Author's response to Reviewers' comments #1

Manuscript reference number: **MS No.: egusphere-2025-292**

Manuscript title: Previous integrated or organic farming affects productivity and ecosystem N balance rather than fertilizer ^{15}N allocation to plants and soil, leaching, or gaseous emissions (NH_3, N_2O, and N_2)

Dear Editorial Team (egusphere), and Dear Referee,

We sincerely thank you for taking the time to carefully review our study and for your constructive and valuable feedback. We have revised the manuscript according to the valuable comments and thank you for the opportunity to submit a revised version of our manuscript. Please find below the detailed one-to-one responses to your comments.

Reviewer #1
1) This is a very important study comparing the N budget and N transformations in organic and integrated farming. 15N tracing method is applied and all the components of N-cycling are analysed , both mineral N and gaseous N forms. This very detailed study allows to almost close the N budget which is a very challenging task and authors manage this exceptionally well. ***Answer****: We thank the Referee for their positive and encouraging feedback. We appreciate the recognition of the comprehensive approach taken in our study, particularly the use of the ^{15}N tracing method and ^{15}N gas flux method and the detailed analysis of N cycling components. We are glad that the effort to close the N budget has been acknowledged and valued.*
The manuscript is very well prepared, provides the summary of results very clearly, although this is a very complex dataset. I've read this manuscript with pleasure and interest, and I definitely support the publication in Biogeosciences. I only have some minor comments which could strengthen some technical aspects of the manuscript and the data discussion. Especially, to enhance your discussion I suggest to keep it quantitative, since you have quantified all the N fluxes you may quantitatively check all of your discussed assumptions (I give examples in the specific comments). I think it is important since your work is very valuable due to tracing and analysing all of the N budget components and, as far as I know, is probably closest to fully close the N budget. ***Answer****: We sincerely thank the referee for their thoughtful and encouraging evaluation of our manuscript. We are pleased to hear that the complexity and scope of the dataset were clearly conveyed and that the manuscript was found to be both interesting and enjoyable to read. We appreciate the suggestion to further strengthen the technical aspects of the discussion by keeping it more quantitative. In response, we have carefully reviewed the discussion and incorporated additional quantitative comparisons where relevant, particularly where assumptions were made based on the data. We agree that this enhances the clarity and robustness of the conclusions and supports the value of our attempt to comprehensively trace and quantify all components of the nitrogen budget.*
Specific comments
1. Title – is very complex and difficult to follow, containing a conclusion, which is discussed in the manuscript, but not fully sure, I would suggest a simplification and more generalisation, like eg. Comparison of N balance in integrated and organic farming: 15N tracing approach with analysis of

all N-compounds (...) - the title should rather contain a method applied and an aim of the study, not the main conclusion

Answer: *Thank you for your comment on title. We agree that the title was complex and have revised it to better reflect the aim and methodology of the study, and management history of the study in line with the recommendation. We replaced the previous title with (Lines 1-3):*

"Effect of preceding integrated and organic farming on ^{15}N recovery and the N balance, including emissions of NH_3, N_2O, N_2 and leaching of NO_3^-"

2. L32 units are missing for IF (-8 ± 15) – not clear what this value means

Answer: *Thank you very much for pointing this out. We have added the missing units (kg N ha^{-1}) to the value for IF to clarify its meaning. The revised sentence now reads as follows (Lines 32-33):*

"Due to the higher productivity, the cumulative N balance across all cultivation period was neutral within the limits of the measurement uncertainty for IF (-8 ± 15 kg N ha^{-1})…"

3. L 34 as above N balance (48 ±14).

Answer: *We thank the referee for pointing this out. We have added the missing units (kg N ha^{-1}) to the value for to clarify its meaning. The revised sentence now reads as follows (Lines 35-36):*

"The cumulative positive N balance (48 ± 14 kg N ha^{-1})…"

4. L 72-74 "The only method for in-situ measurement of N2 is the 15N gas flux method" – this is not true because also natural abundance isotope analyses of N2O can be used to quantify N2O reduction and hence – calculate the N2 flux (please check: https://bg.copernicus.org/articles/14/711/2017/, https://bg.copernicus.org/articles/17/5513/2020/). The method has of course its limitations, but 15NGF also has (e.g. the high detection limit and short time of possible measurements after tracer application).

Answer: *Thank you very much for this important clarification. We agree that the ^{15}N gas flux method is not the only in-situ approach available for estimating of N_2 emissions. As correctly noted, natural abundance isotopologue analyses of N_2O can also be used to infer N_2 production via N_2O reduction, as demonstrated by studies such as Lewicka-Szczebak et al. (2017 and 2020).*
We have revised the statement in the manuscript to reflect this and to avoid overgeneralization. The revised sentence now reads as follows (Lines 73–77):

"Available methods for in-situ measurement of N_2 fluxes, include natural abundance isotope approaches (Lewicka-Szczebak et al. 2017, 2020) and ^{15}N labelling (Micucci et al., 2023). The ^{15}N gas flux method (^{15}NGF) is a well-established approach for quantifying N_2 losses and considering this loss pathway together with the more easily accessible pathways enhances our understanding of N allocation in agroecosystems (Kulkarni et al., 2017; Friedl et al., 2020; Dannenmann et al., 2024)"

6. L 167-168 It would be good to describe the preparation methodology, which peripheral was used, which masses were measured, what was the detection limit and precision of the measurements.

These are very demanding analyses, so these details are necessary. Please add a citation of the preparation method applied.

Answer: *Thank you very much for your comment, and we appreciate the suggestion to provide more detailed methodological information. Here we have provided full information (Lines 180-187) :*

"Gas samples were analyzed using an isotope ratio mass spectrometer (Isoprime PrecisION, Elementar UK Ltd., Stockport, UK), coupled to an isoFLOW GHG GasBench (Elementar UK Ltd.). This setup allows for subsampling of gas volumes (30 µL) for ^{15}N-N_2 analysis by measuring m/z 28, 29, and 30, and for analysis of ^{15}N-N_2O after cryogenic pre-concentration of N_2O of the remaining vial content by measurement of m/z 44, 45, and 46. Gas handling and preparation followed the protocol described in Arah, (1997); Stevens and Laughlin, (2001b); Spott et al. (2006). In-run uncertainty, i.e., the standard deviation determined from repeated analysis of reference gases for isotope ratios r^{29}, r^{30}, r^{45} and r^{46}amounted to 1 10^{-6}, 5 10^{-7}, 7 10^{-5}, and 4 10^{-5}, respectively. At the enrichment of the fertilizer added to the mesocosms (85% at), this standard deviation values relate to 87 µg N_2-N m^{-2} h^{-1}."

8. L 325 "52% for OF and for IF (35%)" – the bracket should be removed.

Answer: *Thank you very much. We have removed the brackets, and the sentence now reads as follows (Line 342):*

"During green rye cultivation, the ^{15}N recovery in the soil was 52% for OF and 35% for IF…"

9. L 433-443 You discuss the possible N2 flux underestimation as the missing component of your N-balance. It would be interesting to make some estimations with real values to check this theory in practise. Eg. if we assume the 50% of N2 underestimation (as literature data suggest) will this really be sufficient to close the N budget? Just looking at the fluxes, I think it isn't. How large should be the N2 flux really to fill the missing budget? Would this amount be realistic?

Answer: *Thank you for this valuable comment. Based on our N balance, the unrecovered N was 15 kg N ha^{-1} for OF and 7 kg N ha^{-1} for IF. Our measured N_2 fluxes were approximately 1.0 kg N ha^{-1} for both treatments. Assuming a 50% underestimation (as suggested in the literature), the actual N_2 fluxes would be 2.0 kg N ha^{-1}, still leaving a gap of 14 kg N ha^{-1} for OF and 6 kg N ha^{-1} for IF. This means that method-inherent underestimation of the N_2-flux cannot close the balance, however we could detect significant fluxes only for approx. two weeks after fertilizer application. Therefore, we were not able to assess the influence of rewetting events on N_2 emissions, which may significantly contribute to N_2 emissions. In addition, some part of the imbalance may be due to underestimation of NH_3 emissions. To take up this suggestion, we revised and rearranged the text to (Lines 459-474):*

"… Despite the amount of N_2 losses quantified in this study agreeing with the past studies, the direct field measurement of N_2 fluxes in addition to all other relevant loss pathways did not result in a closed ^{15}N balance, with the average imbalance being 15 and 7 kg N ha^{-1} for OF and IF, respectively. While some part of the unrecovered ^{15}N may be due to an underestimation of NH_3 loss, N_2 emissions may be underestimated as well (Yankelzon et al., 2024a). Heterogeneous ^{15}N distribution in the microplot soil resulting from surface or slit application of the slurry and $^{15}N_2$ diffusion and storage in subsoil layers contributes to the underestimation of N_2 flux rates. These complications associated with ^{15}N labelling were discussed in previous studies which indicate that fluxes may be underestimated by up to 30-50% (Vanden Heuvel et al., 1988; Arah, 1997; Well et al., 2018; Well et al., 2019; Friedl et al., 2020; Micucci et al., 2023; Dannenmann et al., 2024). Even assuming a 50% underestimation, N_2 emissions after fertilization are in the range of 2 kg N ha^{-1}, indicating that the method-inherent potential underestimation alone cannot close the N-balance.

However, the short coverage of the direct N_2 measurements, which is due to the short period of time during which the isotopic enrichment of the N pool subject to denitrification is sufficiently high so that the N_2 flux can be detected, may be an additional source for underestimation. Consequently, we cannot exclude that additional ^{15}N in N_2 is emitted in the months following fertilizer application, particularly during rewetting events or towards the end of the growing season when plants decrease their water uptake and water content increases, as observed by Almaraz et al. (2024)."

10. L 450 "ratio in this study ranged from 0.01 to 1.00" to 1? Is this a mistake? this would mean no N2, only N2O - I think you do not have such case, N2 flux is always much higher than N2O flux

Answer: *Thank you for pointing this out. After revising the data, we confirm that the maximum $N_2O/(N_2O + N_2)$ ratio was approximately [0.01 to 0.45], not 1.00. The text has been corrected to reflect this and the figure as well. The sentences now read as (Line 301 and line 480):*

"The ratio of total N_2O : (N_2+N_2O) showed a similar progression over time for both sites, ranging from 0.01 to 0.45."

"The N_2O : (N_2 + N_2O) ratio in this study ranged from 0.01 to 0.45 across both sites using different slurry application techniques….."

11. L 519 – 522 Did you try to extrapolate these N2 and N2O losses? It is possible to try some extrapolation and asses if this could explain the missing 15N? Eg. Assuming theoretical values of eg. half detection limit for the further period (after these losses can be detected) ? Would this be significant in the N budget change?

Answer: *Thank you for this comment. We agree that such extrapolation is useful for N_2 as the magnitude of these emissions is relevant for the budget, but cumulative N_2O emissions are much smaller. In addition, please note that we indicate that unrecovered N_2 is not the only reason for an imbalance in the N budget (NH_3 emission, other uncertainties as explained in subsequent section). For this reason, we revised the manuscript as follows (Lines 550-560):*

"The only measurements that don't cover the whole cultivation period are those of N_2O and N_2, suggesting that underestimation of these N losses due to coverage of measurements of only a fraction of the whole cultivation period could explain the unrecovered N losses. Since N_2O emissions are approximately a factor of 10 lower than N_2 emissions (Scheer et al., 2020), N_2 emissions may have contributed the main part to the unrecovered losses. Assuming a background N_2 emission rate of 87 µg m^{-2} h^{-1}, which is equivalent to an emission if measured isotope ratios are increased by one standard deviation compared to the background, 0.7, 2.5 and 3.7 kg N_2-N ha^{-1} are released during green rye, maize and ryegrass cultivation, respectively. Such a background emission together with slightly underestimated NH_3 emissions could explain the unrecovered N_2 losses for IF. Overall recovery for OF was close to or lower than that of IF, suggesting that for the OF, additional N_2 was emitted during the cultivation period, which could be due to more frequent denitrification events caused by higher soil bulk density (Table 1, Luo et al., 2000; Hamonts et al., 2013)."

12. 547 – 549 "reduction of uncertainty for determination of (…), N2 and N2O emission is not in view" - this is not fully true, because there are some ideas of enhancement of the 15NGF for in situ measurements (see https://link.springer.com/article/10.1007/s00374-024-01806-z) , so that maybe better sensitivity for N2 can be attained, but with large costs and efforts.

Answer: *We sincerely appreciate the reviewer's insightful feedback and for directing us to the recent methodological advancements by Eckei et al. (2025). We agree that the improved 15N gas flux method (^{15}NGF+), which combines 15N labelling with helium-oxygen flushing to reduce atmospheric N_2 background, represents a significant step toward enhancing sensitivity for N_2 detection in field-scale studies. Their work demonstrates that lowering the N_2 background to <2% enables more precise quantification of N_2 and N_2O fluxes, particularly when paired with production-diffusion modelling to account for subsoil processes.*

However, as Eckei et al. (2025) emphasize, practical challenges remain, including the high technical complexity, specialized equipment requirements, and labor-intensive protocols (e.g., prolonged gas flushing, isotopic analyses, and model corrections). These constraints currently limit the method's widespread adoption for routine monitoring or large-scale assessments. Consequently, while ^{15}NGF advances our capacity to study denitrification dynamics, its application remains resource-intensive and context-dependent, with unresolved uncertainties in scaling results across heterogeneous field conditions.

We have revised our statement in the manuscript, which now reads as follows (Lines 582-590):

"Following the same line of argumentation, reduction in the uncertainty of the direct measurement of N balance components, i.e., losses through leaching, NH_3, N_2 and N_2O could help resolving differences more accurately. While recent advancements, such as the improved ^{15}N gas flux method (^{15}NGF+) demonstrate potential for enhanced sensitivity in quantifying N_2 emissions under field conditions (Eckei et al., 2025), such approaches are expensive and technically challenging which will delay their use in studies targeting complete N balances. Since the same applies to significant reduction of uncertainty in determining leaching losses, NH_3 volatilization, and N_2O emissions, it appears like these loss pathways were not markedly influenced by management history given the current measurement frameworks."