and is instead either lost deeper in the soil or emitted as gas.

Community comment--

Comment 1: *The authors mention that the long-term experiment is a 26-year-old experiment; however, they mention a significant disturbance about ten years after the experiment was initiated. More information should be provided about why they consider that period and not instead a shorter 16-year-old experiment.*

Response: We considered all 26 years of the experiment given that fires are a natural part of this ecosystem. While aboveground production can be susceptible to disruptions, Burnett et al (2012 Ecosphere) found that belowground processes are highly resistant to fire. Fertilization treatments, our primary interest, were conducted for the period both before and after the fire. Finally, we describe our results as long-term responses to fertilization – both 16 and 26 years would be considered long-term for a fertilization experiment, and the reader has the necessary information should they choose to consider this a shorter-term experiment because of the fire. In revisions, we will add an additional sentence describing why we are considering the entire 26 year period.

December Response: The following text “Since fires are considered a natural part of this ecosystem and below-ground processes in the area have been shown to be highly tolerant to fire (Burnett et al. 2012), all 26 years of the experiment have been considered in this experiment”, has been added to the methods on line 94-96.

Comment 2: *Major conclusions in the paper suggest a significant loss of inorganic N, such that less than 5% of the N-added remains in the system. However, seasonal differences were found, while the fertilization effect remained noticeable. In this context, it is crucial to clearly specify the fertilization and sampling dates for the study year to eliminate any confounding effect on the results.*

Response: Good point, thank you. Fertilization and sampling dates will be clearly indicated on a revised version of the manuscript.

December Response: The newly added Figure 1 now includes both the fertilization and sampling dates, alongside yearly precipitation data.

Comment 3: *Including missing data on species, cover, biomass, and productivity, as well as other above-ground information, could help the reader understand other possible differences between the plots and help authors improve their interpretation of the study results. It is unclear why they only sampled Sporobolus and not any dominant C4 grasses. Is there any evidence of changes in species composition and productivity in the treated plots?*

Response: We appreciate the suggestion and agree that information about species composition could make useful additions to this study; however, the focus of our study was to create an N-budget, and we did not collect detailed information about species composition or productivity. Information on plant biomass was provided by others collecting long-term measurements on these plots. An earlier paper (Ladwig et al. 2012 Oecologia) includes some information on species composition in this experiment.

We also only sampled Sporobolus for our C & N measures of plant biomass because it was the only species which could be found across nearly all plots. A CN comparison across treatments including multiple species was outside the scope of our experiment.

December Response: Our reasoning for the choice of species to sample plant CN is included in the methods (line 104). We have also included text in the methods (line 139) and the discussion (line 256) stating that the species composition measurements were outside the scope of this study and referred to the Ladwig paper described above.

Comment 4: Rainfall for the years of the experiment could also help to visualize the variability and temporal heterogeneity for this limiting resource, as mentioned in the paper's conclusions.

Response: Agreed. This will be included as a supplementary figure of average annual rainfall for each year with data collected by a weather station near the research site. This figure will include annual and monsoon rainfall as a stacked bar graph.

December Response: Our new figure 1 includes both precipitation data and also the dates of samplings and fertilization.

Comment 5: *As general concerns, there are non or very few specific explanations or mechanisms by which authors explain their results. There are no answers as to why the fertilization effect, even though small, is still noticeable in the soil.*

Response: We will expand the discussion section and discuss mechanisms possibly affecting N-retention with particular note of specific references listed by other reviewers. As it is, we were surprised by how little N remained in the system after 26 years of fertilization.

December Response: See expanded discussion, especially lines 275-315

Comment 6: *The terms pre-fertilizer and post-fertilizer in the figures are misleading. They should be changed to pre-monsoon, mid-monsoon, and post-monsoon as mentioned in the sampling protocol unless they are different ideas, in which case those differences should be clearly explained in the methods section.*

Response: Good point. Future versions of this manuscript will contain modified figures to keep names of samplings consistent.

December Response: Naming scheme used in figures was changed to remain consistent with text

Comment 7: Figure 2e lacks statistical significance letters. Please check units in the y-axis from panels e and f in the figure.

Response: Letters will be added, and axes labels corrected.

December Response: Letters were added and axes labels were corrected

Comment 8: The text does not cite references by Báez, Craine, Le Bauer, Lieb, Liu, and Mueller.

Response: Future versions of the manuscript will substantially increase the references used and including the suggested literature

December Response: Reference list has been updated to only include publications referenced in the text.

Comment 9: Fenn et al. 2003a needs to be differentiated from Fenn et al. 2003b both in text and references.

Response: This distinction will be made, as suggested.

December Response: Distinction has been made as suggested.

Reviewer # 1—

Overview Response: We appreciate your positive comments about our study and our manuscript. We respond to individual comments below.

- **Methods**

Comment 1: *Section 2.5 Soil N pools (starting at line 105). Using K_2SO_4 for inorganic N extractions is incorrect. Typical extraction method is 2M KCl. If samples were only extracted with K_2SO_4 and then assessed for inorganic N, then you cannot correctly interpret inorganic soil N as it is presented. Please address this or clarify that perhaps this was a typo. K_2SO_4 paired with K_2SO_4 + chloroform is used for microbial biomass C and N (which you correctly described).*

Response: Although KCl is perhaps a more common extractant for NO_3 and NH_4, particularly when microbial biomass isn't being measured, K_2SO_4 is also regularly used to extract for this pool of N. The extractant for inorganic N is typically a neutral K salt solution, which could be K_2SO_4 or KCl (Keeny and Nelson, 1982, ASA-SSSA *Methods of Soil Analysis*). Although KCl is less expensive, which is perhaps one of the reasons it is used more frequently, when we are working in long-term plots and attempting to minimize disturbance, we try to take the minimum soil required for our analysis. In this way, using K_2SO_4 for both nutrients and biomass both minimizes soil sampling and also decreases the number extractions required. Examples of other dryland papers that use K_2SO_4 to extract for 'available N' are Leitner et al. 2017 Global Change Biology, Schaeffer et al. 2017 Soil Biology and Biochemistry, Fierer and Schimel 2002 Soil Biology and Biochemistry.

December Response: No changes made to manuscript.

Comment 2: *No mention of how aboveground plant biomass (presented as a result in lines 179-181) was measured. This needs to be included in the methods.*

Response: The data was collected by other researchers and was not originally included in our methods – we will update the methods in future versions to describe the methods in more detail.

December Response: Section describing plant cover and aboveground biomass methods has been added (see section 2.7)

Comment 3: *Line 130: data were log transformed. Here, I would then also state that the original data are presented in figures.*
Comment 4: *There is no mention that the ANOVA met assumptions – this should be addressed.*

Response: Agreed. Thank you for pointing this out.

December Response: Now described on lines 162-165.

Comment 5: *Finally, so long as you had equal sampling sizes across treatments (i.e., you didn't lose a single sample), then your statistical approach is great. Just double check you had equal sample sizes; if not, consider generalized linear models or something similar that allows for unbalanced design. I highly doubt your results would change with this approach if it is warranted.*

Response: We were very careful and didn't lose a single sample! Sample sizes were equal.
December Response: Described on line 165

- **Results**

Comment 6: I would present data in order of the methods section – this section felt unorganized at times. The figures themselves were crystal clear and easy to read – nicely done!

Response: Thank you for the nice comment. We will make sure to rearrange the Results and/or methods section(s) to improve the flow of the paper.

December Response: The order of the variables presented in methods and results section were parallel to each other with only few exceptions. Therefore, to increase clarity we have now added sub-headings to the results section which match the subheadings used in the methods.

Comment 7: *Lines 189-200 should mostly go in the Methods section.*

Response: Agreed. These lines will be moved as suggested.

December response: This section was split, with the methods and results portion for each labelled with the same subheading title ("Calculated N content).

Comment 8: *Table 2 - there should be units specific to aboveground pools. For example, Plant N Pools should be presented as g N g-1 m-2 or something similar.*

Response: Thank you for bringing this to our attention – there are a number of errors in Table 2. Both above and below-ground N pools are in g N g-1 m-2 and we will fix this table. Also, the plant-pools should be listed as leaf and root pools, not NH4 and NO3.

December Response: Table 2 has been updated to list the correct labels and units.

- **Discussion**

Comment 9: *I know space is limited, but this section seemed to scratch the surface on the topic of N retention, which was your key conclusion to explain "missing N" (that drylands don't really have the capacity to retain excessive N). I would like to see further expansion on the potential loss pathways in the discussion as a way to explain the "missing N." Be sure to check out the concepts in the N saturation literature, in particular around lines 52-53 and lines 233-234: Aber et al. 1998 (DOI: 10.2307/1313296), Lovett and Goodale 2011 (DOI: 10.1007/s10021-011-9432-z), Homyak et al. 2014 (DOI: 10.1007/s10021-014-9792-2). Work done in California grasslands address extensively the idea of N retention as it interacts with timing of a) N deposition and b) rainfall, which might greatly help your discussion content around this topic.*

Comment 10: *In addition, here are some additional examples of varying responses of drylands to N additions: Hooper and Johnson 1999 (DOI: 10.1007/BF01007582), Yahdjian and Sala 2010 (DOI: 10.1007/s10021-010-9341-6).*

Response: Thank you so much for the detailed suggestions and additional references. We will definitely expand the discussion section based on these references, paying particular attention to potential loss pathways for N.

December Response: See expanded discussion, especially lines 275-315, the majority of which are new to this revision.

- **Other**

Comment 11: *Throughout the manuscript, spacing between numbers and units is inconsistent. Add a space between all numbers and units (some examples are in the line-by-line comments below, but I did not catch them all).*

Response: We appreciate the detailed review and will correct the grammatical errors pointed out here and below in future revisions.

December Response: All spacing was edited in update, although track changes was not used because the revision became quite cluttered

- **Line-by-Line Comments**

Comment 12: *Line 2: "Grassland" should have lowercase g*
Comment 13: *Line 38: Püspök et al. 2022*
Comment 14: *Line 41: Another recent paper to add here is Krichels et al. 2022.*
Response: Thank you – we will include this reference.

December Response: References were added and edits made.

Comment 15: *Line 43-44: This sentence is a little bit awkward. Instead of wording these processes as "either N is stored, or it is taken up" it might be more useful to describe the "leaky pipe" concept. All of these pathways are occurring at the same time; maybe not necessarily simultaneous, as loss or uptake (and respective magnitudes) will depend on so many factors like soil moisture content.*

Response: Thank you. We will reword the sentence as suggested.

December Response: Additional text has been added to this sentence, describing the seasonal variability in the destination of N-inputs, and also this concept has been described in more detail in the newly expanded discussion.

Comment 16: *Lines 82-83: "other perennial C4 grasses" should be listed, especially since the paper focuses solely on Sporobolus. In fact, a bit of natural history about Sporobolus could be nice to include (so many papers from this region and further south in Jornada focus on the grama grasses).*

Response: Vegetation in the plots was very sparce. We decided to focus on *Sporobolus* since it was the only species present in all 20 plots, as opposed to grama grasses. We will include a few sentences on the natural history of *Sporobolus* in the revised text, along with a bit more information about species composition in these plots.

December Response: A short history of Mesa dropseed and reference to a publication that focuses on species composition was added to text, see lines 104-106.

Comment 17: *Line 78: MAP is presented, but only one sampling year (2020). What was 2020 precip amount?*

December Response: New figure explaining daily precipitation patterns, as well fertilization and sampling dates (Figure 1) has been added. The yearly total precipitation amount can be found in caption.

Response: Yearly precipitation will be included in supplemental materials. See response to an earlier reviewer.

Response to comments below: We will update text to reflect the comments below in our revision. Thank you for your detailed review.

December Response: All text has been updated as suggested.

Comment 18: *Line 94: space in between 2 and mm.*
Comment 19: *Line 102: space in between 5 and g*
Comment 20: *Line 134: "stats" R package should be cited*
Comment 21: *Line 179 and Table 2: no units presented for above-ground biomass.*
Comment 22: *Line 265: sentence starting "Thus, because N is not being immobilized…" would fit better near line 234.*
Comment 23: *Line 276: subscript the 4 and 3 in NH_4NO_3.*

Reviewer #2—

Comment 1: *I believe the methods and statistical analyses need to be more thoroughly explained and more clearly aligned with the figures shown. Specifically, pre-fertilizer values were shown in almost every graph, but there is no explanation of pre-fertilizer sample collection or how they were considered statistically with your analysis of variance. The methods say the ANOVA included sampling date, but the figures compile sampling dates within three different time points (pre-fertilizer, post-fertilizer and peak biomass) without an explanation of how these statistics were performed. For example, I would assume that peak growth was only a single time point, which would mean the model would have to be different than the explanation given. Further, I can't find additional information about when peak growth is and how it relates to the pre-monsoon, mid-monsoon, and post-monsoon months. It would be helpful to see* *the post-hoc results, which could be included as a supplemental table. In addition to clarifying the methods, expanding on the figure legends would help the reader understand the figures.*

Response: Sample collection only occurred at three points in time: pre-monsoon = pre-fertilizer, peak biomass = late-monsoon (where plants are believed to have reach peak growth), and post-monsoon Fertilizer was applied around 1 July after the first samples were collected and prior to the start of the summer monsoon. But, as addressed in a previous response, we will modify the sampling naming scheme to be more consistent and less confusing.

December Response: New figure explaining daily precipitation patterns, as well fertilization and sampling dates (Figure 1) has been added and sampling naming scheme has been updated in methods section.

Comment 2: *The authors should provide more information about aboveground biomass. There is almost no mention of aboveground biomass in the methods and results, but the authors include the finding about aboveground biomass in the third sentence of their discussion and expand on it throughout. This feels like a central message and the methods and results need to be expanded upon. The discussion also mentions plant communities, but those were not measured in this study.*

Response: We did not collect plant biomass ourselves, but instead relied on measurements conducted by those maintaining these long-term plots. We will work with these researchers to include the methods in our manuscript and thus be able to refer to the data directly.

December Response: As mentioned in previous response: section describing plant cover and aboveground biomass methods has been added (see section 2.7)

Comment 3: *Lines 20 – 30: The introduction references "arid ecosystems", "aridlands", "the southwest US", and "drylands" without a clear definition of each of these land types. Because the authors' study takes place in a semi-arid dryland, I would recommend introducing the concept of drylands at the beginning of the introduction and then using drylands when talking about larger concepts and semi-arid when talking specifically about studies/information specific to the authors' study site.*

Response: We appreciate the suggestion and recognize that the multiple names used for dry ecosystems can get confusing. We will edit the manuscript to include your suggestions, referring to drylands in the general sense and semi-arid ecosystems when authors are specific or when talking about our own ecosystem.

December Response: We have updated the paper, referring to drylands in the general sense and semi-arid ecosystems for our own ecosystem.

Comment 4: *Line 257: Because the authors highlight the limitations of the Sinsabaugh study, it would be helpful to read about other studies that observed a positive response to N addition.*

Response: The discussion section will be expanded in response to Reviewer 1 and the community commenter. Another reviewer suggested a number of additional studies, and we will make sure to include studies showing different responses to N addition in the text. We can also draw upon some recent N results in a different experiment (Brown et al. 2022 JGR-Biogeosciences).

December Response: The discussion has now been substantially expanded, particularly focusing on aridland models for N saturation and potential seasonality with timing of precipitation.

Comment 5: *Lines 255 – 270: There is no mention of the role of limiting organic C when exploring the lack of microbial response to N addition. This might be an important part of the co-limitation or serial limitation story and could be explored further. The authors could also look at stoichiometric relationships between C and N in the soil and microbial biomass.*

Response: We appreciate this suggestion and will make sure to describe that different factors often limit the above and below ground community, including the possibility of C-limitation by the microbes, which has been described by others.

December Response: We have added additional text into the discussion describing that the soil microbes in many ecosystems are limited by Carbon, and that this may be especially true in drylands with low organic content. We also describe that because we did not measure microbial efforts to access C or other nutrients via extracellular enzymes, and also because there was no change in microbial C and N stoichiometry with fertilization (i.e., no effect on either element with fertilization), our suggestions remain speculative.