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Interactive comment on "Diel patterns in nitrate concentration suggest importance of microbial pathways for in-stream processing" *by* Jan Greiwe et al.

Anonymous Referee #1

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Summary

This paper examines patterns and sources of diel variation in stream NO3 concentration along a lowland river in Germany. The authors show that diel patterns of stream NO3 concentration vary over the growing season, yet most days show similar diurnal oscillations. Further, by combining different statistical techniques, the authors convincingly show that diel patterns are mostly driven by in-stream processes. Finally, the authors analyze diel and seasonal patterns of several environmental variables to discuss which in-stream process is driving diel NO3 cycles.

General comments

Printer-friendly version

Discussion paper

This paper makes a significant contribution to watershed and stream ecology through its assessment of patterns and controls of diel variation in stream NO3 concentration. However, I have some major issues that need to be addressed. All comments are made in the spirit of increasing the potential impact of this interesting research.

1. While most of the findings presented in the paper are original and compelling, the conclusions raised from them are sometimes speculative and inaccurate. For instance, the authors concluded that "the magnitude of microbial NO3 processing may be large compared to plant uptake", but they did not measure any in-stream process (GPP, denitrification, nitrification) nor NO3 uptake rates. Hence, it is impossible to know, based on their data and results, which in-stream process was contributing the most to NO3 uptake rates over the study period. Similarly, they stated that "diel patterns in NO3 concentration suggest the importance of microbial pathways for in-stream processing", but the 70% of diel patterns seem to be driven by photoautotrophic uptake (not microbial pathways). My suggestion is to focus the objectives and conclusions on the compelling results and only speculate about the relative importance of different in-stream processes in the discussion.

2. I missed some results regarding lateral inputs. In the discussion, the authors mentioned that lateral inputs may not affect diel NO3 patterns because they did not observe diel variations in discharge. While I agree with this statement, lateral inputs should be included in the hypothesis, methods and results (see Flewelling et al. 2014 or Lupon et al. 2016). Also, the authors mentioned that there was a tributary entering to the upstream reach. Does the tributary show diel variation in NO3 concentration? How this may influence stream NO3 concentration in S2?

3. I was confused by some of the approaches used. For instance, what is the point of the mass balance? It has many uncertainties (e.g. groundwater, tributaries) and the results derived from it are difficult to interpret. My suggestion is to delete this whole section. Instead, I will focus on analyzing (i) if all sites showed similar seasonal patterns in diel NO3 variation (i) if the effect of longitudinal propagation differed across

clusters; (iii) if there was a lag time between diel patterns of drivers and stream NO3 concentration (see my specific comments for more info on this regard).

4. The discussion is a little bit puzzling. My suggestion is to delete all sub-headings and focus on how different sources shape stream NO3 concentration. You can start with a paragraph discarding longitudinal propagation and lateral inputs as factors causing diel NO3 patterns. Then, move to the most obvious process: photoautotrophic uptake (clusters A-B) and how it varies over time depending on light, temperature, discharge. Finally, you can suggest potential explanations for the other clusters: denitrification (cluster C), nitrification (cluster D), storm flow (cluster F).

5. While I like the figures, most of them (and their captions) need some improvements (see my specific comments). Also, I missed a figure showing the raw data (i.e. diel patterns of NO3, discharge, light and temperature over the whole study period). This figure is key to understand some of the points discussed (e.g. no diel variation in discharge); and it will be very helpful to the readers.

Specific comments

Ln 1. The title is a little bit speculative. Perhaps something focused on in-stream processes vs longitudinal propagation would be better.

Ln 21. This sentence is not accurate. What your results are telling us is that different in-stream processes might generate diel patterns in NO3 concentration, and that the relative importance of such processes may vary depending on the season.

Ln 37-44. This rationale is correct, but does not engage with the objective of the paper (i.e. you don't quantify any in-stream process). My suggestion is to delete this part and merge this paragraph with the following one.

Ln 46-55. As it occurs with the previous paragraph, this section goes beyond the objectives of the paper. My suggestion here is to shorten it to something like "Previous studies have suggested that seasonal patterns of diel variation in stream NO3 concentration are related to in-stream photoautotrophic uptake (refs). Due to photosynthetic light requirements, photoautotrophs take up NO3 mostly during the day, with minimum and maximum NO3 concentrations occurring at X and Y (refs). However, there is evidence that diel variation (. . .)."

Ln 67. What is the difference between the two hypothesis? They look exactly the same to me. Be explicit with the hypotheses you are testing and how you evaluated them (e.g. relevance of in-stream processes vs. other watershed compartments, such as downstream propagation or lateral inputs.

Ln 80. Just for curiosity, did you expect to observe differences between reaches or among sites? As it is written, it seems so; but you did not mention anything about that in the introduction nor discussion.

Ln 94. Longitudinal profiles were only used to validate the probe measurements, right? If so, I would simplify these sentences (i.e., "In addition, biweekly grab samples were collected at each site to validate probe measurements"). Also, it would be nice to show the uncertainty associated with these measurements.

Ln 97. How confident you are with your rating curve?

Ln 109. In my opinion, there is no need to use two travel times. I would use only nominal water residence time. However, the authors can easily convince me of the opposite.

Ln 111. Did you assume the same discharge at all sites? Is this assumption reasonable given the length of the stream section and the tributary? Also, why did you choose these widths?

Ln 115. I suggest to change the order of sections 2.3.1 and 2.3.2. First, you identified types of diel cycles; then, you investigated the processes involved in such patters. This suggestion also goes for the results section.

Ln 130. Did you analyze the relationship between the amplitude in diel variation of T, S, h and stream NO3 concentration? May be worth to try.

Ln 134. Sorry, I did not follow this rationale. Several studies have related Cobs or Cres with diel patterns of environmental variables. Is it really necessary to use the first derivate? Using Cobs or Cres will simplify the results.

Figure 2. I would only plot those cases when $r < 0.75$ because, as you mentioned, cases with low r are difficult to interpret. If you do so, then you can color the data based on clusters. Finally, the caption should define all the elements (X-axis, legend, dashed horizontal line).

Ln 156. So, lag times (those with $r > 0.75$) are close to zero, but different from zero. Is that right? How do you explain it? Is it possible, then, that diel variations are a combination of in-stream processes and downstream propagation? Relatedly, have you check if the lag times vary across clusters? This may partially explain some of the observed patterns.

Ln 161. I missed some information in this section. For instance, which cluster dominates in each site? Some of this info is available in Figure 5, but should be more clearly stated here. Also, move Figure 5 here.

Figure 4. This figure has a lot of information and it is difficult to digest. Some ideas that came to my mind to improve it: (i) Panels A-C can be a table (Table 1). If you do so, then you can add some statistical test (e.g. Wilcoxon test) to show if clusters had different environmental conditions. (ii) Panels E-G can also be a table (Table 2). Here, you can report, for each cluster and relation, the mean r, the IQR of r, and the proportion of cases that has a significant relation (p-value < 0.05, or $r > 0.5$). In this way, the reader will easily see in which clusters these relations were consistent over time. (iii) It will be nice to show if there was a relationship between seasonal patterns of environmental variables and diel NO3 variability. If so, you can make a new figure showing these relations (similar to Fig 6 Heffernan and Cohen, or Fig. 6 Roberts and Mulholland 2007).

Figure 5. Given that the sensors were not allocated in all sites at the same time, perhaps it is better to report the relative values (e.g. days cluster 1/days with measurements) for each month. Also, I guess that the lack of values in S1 from April to June is due to missing data. Finally, it will be better to show the results in bars (not areas), as months is a discrete variable.

Ln 241. I agree that cluster F enclosed a wide range of diel NO3 patterns and environmental conditions; and thus, may be a box with all the "weird" days (i.e. storms). However, cluster E looks more consistent in terms of diel patterns and they may be related to in-stream processes (i.e. nitrification). My point here is that, based on your data, you cannot discard any hypothesis rather than longitudinal propagation; at least for clusters A-E.

Ln 243. Another possible explanation is that there is a lag time between light inputs and NO3 uptake (see Heffernan and Cohen 2010 discussion). A cross-correlation analysis can be a good way to test if there was a decoupling between light and stream NO3 concentration at daily scale.

Ln 252. Seasonal changes in light inputs occur even if there is no forest (i.e. the duration, timing and amount of sunlight varies over the year). Also, there are seasonal changes in the N demand by plants (see Heffernan and Cohen 2010).

Ln 258. Yes, phosphorous limitation may affect NO3 uptake. However, the relation N:P of this streams is < 16; suggesting that there is N limitation. Perhaps you don't need to go that far here (sometimes is better to keep the discussion simple and straightforward). One sentence stating that other factors, such as seasonal changes in nutrient availability, photoautotrophs stoichiometry, or temperature may further affect diel NO3 cycles is enough to make your point here.

Ln 278. Here, we are mixing apples with oranges. On one hand, some studies showed that diel patterns of NO3 concentrations changed during late-summer and fall, and that this phenomenon may be related to in-stream nitrification (e.g. Laursen 2004, Lupon

2016). The causes of this phenomenon is, as far as I know, under debate. It may be due to higher DOC inputs, or due to changes in pH and temperature. Curiously, this phenomenon seems to occur at S2 in September. On the other hand, Lupon 2020 showed that in-stream processes may vary along rivers. This may explain, for example, why S1 and S2 showed different diel patterns in September, or why the three sites did not show the same seasonal patterns. I would separate this two stories in two paragraph; one focused on in-stream processes and another one focused on why the three sites behave differently.

Technical notes

Ln 11. “sites” instead of “locations”

Ln 23. Better to say “in-stream processes can significantly influence loads and concentrations of nutrients”. Further, Peterson et al. 2001 may be also a good, general reference for this sentence.

Ln 27: nitrogen (N)

Ln 27. Nitrate (NO3-). From hereafter, use NO3- instead of nitrate.

Ln 32. “Carbon dioxide”

Ln 47 (and hereafter). The proper name of this process is “photoautotrophic uptake”, not “autotrophic uptake” (nitrifiers are also autotrophs) nor “plant uptake” (mostly used for terrestrial systems). Also, the use of Ua made sense in Cohen’s papers, but not here. Use “photoautotrophic uptake” instead.

Ln 51. Nitpicking, but “microbial net depletion” sounds weird; perhaps “other in-stream processes”?

Ln 55. Same here. “Such diel variability in these other in-stream processes would cause. . .”

Ln 72. Technically, you are studying a stream section that is divided in two reaches.

Figure 1. The map should show the contributing catchment to S3. Also, I would delete the longitudinal profile, as you don't use this data in the current manuscript.

Ln 79-82. I would divide this sentence into two: one for each reach.

Ln. 80. Delete "and in this sense it (. . .) southwest Germany"

Ln 87. I missed some information about stream biotic compartments (e.g. emergent and floating macrophytes, algaes, biofilm). This is important to understand the role of photoautotrophic uptake.

Ln. 105. I would move this whole sentence to the introduction, when you state your expectations.

Ln 107. "patters, we determined (. . .) cross-correlation, which is (. . .)"

Ln 121. I understand why you named it "C residual". Yet, it may be more intuitive for the reader to refer it as "C corrected" or something like that.

Ln 129-141. Move this paragraph to the "Assessing the origin of diel nitrate variation" section.

Ln 137. This statement is not entirely true. Discharge can also affect in-stream processes (see Seybold and McGlynn 2016). Anyway, as I mentioned earlier, I would relate all environmental variables with Cres.

Ln 149. Nitpicking, but this heading does not seem right for the results. What about "Sources of diel patterns "?

Ln 152. Move this sentence to the methods section.

Ln. 168. Delete "a quarter of a period (0.5 travel time)"

Ln.169. Delete the whole sentence "Note that (. . .)."

Ln 171. Move everything related to drivers to another section and keep this one strictly to diel patterns characteristics.

Figure 3. Please, describe what the black dots and the shaded area represent (mean and standard deviation?).

Ln 214. Delete "However, (. . .) lag estimation."

Ln 223. What is the point of this paragraph? I might missed something. Do you mean that the observed diel pattern may be as a result of longitudinal propagation and in-stream processes?

Ln 241. "in-stream processes"

Ln 306. Clusters A and B, right?

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