

## ***Interactive comment on “Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland” by L. Fuchslueger et al.***

**Anonymous Referee #2**

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The manuscript titled “Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland” presents an interesting manipulation whereby two alpine grasslands under different management strategies are subjected to drought conditions in situ in order to observe effects on key N cycling processes related to the generation and consumption of reactive N. Overall I found the approach to be innovative, the methods to be well executed, and the results to be of interest to the readership of Biogeosciences if couched in the proper context. However, similar to reviewer 1, I found some fundamental flaws in the justification for their hypotheses. The incorrect use of references aside, the hypotheses are completely lacking in substance and provide no mechanistic framework to build upon the ideas presented in the

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introduction. Moreover there seems to be a bit of contradiction in the formulation of the authors’ arguments. From previous work, we understand that the kinetics of AOA activity indicates a higher affinity for ammonia as demonstrated by a half saturation constant in the nM range, and the authors even acknowledge that AOA should have a clear advantage in environments with low ammonia concentrations. Therefore, under drought conditions, we would expect ammonia concentrations in porewater to increase over time. Yet, the authors predict that drought would have ‘stronger effects on bacterial than archaeal amoA gene copy numbers’. If the authors had alluded to the hypothetical mixotrophic nature of AOA (sensu Jia and Conrad, 2009, or Sims et al. 2012), then perhaps this hypothesis would make sense; AOA weathers drought more effectively than AOB by employing alternative metabolic pathways. Granted I don’t believe this to be the case, but perhaps the idea has a better foundation than the one provided in this manuscript. The authors’ would do well to revise their hypotheses for clarity, so the reader better understands their reasoning for why drought affected AOA communities should outperform AOB, or what exactly from a land management perspective is meant by ‘stronger impact’. As an example of my meaning, I draw the authors’ attention to a recent publication on the same subject matter (doi: 10.1111/1574-6941.12395). In their presenting their hypotheses, Thion and Prosser (2014) provided a clear rationale as to why they believe a particular group within the AO community would perform better under drought conditions. I think the authors should give additional consideration to Thion and Prosser (2014), because like their own study, this research was conducted on non-adapted AO communities, meaning that microbes in these soils rarely experience drought. Some of the literature cited in the introduction as a ‘case in point’ stems from research in Mediterranean climates, e.g. California grasslands, where microorganisms are well-adapted to seasonal drought.

I think the approach of using rainout shelters rather manipulating soil moisture in mesocosms was a good choice; however, it exposed the desired treatment effect to number of modulating factors likely related in part to differences among vegetation communities (primarily grassland vs. grassland populated with ericaceous shrubs, and a legume),

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e.g. litter production, root density, etc. This may have robbed the study of some of its statistical inference, and perhaps a power analysis a priori would have helped in improving the design. I find it interesting that in choosing this design, the authors focused solely on microbial dynamics and gave no consideration to plant contributions (e.g., N uptake preference, timing of maximal root growth, etc.) to N cycling dynamics in their discussion, despite that plants contribute substantially to below ground processes. Presumably, the plant component, unlike the prokaryotic component, straddles the treatment effect (rainout shelter). Perhaps some of the spikiness in pool dynamics among control plots could be related to differences in plant phenology.

Minor points:

How does measuring abundance of AOB vs. AOA really get at the functionality of AO in response to drought, particularly from a climate change perspective, since these data may provide a framework for process models? Several studies indicate that, at least in certain environments, population abundance alone is a poor predictor of relevance to ammonia oxidation.

C<sub>tot</sub> and N<sub>tot</sub> were determined on an EA coupled to an IRMS. Why then not report isotopic values for these elements in Table 1? Some of the readership might draw inference from these values.

How was the efficiency for the <sup>15</sup>N microdiffusion determined, and why is it not reported?

Regarding linearization of plasmid DNA used to standardize qPCR, I agree with reviewer 1, but here are two publications presenting mixed results to help you make your own determination.

1. Oldham AL, Duncan KE (2012) Similar Gene Estimates from Circular and Linear Standards in Quantitative PCR Analyses Using the Prokaryotic 16S rRNA Gene as a Model. PLoS ONE 7(12): e51931.

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doi:10.1371/journal.pone.0051931

2. Hou Y, Zhang H, Miranda L, Lin S (2010) Serious Overestimation in Quantitative PCR by Circular (Supercoiled) Plasmid Standard: Microalgal *pcna* as the Model Gene. PLoS ONE 5(3): e9545. doi:10.1371/journal.pone.0009545

Throughout, either/or and neither/nor.

Throughout, please note archaeal, not archael

In the Discussion, comments concerning mowing effects are overly speculative and should be removed.

'in accordance with' not 'in accordance to'

'respond more sensitively' not 'more sensitive'

P9197 L3-9 Clunky and redundant, please consider revising.

Soil acidity (~5.5) may play a role in overall nitrification potential, particularly with regard to competitiveness of AOA to AOB. Why was this never really discussed?

Pg 9197 L17-21 Please clarify, AOA and AOB were extracted to determine nitrification potential? Are the authors referring to abundance as a potential? If so please refer to the first comment in this section.

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Interactive comment on Biogeosciences Discuss., 11, 9183, 2014.

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