

Referee #1

"I found the approach very interesting, but I thought a little bit more experimental detail should be provided"

The experimental detail given about the field work preceding the lab work has deliberately been kept to a minimum as this is fully described in the paper 'Carbon cycling in temperate grassland under elevated temperature' by Jansen-Willems et al. (in press). However, we could add some details, for example on how plots were assigned as described in comments to reviewer 2. For details regarding the IRMS analyses we referred to Stevens *et al.* (1993), however, more details are given below. For details regarding the ¹⁵N tracing model we referred to Müller *et al.* (2007), to keep the manuscript concise, but we've given some extra information in the 2nd comment of reviewer 2.

"In my opinion the authors nicely combined field/lab work and modelling, however, when I read through the article at first I was a bit confused about the modelling component, because I found it presented as an "add on". I think it could be a bit more put forward in the abstract already'

We agree with the reviewer that it would be better to make this already clearer in the abstract as the modelling is a major component of this paper. We would therefore suggest that the sentence starting with To evaluate... in L17 could be replaced with: "To evaluate the legacy effects of increased temperature on both nitrogen (N) transformation rates in the soil and nitrous oxide (N₂O) emissions, an incubation experiment and modelling approaches were combined. For this, soils were taken from a long term in situ warming experiment on temperate permanent grassland." And the sentence finishing with "...NO₃¹⁵NH₄ Gly, ¹⁵NO₃NH₄ Gly or NO₃NH₄ ¹⁵N-Gly." In line 23 could be followed by "Soil extractions and N₂O emissions were analysed using a ¹⁵N tracing model and source partitioning model."

"I would also have liked to be provided with more detail on the calculation of N₂O fluxes (which factors were taken into account for the calculation, which equations were used?)"

N₂O flux sampling and calculations were explained in 182-191. For this the ppm and time difference between t₀ and t₁ were taken into account and also the volume of gas in the jar, and the grams of dry soil. The grams of dry soil were determined by drying subsamples at 105 degrees Celsius for 24hrs as described in L141. The volume of gas in the jar (ml) was determined by subtracting the grams of water and the grams of soil divided by 2.6 from 750 (the empty volume of the jar). To convert the N₂O from volume to mole, the temperature was assumed to be 20°C (the temperature of the incubation room) and the pressure was assumed to be 1 bar. We could, if necessary, add all used equations as supplementary info.

"Moreover, I would be interested in a little bit more detail on the IRMS analysis (e.g.: how were samples returned to ambient pressure?, what was the precision of your IRMS analyser?, how were δ-values defined?)"

Bringing samples to ambient pressure: a double ended needle is fixed vertically in a clamp stand with the ventral needle submerged 3-4 mm in a beaker of water and the gas sample is held upside down and pushed onto the dorsal needle. The excess pressure in the sample vial is thus released causing the water to bubble until the pressure inside the vial equals that of the ambient atmospheric pressure. Cessation of bubbling implies equal pressure has been reached. Regarding the detection limit, the s.e.m for atom% ¹⁵N of a 50 ppm N₂O std gas was 0.00003 (n= 10), stdev was 0.00009 atom% ¹⁵N. Respective values for a 0.4 ppm N₂O std were higher: the s.e.m was 0.00084 (n= 10), stdev was 0.003 atom% ¹⁵N.

“My last specific comment refers to figure 4. Maybe it’s just me but I did not really get figure 4. In the figure caption is says: “Modelled vs measured data”, but, to me it seems like either modelled or measured data is presented. I would be very grateful for an explanation.”

Figure 4, does give both the modelled and measured data. The lines are the modelled data and the squares, circles and triangles are the measured data including their standard deviation. This could be further described by changing the figure caption to: “Fig. 4. Modelled vs measured data. The lines are modelled data, and the squares, circles and triangles are the measured data points. Error bars are standard deviations. Time is the time in days from the moment of label addition.

Technical corrections

- L48 should be changed
- L96 should be changed
- L132 should be changed
- L137 should be changed
- L137&150 This could be removed. L137 could be changed to: “On the day the heaters were turned off, all soil within.....”. L150 could be changed to: “Two days after soil sampling all jars were put in a dark climate chamber...”
- L329-332 Could be changed to: “However, the overall gross mineralisation of organic N to NH_4^+ did not differ with the previously imposed warming treatments. This was because, the mineralisation of labile N was the major contributor to total mineralisation, and this rate was not significantly affected by previous warming (Table 2).”
- L407 comma should be added
- L426-429 Could be changed to: “This could be due to a decrease in the rate of denitrification. However, it is also possible that under treatment T_2 and T_3 more of the NO_3^- was converted into N_2 as opposed to N_2O . As denitrification, in contrast to nitrification, can also lead to production of N_2 as well as N_2O .”
- L460 Could be changed to still not
- L473 dot should be added
- L483 Should be changed to: were decreased
- L704 Space should be added
- L 716 Space should be added

Referee #2

“The summary of my suggestion for the authors is to clarify the role and importance of the model and the source partitioning in the hypothesis and discussion. Like the first reviewer, I find that the important role that the model and the math play in this paper is not sufficiently emphasized”

As mentioned in our response regarding the comments of reviewer one, we could add more detail to the abstract. We feel that the reason and importance for development of the source partitioning model is sufficiently explained (L91-103). The use of the ^{15}N tracing model in this paper is for application purposes, and not model development. Therefore we think that the interested reader can also go to the original publication for further information..

“Regarding the model: I found it difficult from the included text to understand how this model differs from the one published in Müller 2014. I would be helpful to the reader is more detail were provided here.”

The model used is an extension of the Müller *et al.* (2007) model. The only change is the inclusion of an amino-acid component (As described in L201-203). If necessary it could be made more clear in this sentence that they only addition compared to the 2007 model is the addition of an amino acid glycine pool, and the transformations to and from this pool. Our aim was to present a simplified approach to analyse the ¹⁵N tracing data set (without nitrite). We therefore applied a two-step approach, where we first determined the simultaneously occurring gross N transformation, and then taking the MS ratios to identify the N₂O processes.

“I am also not certain if those differences require further support; there is an addition of amino acids in the model (which is based on glycine additions, not the extant AA pool, unlike the NH₃ and NO₃ calculations), there are less time points, no nitrite measurements and no N gas output. I not that previous iterations of this model have received extensive verification. How can we evaluate the robustness of this new model?”

We agree that in the model it is not the total amino acid pool but Glycine is a major part of the AA pool in soil (see also Barraclough (1997)). The previous publication the reviewer refers to, were mainly concerned with the development of the model itself which obviously included more rigorous evaluations. Here we applied the model (with just one extension) to a data set. Since it is an analysis tool which was verified before we did not see the need to further evaluate and verify it in detail, as the conceptual ideas included in the tool were verified before. Furthermore, since the publication of the basic model by Müller *et al.* (2007), more than 50 peer-reviewed papers have been published which used the basic model or modifications of the basic model. This shows the robustness of the tracing approach to various soils, ecosystems and climatic conditions. Thus we believe that the conceptual idea using a numerical tracing model together with a MCMC method is robust enough to analyse even extended data sets. We should also bear in mind that a tracing model, unlike a simulation model, only takes into account the most basic kinetic settings to describe the underlying N cycle. Based on this outcome, we are confident that the Ntrace model approach is robust and flexible enough to be used as an analysis platform for ¹⁵N tracing data sets.

“Is it an improvement of the previous model or has crucial information been compromised by the data omissions?”

This is not an improvement of the model, but just an extension to the 2007 model. In a separate step the N_{gas} dynamics were analysed without the need to know in detail the Nitrite dynamics in the soil. Thus, the two step approach presents a new approach to analyse underlying gross N transformations and the N₂O dynamics.

“Do the good curve to empirical fits in fig. 4 imply that the more complex data collection of Muller 2014 is not necessary?”

No, it is only a more simplified approach. However, the value of the 2014 model is that the N₂O dynamics are based on the underlying nitrite dynamics, which are themselves evaluated as a sub-pool approach. Thus our model does not take into account N₂O consumption (see comment below)

“It is not clear how the tarting point recalcitrant and labile N parameters were determined (it is probably in Muller 2014, or 2004 or 2007, but would like to see it in here), or even what these pools include (chemically/physically, because the functional implications are self-evident).

It is based on measured soil organic N data from the field site (as used in other publications Müller *et al.* 2007, Müller, *et al.*, 2014). We do not know the specific constituents of the N_{org} pools. In the model we used a conceptual pool approach which did not present actual chemical composition but only take into account differences in recalcitrance, and associated turnover times. Therefore it is an approach

which is comparable to conceptual models representing C transformations by two or three-pools (Knorr *et al.*, 2005). By the way the exact initial value is not critical because for the recalcitrant pool a zero-order reaction has been applied (i.e. independent of the initial pool size) and for the labile pool the quick turnover time ensure that a small pool will be governed quickly by the dynamics of the in and out-flowing rates.

“Finally, why is there so little evaluations of the model itself (aside from the results) in the discussion? As described in a previous comment. This model was merely an extension of a very well evaluated model, that has been used in many previous publications. It was therefore not deemed necessary to evaluate it again. In this paper it was about the model applications, and therefore only the results were evaluated. Furthermore, we should bear in mind this is an analysis tool which should include basic kinetic structures to quantify the underlying gross transformations. As mentioned above this aspect has been evaluated by using this tracing tool for many applications.

“Regarding the source partitioning: I really enjoyed this method and results (fig. 5), a very nice illustration of the importance of organic N as a source of N₂O, which in turn supports the model results. However, since the N_g process has been removed in the model, and N₂O consumption is not in the source partitioning (see my comment for Fig 6 below), could the authors please comment on whether the partitioning math is sensitive to N₂O consumption?”

One of the shortcomings of this simplified model is that consumption of pathway specific N₂O rates cannot be evaluated. This requires a more complex approach such as the 2014 model. However, since we carried out the experiment at the WFPS of 64% we do not believe that N₂O consumption which would mainly happen under fully reductive conditions has played a major role.

Comments by line

- L24 At all times, so both pre and post incubation.
- L74 It was 17.6%, sentence could be changed
- L130 Treatments were assigned according to a latin square design. This is fully explained in the Jansen-Willems et al paper.
- L136-166 A timeline could possibly be added, but we think it could already be enough if we would use day 0 as the day of label addition, and change all other number accordingly. The changes should be L150 (day -55), L156 day -53, L157 day -43 and -5, L161 day 0, L172 day -2.
- L179 As the label addition is the main event during the incubation, we actually think it makes sense to call this day 1.
- L204 Based on C-N measurements and the dissection into the pools as explained in the previous publications (e.g. Müller *et al.*, 2004)
- L308 How do authors think that such a large substrate input (relative to background levels of NH₂) altered rates and pathways? How did glycine additions compare to background amino acid concentrations? We did not measure glycine concentrations, so we don't know how they compare. However, based on the model outcome (including the dilution of the NH₄⁺ when added glycine had reduced quickly) we can assume that the glycine concentration was very low and must have approached concentrations at the detection limit within the first two days. So we think it had an effect but only for a very short period of time (as seen on the output of the glycine concentrations, data not shown)
- L331 This should indeed be changed to labile organic N

- Fig 3. There is a legend underneath the graph that applies to all three panels. We do not really understand what other legend the reviewer would like to see.
- Fig 4. This was also commented on by reviewer 1, and we suggest a new caption as given in the previous comments.
- Discussion Fig 6. Thank you, this is a good point and we should include something on this in manuscript. For NO_3^- and NH_4^+ this is indeed due to the addition of substrate. The higher absolute rate of organic N oxidation at the start of the incubation does not seem to solely come from the glycine addition. If this would be the case you would expect the highest ^{15}N atom% of N_2O in the first measurement after addition of the NO_3NH_4 ^{15}N -gly label. However, for all treatments the highest ^{15}N atom% in N_2O was found in the second measurement after label addition. The lower net rates at the end of incubation could possibly have something to do with N_2O consumptions, however this would lead us to more speculative discussions. We realise that with this method we will not capture all possible processes but still we are confident that the majority of N_2O process rates are taken into account. This shortcoming could be included in the manuscript.
- Table 2 N_{aa} should indeed be added, it is the amino acid glycine pool. Division format could be changed to make it clearer.

Barraclough, D., 1997. The direct or MIT route for nitrogen immobilization: a ^{15}N mirror image study with leucine and glycine. *Soil Biology and Biochemistry* 29, 101-108.

Knorr, W., Prentice, I.C., House, J.I., Holland, E.A., 2005. Long-term sensitivity of soil carbon turnover to warming. *Nature* 433, 298-301.

Müller, C., Rütting, T., Kattge, J., Laughlin, R., Stevens, R., 2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. *Soil Biology and Biochemistry* 39, 715-726.

Müller, C., Stevens, R., Laughlin, R., 2004. A ^{15}N tracing model to analyse N transformations in old grassland soil. *Soil Biology and Biochemistry* 36, 619-632.

Stevens, R., Laughlin, R., Atkins, G., Prosser, S., 1993. Automated determination of nitrogen-15-labeled dinitrogen and nitrous oxide by mass spectrometry. *Soil Science Society of America Journal* 57, 981-988.