Interactive comment on “Seasonal variation in nitrogen pools and $^{15}$N/$^{13}$C natural abundances in different tissues of grassland plants” by L. Wang and J. K. Schjoerring

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We thank Referee #1 for the positive comments and constructive suggestions which we find useful for improvement of our paper.

In the revision we have been able to address all the questions and to incorporate all the suggestions of Referee #1 as explained below:

Referee comment #1. One question I would have is whether the absolute values of metrics (like delta 15N or 13C) should be analyzed or the first derivatives (changes in values). Interpretation of some of the 15N patterns are hard because there are multiple causal pathways that cannot be separated. The authors need to go through
the paper and make sure that (reasonable) competing hypotheses are acknowledged. For example, when they state that “Peaks in gamma also appeared during the winter, coinciding with increasing delta15N values, indicating absorption of N derived from mineralization of soil organic matter” is there really any way to be certain that this is the mechanism? Aren’t there competing mechanisms?

Response: Several sources of 15N were contributing the pool of plant available N in the present study, viz. mineralization, mineral fertilizer and animal urine and faeces. During the winter period, there were no N fertilizer application and no grazing animals in the fields which make the input from mineralization more dominant. In line with this we observed that delta15N values increased during the winter, indicating absorption of N derived from mineralization of soil organic matter, and that this coincided with a peak in the gamma value (ratio between tissue ammonium and proton concentrations). Our statement should only be interpreted as a qualitative, descriptive observation which does not prove a causal quantitative relation between the two parameters. The data material does not allow for such quantitative deductions and for the same reasons we have not conducted a more comprehensive analysis of the first derivatives of the time course relations. Nevertheless, we think that the qualitative observation is interesting and hope it can lead to more frequent measurements during periods with low temperatures, where very limited information is available.

Referee comment #2 Remove “very” from the text. Either be quantitative, or just state something is high/low.

Response: All the “verys” have been removed.

Referee comment #3 P12319. L21. Can you define what constitutes events here?

Response: “50% of the time” has been added: “In non-fertilized agricultural grassland in The Netherlands, the frequency of NH3 emission episodes was about 50% of the time during a warm and dry summer period of 28 d.”
Referee comment #4 12327 L5. Temperature, not temperate.
Response: The spelling mistake has been corrected.

Referee comment #5 Discussion 4.1 first paragraph feels unnecessary. Actually, the whole section can be removed without any detriment to the paper.
Response: This paragraph describes the vegetative and productive growth of the ryegrass characterised by biomass constituents, water content and chlorophyll. The aim is to relate the developmental stages and different biomass constituents to the NH3 exchange potential in the following discussion. If the whole paragraph is removed, readers will miss information about the overall growth pattern.

Referee comment #6 In the discussion, could the authors discuss a little the role of tissue C:N in determining source/sink potential for litter. Once decomposition starts, this should be a control on NH3 concentrations. This would help connect the work with others.
Response: A correlation analysis between C/N and gamma values in litter and senescent leaves was performed, but did not reveal significant interdependences. Therefore, it seems that the C/N ratio does not control the NH3 source/sink potential.

Referee comment #7 Can the authors state why chlorophyll was measured?
Response: The reason for presenting chlorophyll data is to characterise the degree of senescence of the ryegrass leaves since their source/sink potential for NH3 is related to the developmental stage.

Referee comment #8 The authors should also discuss Frank and Evans 1997 more. There, they make clear statements about NH3 volatilization and plant delta15N. Are the authors results consistent with their interpretations? That would help others who think about grassland N dynamics.
Response: In the paper of Frank and Evans 1997, it was observed that grazers in-
creased soil delta 15N values, while plant delta 15N values decreased in grasslands. These contrasting effects of grazers were suggested to be related to the fact that grazers on the one hand promote loss of 15N depleted products from soil via nitrate leaching, ammonia volatilization and/or denitrification, while on the other hand there is a higher plant uptake of isotopically light soil nitrate relative to soil ammonium in grazed fields compared to ungrazed fields. In our work, the experiment was not designed to investigate the effect of grazers on N cycling and we lack an ungrazed control field to evaluate the possible effect of grazers. A paragraph discussing these aspect has been added after the second sentence in section 4.4.

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