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Interactive comment on "Amino acid and N mineralization dynamics in heathland soil after long-term warming and repetitive drought" *by* L. C. Andresen et al.

Anonymous Referee #1

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Review of SOIL Discuss., 1, 803-826, 2014 Amino acid and N mineralization dynamics in heathland soil after long-term warming and repetitive drought, by L. C. Andresen, S. Bode, A. Tietema, P. Boeckx, and T. Rütting

General comments: 1. This manuscript touches a topic of great relevance, i.e. how does global change (here increasing temperature, intensifying drought-rewetting cycles) impact the soil N cycle, with an emphasis on gross rates of soil organic N cycling and N mineralization. This is novel. They also discuss how organic N is mineralized, via the direct pathway (organic N uptake by microbes and release of excess of N as ammonium $\sim$ gross N mineralization) versus the MIT route (characterized by extra-

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cellular deamination of organic N to ammonium, which then is taken up by microbes). This has strong repercussions on our understanding of the soil N cycle and its controls. 2. At the downside of this manuscript is the deficiency of statistical replication to allow statistical evaluation of free amino acid (fAA) production rate, or was it analytical failure that obviated this? Nowhere in the manuscript I found a clear description of how many samples were analyzed for fAA dynamics. As far as I understood 3 soils samples were taken at three sites within each treatment plot, and all of these samples were bulked to one composite sample per plot. This means with three treatments, that there were nine plots and nine samples? But for fAA pool dilution there are only two values per treatment. Obviously the authors measured fAA mineralization in all three samples per treatment, allowing simple statistical tests of fAA mineralization but did not so for fAA production. On page 9, lines 7 they mention that "each treatment had two replicates at each time step, both numbers are reported in addition to the average"! This makes no sense to me – see above. Moreover in several parts of the manuscript the authors point out and even discuss non-significant results or results that could not be statistically tested (e.g. page 10, line 26, page 11, line 2-3, page 2, line 22). 3. The mirror image isotope approach was developed to measure contributions of added residues or of organic N to gross N mineralization in soils. In all of these approaches as also cited in the manuscript large additions of organic N (labelled or unlabelled, alongside amendments of unlabelled or labelled ammonium) were used to study the fraction of N mineralization deriving from e.g. residues, proteins or amino acids but these were long-term incubations running over several days where 15N tracers and tracees could equilibrate. In this study the duration of the mirror image isotope pool dilution assays (i.e. mineralization of 15N-labelled amino acid mix to ammonium) was followed only over 30 minutes. The low (34%) contribution of fAA mineralization to N mineralization, if by the direct route i.e. microbial amino acid uptake and release of excess N as ammonium (the other studies showed that this is the major pathway of N mineralization) was most important or dominant, therefore was clearly too short to arrive at reliable estimates of fAA contributions to gross N mineralization. In their results/discussion the

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authors show that fAA production rates outweigh N mineralization by at least 8-fold (gross fAA uptake by microbes usually balances fAA production), pointing to the direct route as the major contributor to N mineralization, and then say that fAA mineralization to ammonium contributes only 34% to N mineralization, and shifts through climate change point to shifts in mineralization of other organic N sources. Given the reasoning above this is clearly not backed up by their data. 4. In the 15N-fAA labelling assays they applied ultrasonication but do not refer to the intensity applied. Ultrasonication at high intensities not only breaks aggregates but also microbial cells. If microbes had taken up 15N-fAA and are broken by this measure the release of 15N-fAA from microbes would grossly bias the isotope pool dilution assay, causing underestimation of the rates of production and uptake of fAA.

Interactive comment on SOIL Discuss., 1, 803, 2014.