

General comments

The article examines the nitrate dynamics in an agricultural watershed in the USA using nitrate isotope techniques combined with other tracers. The paper is interesting and the overall quality is very good. However, there are some points, particularly related to the assessment of the biogeochemical processes, that need to be addressed prior to publication.

Scientific comments

Page 3 lines 65-70: My understanding is the hypothesis regarding the range of $\delta^{15}\text{N}$ values expected from fertilizers comes from literature (Kendall, 1998) although the local source values would better support the hypothesis. Is there some additional evidence that these values are expected in the study area apart from the global dataset of Kendall (1998)? In addition, and in relation to the H1, it is not very clear why the expected range for the $\delta^{18}\text{O}$ of NO_3 values in the study area would be between +15 and +25 ‰ although it is stated later in the text that the fertilizers applied are ammonium-bearing. Would it make sense to adapt the H1 to these types of fertilizers instead of the nitrate-bearing ones?

Page 3 lines 70-75: The authors use literature data ($\delta^{18}\text{O}$ of H_2O , $^{36}\text{Cl}/^{35}\text{Cl}$ and $^{87}\text{Sr}/^{86}\text{Sr}$) to support the H1. However, I think it would be necessary to mention that somehow in the text, particularly in point (2), and also explain what would be added value by combining nitrate isotope with these tracers.

Page 5 Lines 118-121: Nitrate concentrations are already reported in the paper of Frisbee et al. (2022). Are these values same or different compared to those reported in this paper? Were these nitrate values used for the analysis of water samples for ^{15}N ? In addition, the authors mention that the sites were sampled in October – November 2015. When were the samples analyzed for ^{15}N in relation to the sampling period? Preservation measures should be better explained.

Page 8 Lines 169-171: As mentioned in the previous comments is there some additional evidence that supports this statement (i.e., that the endmembers reported by Kendall (1998) and Kendall et al. (2007) are representative of the study area)? Because this is a global dataset.

Page 9 Line 211: I would recommend removing this sentence because I do not really see the meaning behind presenting an average value for the whole river.

Page 10 Lines 232-234: what does "different sampling times" refers to?

Page 13 Lines 251-257: Given that the watershed is agriculture-dominated I do not see any meaning in including a graph depicting the correlation between $\delta^{15}\text{N}$ and land use within the contributing area of each sampling site. However, given that point sources related to N-sources may be important I would recommend exploring the presence of these activities per site contributing area.

Page 13 Lines 259-267: The authors examine the relationships between variables to investigate the processes. I would recommend including plots of $\delta^{15}\text{N}$ vs NO_3 and vs DO even in the appendix despite the occurrence or non-occurrence of significant correlations between them.

Page 17 Lines 305-306: I think the authors jumped into the conclusion of denitrification influence in those samples prior the discussion part.

Page 17 Lines 322-324: But WR5 is point is plotted far from the fertilizers range in Fig. 6. And given that the watershed is agricultural I suspect that the same fertilizers are applied in it, why isn't point WR2 (or WR4) used a reference point in this regard?

Page 17 Lines 325-329: Tile drainage is expected to create fast flowpaths and short residence times which may limit denitrification instead of modifying the original $\delta^{18}O$ of NO_3 signal which most likely is the result of nitrification. So is it possible that the sites influenced by tile drainage are indicative of nitrification?

Page 19 Lines 340-342: What is the $\delta^{15}N$ values of TRB1? Does the mixing of WR6 and TRB1 justify the $\delta^{15}N$ of WR7?

Page 19 Lines 361-364: WR2 and WR4 sites are the ones closer to soil signatures although partly overlapping with the manure range. How is this explained? In page 13 Line 253 the authors mention that WR2 results suggest soil as a potential contributor. Is it possible that other animal breeding facilities may be responsible for ^{15}N enrichment observed in other sites?

Page 20 Line 390: Not clear how these numbers (20-25‰) were derived.

Page 20 Lines 394-397: Based on Denk et al. (2017, <https://doi.org/10.1016/j.soilbio.2016.11.015>) nitrification may have an opposite isotope effect depending on the nitrification step. For example, $NH_4 \rightarrow NO_2$ leads to -29.6 ± 4.9 ‰ whereas $NO_2 \rightarrow NO_3$ leads to $+13.0$ ‰. Maybe this needs to be stated in the text. Based on this please also check Lines 411-412.

Page 20 Lines 400-403: But WR1 falls along the denitrification line.

Page 21 Lines 412-413: The statement about WR3 is very generic and should be complemented by evidence.

Page 21 Lines 422-423: Is it possible that the denitrification signal as reflected in the slope can be influenced by the extend (progress) of denitrification?

Page 21 Lines 433-435: does DO confirm this hypothesis or is it possible that denitrification is occurring at the interface with the sediments? Similarly in Lines 441-442 do the physical-chemical conditions support this statement?

Page 26 section 4.7: Is it possible that flood events contribute towards reduced denitrification? And if this is the case is it expected that this has influenced the values recorded in the study area and to which extent?

Technical comments

The abbreviation "IN" (and other abbreviations) used throughout the manuscript to refer to Indiana (USA). While this may be familiar to U.S. readers, it may not be immediately clear to an international audience. I recommend defining it at first use (e.g., "Indiana (IN), USA") or using the full state name consistently throughout the text. The same applies to other State abbreviations.

Page 3 Line 84: The authors mention HDW, UWR and MWR for the first time. Although it is clarified what these abbreviations refer to later in the text, I would recommend clarifying it here.

Page 3 Lines 85-90: The authors mention the point sources of pollution which might be important in terms of influencing the nitrate isotope signals in the watershed. I would recommend including them in figure 5.

Page 8 Line 168: The term "denitrifier method" is used, which typically refers to the biological reduction of NO₃ to N₂O using cultured bacteria. However, for clarity and reproducibility, I recommend explicitly stating that a biological denitrifier method was applied and, if possible, citing standard references (e.g., Sigman et al., 2001; Casciotti et al., 2002).

Page 9 Lines 201-205: the authors present the minimum and maximum values twice in the text. Actually there is some inconsistency between the minimum values presented (6.7 ‰ vs 6.2 ‰). I would recommend presenting these values once.

Page 9 Line 206: TRB1 refers to tributaries. However, in the figures only the number is used to represent the tributaries' sites. I would recommend naming using the same coding (e.g., TRB1 instead of 1) in the figures or explaining it in the caption of the figures.

Page 10 Lines 217-220: For consistency purposes, I would recommend using past tense for presenting the results. Because sometimes present tense is used.

Page 10 Lines 235-237: I would recommend moving this statement to discussion.

Page 10 Lines 238-244: I would recommend including a figure of boxplots for each river part in the appendix so to be able to clearly see the spatial variability pattern. Because it is difficult to trace by the spatial interpolation maps.

Page 13 Line 252: In Figure 6 there is no Fig6A and Fig6B.

Page 13 Lines 269-279: Given that some similar plots were presented in the paper of Frisbee et al. (2022) (e.g., Fig 7C and 7D compared to Fig. 8 and 9 of Frisbee et al.) I would recommend either referencing them in the captions of the figures or removing them and maybe stick only to the relationship of the literature dataset with nitrates, which was not covered in the previous paper. The same applies for Figure 8 in relation to SO₄.

Page 15 Lines 288-299: In a similar way the presentation of these results should be in relation to nitrate isotopes. If the authors consider the information obtained from these tracers important even outside their relationship with nitrate and nitrate isotopes, then it should be presented as literature data and findings of the previous paper. However, even in the latter case it should be clear how it complements the H1 investigation.

Page 17 Line 303: SO₄ and δ¹⁵N, formatting issues (superscripts and subscripts).

Page 17 Lines 309-311: I would recommend removing this sentence because it is already stated in the beginning.

Page 20 Lines 375-384: The paragraph largely summarizes general information on δ¹⁵N values of fertilizer sources and associated fractionation during assimilation, which reads more like background material. I suggest moving the theoretical aspects (literature ranges and general processes) to the Introduction and retaining only the parts directly used to interpret the study results in the Discussion.