SOIL Discuss., 1, C673–C681, 2015
www.soil-discuss.net/1/C673/2015/

SOIL Discussions Open Access

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.

L. C. Andresen et al.

louise.andresen@bot2.bio.uni-giessen.de

Received and published: 18 February 2015

Dear Editor

Thanks for the reviewer comments and corrections. Here we present a structured reply with referee comment followed by author reply and corrected text. At the end of the reply letter follows the entire manuscript wit marked up corrections, including tables and one corrected figure. The manuscript has been checked by a native for correct English language.

Kind regards Dr. Louise C. Andresen, on behalf of all authors

Referee #1

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 _ gross N mineralization) versus the MIT route (characterized by extracellular 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.

Author reply: Each climate treatment is 3 times replicated at the field site, which are used as replication in the present study. From within each replicate plot a total of 9 soil cores (3 cores at 3 different locations within each plot) were taken. Due to analytical failure, in some cases only 2 replicates per treatment could be successfully analyzed. We clarified this in the manuscript: P9 L5: ' . . .0 and t respectively. Gross N mineralization had three replicates per treatment analyzed at each time step.', and P9 L7: 'Some treatments had only two replicates successfully analyzed at each time step',

and P9 L10: '. . .was obtained by measuring 15N-NH4+ production in three replicates per treatment at the time steps 10. . .'

Referee #1: 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).

Author reply: First, we think it is important to keep in mind the difference between 'statistical significant' and 'biological meaningful', i.e. even results that are not statistically significant might well be biological meaningful. This is particularly the case for studies with low field replication combined with complicated lab essays (as in our case). We did not apply statistical models to data with only 2 reps. as this this is not meaningful. Therefore, we discuss (at least as trends) the results and have modified the text as follows: P10 L 24: 'In drought treatment FAA mineralization was reduced (P = 0.006; Table 2), and gross FAA production seemed to decline with drought and warming (Table 2,) though, due to limited amount of replicates this could not be test statistically.' Here we furthermore correct from 'Fig 3' to' Table 2'. P11 L2; we delete the line: 'Turnover times were not significantly affected by climate manipulations', because this was in fact not statistically tested. P2L 22 the sentence was modified: '. . .., even though gross FAA production declined.' Furthermore, we have specified: P12 L7: ' . . .unlike our hypotheses, no significant effects on any. . ..' P12 L 19: '. . .the relative importance of FAA mineralization for gross N mineralization rate.'

Referee #1: 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, alongsideamendments 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 tracers 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.

Author reply: It seems that the reviewer has misunderstood the time duration set-up. For studying FAA mineralization and gross N mineralization rates we used a time frame of 7 hours, not 30 minutes. This is of the same length as all but one of the experiments with mirror approach cited in Table 3, and was chosen because AA turnover is fast, in our case 3 hours.

Please correct in Table 3 the error in line 3, the Hadas et al 1992: move 7 out to the 't' column, 4.93 out to the 'FAA' column; 5.59 out to the 'gross min.' column and 88 out to the 'alfa' column. Furthermore, we correct in Table 3 by deleting the two words 'straw' in second last and third last line. This was a mistake as the data was from the part of the experiment without any straw. Hence all experiments in Table 3 investigate amino acid mineralization, not residues. Furthermore we have in Table 3 all duration now by the unit hours (change from 1 week to 168 hours).

Referee #1: 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 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.

Author reply: Again it should be noted that the AA mineralization was investigated over 7 hours, similar to earlier studies. This is of the same length as the turnover of the AA pool (2.9 to 11.5 h, depending on climate treatment). Therefore, we do think that this

is the appropriate duration for investigating AA mineralization, as otherwise we would lose the 15N signal. See furthermore our reply to reviewer #2 below.

Referee #1: 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.

Author reply: Indeed sonication can break microbial cells however, the sonication was done with a sonication bath (Elma S 100 H) which has Ultrasonic power of 200 W and capacity of 9.5L ($\pm$ 20mW/cm3), according to the work of Feliu et al. 1998 (Feliu, J.X., Cubarsi, R., Villaverde, a., 1998. Biotechnol. Bioeng. 58, 536–540. doi:10.1002/(SICI)1097-0290) on E-coli (strainGSCG5073) this energy density would result in β-galactosidase activity release rate of 0.0035min-1 (i.e. 0.02% after 30seconds). Of course the release rate, determine by Feliu and co-authors is only applicable for this specific strain and cannot be generalized for the complex microbial community present in soils. However this seems a good indication for the fact that the cell lysis was probably very minimal. Furthermore as the samples were inside plastic tubes they will have experienced even less sonication power. Finally, we actually did not assess the FAA uptake, but only looked at the FAA production out of SOM, using the dilution of the 15N labelled FAA pool. To our view the release of FAA taken-up by microorganisms would actually not cause such a problem here. We have modified the manuscript: 'The subsample was hand-shaken, sonicated by ultra sound (20 mW cm-^{3} by Elma S 100 H) during 30 sec, and then. . .'

Referee #2 General comments: The manuscript describes the results of a 15N flux rate experiment on soils sourced from a long-term climate manipulation experiment in heathland in the Netherlands where slight warming, and severe early-season drought have been imposed in-field since 1999. The main focus of this MS is whether mineral-

ization of amino acids constituted a significant proportion of gross N flux as measured by pool dilution, and whether gross N mineralization is affected by drought and warming. The manuscript is concise, and most relevant variables are quantified. However, the standard of English is in places poor, so much in fact that parts of the discussion are very difficult to disentangle. I appreciate the difficulties in writing formally in a language other than your mother tongue, but nevertheless, it is important that results are communicated effectively.

Author reply: I have asked a native speaker to improve the language of the manuscript.

Referee #2: I also have to question why the paper ends with speculation on the concept of niche specificity when this hasn't previously been brought into the context of the work presented here (and could not be [dis]proven in this study in any case).

Author reply: To prove or disprove the concept of plant nitrogen utilization niche is not the purpose of the study. We have decided to omit this discussion as we do not bring any analysis of plant data and plant N uptake. And we delete this in the discussion, the sentences: 'This will have consequences for the N availability for vegetation at future frequent drought events.', and: 'A resource based N-niche differentiation of co-occurring species would result in a drought induced shift from species relying on free amino acid N uptake to species relying on inorganic N uptake (McKane et al., 2001; Nordin et al., 2004), which may threaten the heathland ecosystem.' are deleted.

Referee #2: Specific comments: 1) The specific question buried underneath all this: how much of the mineral N pool comes from amino acid mineralisation is a very interesting one, I find the introduction and discussion muddled. The contrasting concepts of internal vs extracellular mineralization are introduced and discussed, yet the methods used in the present study would never allow these questions to be answered directly. The main reason for this is that free amino acids (FAAs – and it should be a capital 'F' in the MS for this acronym) only present a portion of the total N that is able to be taken up intact by microorganisms. Recent work highlights that not only are FAAs one of several

organic N species to be taken up by plants and microorganisms, peptides in particular may be responsible for a much larger N flux than monomeric FAAs. Consequently, the authors need to either expand their introduction to better cover the recent literature on non-FAA-N, or better, focus more on the effects of climate manipulation per se, rather than heading into an area that cannot be answered by the techniques used. I would prefer the latter option.

Author reply: We have corrected FAA throughout the manuscript.

We concur that we can not conclude about the relative importance of the direct route and MIT. For this reason we modify the text as follows (also in response to reviewer #2, see below): We delete text from P 3 L 17: 'Two alternative pathways. . .. . .; Giessler et al 2009).', on page 4 L1. Furthermore, we delete text from P11 L 18: 'Immobilization of fAA. . ...Bennett, 2004).', on P11 L23. And delete the sentence P4 L12: 'Thereby, investigating the relative importance of direct mineralization versus MIT is now possible by focusing on the relative nitrogen fluxes.'

The reviewer has in the comment a focus on plant and microbial uptake of intact peptide and other large N compounds. However with our study we do not go to the detail of organism consumption of FAA, peptides etc. but merely the production in the soil solution of free amino acids and ammonium. We have added the viewpoint to the text: P4 L2: '. . .FAA production rate. However, FAA are not the only source of gross N mineralization.', and further more acknowledge the importance of protein and peptides on the same page: 'Methodologies using 14C to study FAA turnover have revealed that the transformation of N from proteins to ammonium was much slower than from amino acid to ammonium, which suggest that the depolymerization rate is the main important constraining factor of N availability in forest ecosystems (Jones and Kielland, 2002; 2012).' To focus the text on climate change effects we have added to the introductions: P 4 L 17: 'Changes in soil nitrogen dynamics occurring in response to these conditions diverge for the two factors warming and drought.'

Referee #2: 2) Following on from this, there are several parts of the introduction (such as P806 L1) that are factually incorrect due to the absence of any acknowledgement that FAAs are not the only form of bioavailable organic N.

Author reply: We have clarified why amino acids are important for ammonium and corrected P806 L1: 'Gross mineralization is depending on the availability of FAAs, because FAA mineralization is the main pathway of ammonium production (Barraclough 1997; Stange & Döhling 2005; Geisseler et al. 2012), hence, gross N mineralization depends on the FAA production rate.'

Referee #2: 3) The statistics and subsequent results in part make little sense. In the sampling section,a rigorous approach using composites of 9 reps per plot (field reps) is described. However, in the calculations section, it is stated that each treatment had two reps. Which is it, and if it's the latter, why?

Author reply: See our reply to reviewer #1

Referee #2: Also, why were t-tests carried out when this is surely a simple 1-way ANOVA design?

Author reply: We disagree here, a simple 1-way ANOVA design will compare all treatments and we think that it is not appropriate to compare the warming and drought treatment. Therefore, the t-test was applied in the current study.

Referee #2: This is further made difficult by discussion of non-significant differences in the discussion – if there is no significance, there is no difference!

Author reply: See our reply to reviewer #1

Please also note the supplement to this comment:
http://www.soil-discuss.net/1/C673/2015/soild-1-C673-2015-supplement.pdf

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

Figure 1

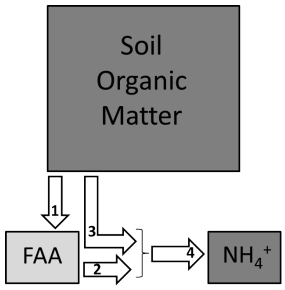

Fig. 1.