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Wickramasinghe and co-authors have presented an interesting manuscript that investigates sources and processes that control riverine nitrogen fluxes in a large agricultural watershed with mixed land use in Indiana, USA. The authors have obtained surface water samples from 18 mainstem and 6 tributary sites under baseflow conditions in the fall of 2015 (e.g., more than a decade ago) and present a very comprehensive data set for their surface water samples that includes amongst others nitrate concentrations, nitrate fluxes, nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios of nitrate, as well as less commonly used tracers for assessment of nutrient-containing fluids such as $^{36}Cl/Cl$ and $^{87}Sr/^{86}Sr$. The manuscript addresses an important environmental problem, namely nutrient loading of aquatic ecosystems in large agricultural watersheds and is based on a very solid data set from 24 surface water sampling sites using a combination of established techniques and more novel tracers. Hence, the manuscript is in my view highly suitable for Biogeosciences and presents novel ideas that may be of considerable interest for the readership of the journal.

The current version of the manuscript contains, however, several major deficiencies that should be addressed prior to reconsideration of this manuscript for publication. They include the following:

- The authors appear to suggest that the isotopic composition of select nitrogen inputs to the agricultural system, such as nitrate from atmospheric deposition or nitrate-containing synthetic fertilizers may percolate through the agricultural soil-plant system without changes of the isotopic composition of these sources. Research throughout the last three decades has clearly shown that this is (almost) never the case. Repeated soil nitrogen cycling processes including uptake, ammonification, nitrification and potentially denitrification not only reset the oxygen isotope ratios of nitrate within soils, but usually also cause a shift to higher $\delta^{15}N$ values in soil seepage water even in agricultural systems that are dominated by input from only synthetic fertilizers. Hence, this study would have greatly benefitted from sampling seepage water nitrate below the soil zone (e.g. tile drains). If such data exist, I strongly recommend adding them to the manuscript.
- The overarching hypothesis H1 is, in my view, very poorly chosen for the following reasons: a) The cited range of $\delta^{18}O$ values of +15 to +25 ‰ (line 69) is only valid for nitrate-containing synthetic fertilizers but as outlined above, this isotopic composition never percolates conservatively through the soil-plant system. b) Nitrate-containing products are never the sole source of synthetic fertilizers in agricultural watersheds, and the authors indicate that this is also not the case in the study area (see lines 319-322). Hence, the key hypothesis that is tested appears very flawed and should be re-evaluated.
- A very helpful indicator for the approximate level of anthropogenic nitrogen (N) inputs into the watershed is the number of humans living in the catchment (as a proxy for N inputs via wastewater effluents) and the number of animals (e.g. chicken, pigs, cattle) as a proxy for manure produced and likely applied to agricultural fields. The authors should enhance their text on lines 85-89 by providing this additional information. It would be important to know how many of the 400 wastewater treatment facilities release their effluent directly into the studied riverine systems, and what the treatment levels are (e.g., primary, secondary, tertiary). This

additional information on the major anthropogenic N sources in the watershed has the potential to greatly improve the manuscript.

- One of the key findings of the manuscript is that the isotopic composition of riverine nitrate is markedly affected by denitrification and in the abstract the authors state "… we infer that denitrification occurs in the river or subsurface rather that in fields or riparian zones". In my view, the data base for determining where denitrification occurs in this manuscript is weak and the authors admit that distinguishing the locations where denitrification occurs was beyond the scope of this study (line 615). For assessing "in-river" denitrification the authors could have used the dissolved oxygen data to reveal whether denitrification in the water column is even feasible or whether benthic denitrification needs to be invoked. Again, this study would have greatly benefitted from sampling seepage water nitrate below the soil zone (e.g. tile drains) to prove that the soil zone is not an area of significant denitrification. And finally, adding some information on the extent of denitrification in aquifers would be desirable if such information exists given that the study was conducted during baseflow conditions. Figure 4 list % N loss in yellow bars, but I did not find much discussion of these data in the manuscript.
- And finally, any information on the extent of groundwater contributions to riverine flows in the various portions of the watershed would be helpful, given that sampling occurred during baseflow conditions. Based on the various tracer studies previously conducted, one would hope that some of this information is available. Also, information on how deep the groundwater table is typically below the agricultural fields would shed some insight on the potential connectivity between groundwater and surface water in the study area.

Apart from these major issues, there are a number of technical decencies throughout the manuscript that should be addressed in a revised version of this manuscript including sloppy to or incorrect use of stable isotope terminology. A list of minor suggestions is provided below.

From a technical viewpoint the manuscript is quite good. The high-quality data are well presented in effective Tables and Figures that are of good quality. Previous literature is extensively considered and well cited although some similar regional riverine nitrogen studies appear to have been overlooked (e.g., Kruk et al. (2020): Tracing nitrate sources with a combined isotope approach ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$ and $\delta^{11}B$) in a large mixed-use watershed in southern Alberta, Canada. – Science of the Total Environment, 703: article 135043). The majority of the findings are reasonably well supported by data, although some findings remain rather speculative.

At the end of the abstract, the authors state as a key conclusion that "… N dynamics in large agricultural watersheds are complex …". This is neither a new nor a very innovative finding. Based on the experience made in this study, the authors should make a clear statement to what extent it is possible to accurately identify the sources of riverine nitrate given that the isotopic compositions of riverine nitrate have been modified by denitrification. Also, a clear list of suggestions of what should be improved in similar future studies would be very helpful, so that the key unresolved issues can be resolved in future studies. If that can be done, and the issues listed above and below can be resolved, then this manuscript may become of considerable interest for the readership of Biogeosciences.

Minor technical aspects that should be addressed:

Line 37: are these health effects due to excessive nitrate in drinking water? If so, that should be stated.

Line 46: Given that isotope ratios are analyzed, I suspect you deal with 4 stable isotopes: $^{15}N/^{14}N$ and $^{18}O/^{16}O$. Same issue in lines 165 and 168 and elsewehere.

Line 66: do you mean $\delta^{15}N$ value of dissolved nitrate in water?

Line 67: when you mention "fertilizer", specify whether you only mean synthetic fertilizers, or also manure?

Line 68: +7 ‰ appears far too high if you refer to synthetic fertilizers only.

Line 75: Why is this not section 2 = study area?

Line 88: How many of these wastewater treatment plants release their effluent directly into the river?

Line 151: from which water depth was the water pumped?

Line 168: provide a reference for the denitrifier technique and explain a bit more what it is (e.g., which measurement gas is used etc.). Also, how were the measurements normalized to the international measurement scale?

Line 177: what geologic origins of N compounds do you have in mind? I do not recall that this topic is addressed later in the manuscript.

Line 230: for which dissolved constituents are these concentrations and isotopic compositions?

Page 12, figure caption: explain what the yellow bars are.

Line 262: You cannot enrich a delta value since it refers to an isotope ratio. If you use "enrichment", state which compound is enriched and in which isotope (e.g., either ^{15}N or ^{14}N). Same in line 265 and various other occasions throughout the manuscript.

Page 15, Figure 6: make the denitrification line more clear and visible, e.g., by not using yellow.

Line 303-5: some formatting issues

Line 312: $\delta^{15}N$ value rather than "concentrations". Also, in line 68 the range was up to +7 ‰, but here it is up to +5 ‰, which is inconsistent. In my view, both values are too high.

Line 360: were nitrate concentrations and isotope compositions of nitrate in any of these discharges measured?

Line 367: you cannot fractionate a delta value and hence this heading should be revised; si,milar issues elsewhere throughout the manuscript.

Line 381: isotope fractionation during which process?

Line 409: delete delta sign: enriched in ^{18}O

Line 434: do your dissolved oxygen measurements indicate low enough DO levels to allow for water column denitrification?

Line 476: Also, many groundwater studies have identified denitrification. Should this have not also been mentioned?

Line 477: Do you mean “locations” or “zones” of denitrification. Denitrification is a process, not a source.

Line 480: delete “subsurface”. Aquifers are always in the subsurface.

Line 587: It is more than 10 years that the original samples were obtained. Has any of this desirable repeat sampling occurred in the meanwhile?