Author’s response to comments by anonymous referee #1

We would like to thank referee #1 for taking the time to read our manuscript carefully and for providing very constructive feedback. The helpful suggestions will improve the quality of our manuscript. Our responses to the individual comments are shown in blue below.

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

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.

> Reply: We agree that our statements on the relative importance of biochemical processes require some assumptions and should not be presented as key findings. We are happy to shorten that section and shift the focus of this paper towards the role of in-stream and transport processes as suggested by referee #1 below.

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?

> Reply: Unfortunately, we lack information about diel patterns in the tributary. In a revised manuscript, we will discuss the potential impact of lateral inputs and diel variation in the tributary based on its size and the transformation of the concentration signal between S1 and S2 (the sampling points between which the tributary enters the main stream).

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).

Reply: We agree that the mass balance has many uncertainties. Considering our shifted focus, the mass balance will be less important for our main statements. Nevertheless, mass balances are important tools in hydrology and readers may expect to find it in our manuscript given the experimental setup. In order to provide a complete analysis we will include it as supplementary material. We are grateful for the suggestions regarding data analysis. In the original manuscript we already compare seasonal patterns (i) at the monitoring sites in figure 5. The effect of longitudinal propagation (ii) will be assessed for the different clusters in a revised manuscript by modifying figure 2 as suggested by referee #1 below. A lag between drivers and concentration (iii) was in fact observed and is evident in the clusters we found. While timing of irradiance and stream temperature was more or less constant throughout the year, the nitrate clusters reflect different lags relative to these drivers. We will revise the manuscript to make this more clearly.

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).

Reply: We appreciate this suggestion and will revise our discussion accordingly.

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.

Reply: The figures will be revised according to the comments below and a figure showing the raw data will be added.

Specific comments

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

Reply: We will change the title to "Diel patterns in nitrate concentration produced by in-stream processes".

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.

Reply: This sentence will be deleted.

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.

Reply: We agree that this section should be shortened and merged with the following.

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 (: : :)."

Reply: We agree that this and the previous paragraph include unnecessary information and will merge them in order to streamline the introduction.

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.

Reply: We thank referee #1 for pointing out that our hypotheses need improvement. Rethinking this, we decided to take up the suggestion by referee #1 below. This means we move the pattern identification to the front and then test possible explanations. Our research questions would then be: What patterns in nitrate concentration do we find in the studied system? Are these patterns produced by in-stream processes or transport? And, finally, how are they related to drivers of biochemical processes?

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.

Reply: In fact, we expected to see differences between the two reaches as a result of the revitalization measures in the downstream reach. However, during the course of the monitoring it became more and more obvious that it would be extremely challenging to separate these effects from other influences (e.g. groundwater, tributary). We think we should include these expectations in the introduction and state that we were unable to separate potential effects of the revitalization from other influences.

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.

Reply: The reference to longitudinal concentration profiles is, indeed, unnecessary and will be removed.

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

Reply: The rating curve could have more data points – as always - but is considered sufficient to provide the discharge corresponding to our water level measurements. The validity of the rating curve is limited to the range of the tracer injections and to the corresponding monitoring site (S3). Despite its uncertainty, our field rating curve certainly reflects reality much better than discharge data from a official gauging station tens of km upstream.

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.

Reply: Maybe using nominal residence time would be sufficient. However, nominal residence time strongly depends on channel geometry and discharge which both are subject to uncertainties. In fact, both independent approaches produce similar results, which increases our confidence in travel time estimates and we would like to keep both in the revised manuscript.

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?

Reply: In fact, our discharge measurements at S3 may not be representative for the entire stream section due to unknown contributions from the tributary (which we estimate based on visual judgement to account for a maximum 10 % of stream flow) and possibly groundwater. These uncertainties mainly impact our mass balances. In the remaining part of our analysis discharge or water level data from S3 is used to generally characterize discharge conditions.

Channel widths are roughly based on aerial imagery. However, channel geometry has further sources of uncertainty (e.g. distribution of water depth and discharge) that we were unable to accurately account for. For estimating nominal travel time, we therefore selected a generous range of possible stream widths and used independent travel time estimates from our tracer injection to validate our assumptions.

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.

Reply: We appreciate this suggestion and will restructure the manuscript accordingly.

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.

Reply: Good point. In order to assign clusters to drivers, it would be nice to show that these variables are not only related to nitrate concentration in terms of timing (which we have shown already) but also in terms of magnitude. We actually did that, but amplitudes of environmental variables were not clearly correlated with nitrate concentrations. We will mention this in the revised manuscript.

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.

Reply: We are indeed convinced that drivers of biochemical processes are only indirectly related to solute concentrations. If we e.g. assume irradiance as the main driver of photosynthesis and associated nitrate assimilation, we would expect the strongest effect on nitrate concentrations, when irradiance is strongest (ignoring that increasing irradiance may actually inhibit photosynthesis at some point). Or in other words, maximum irradiance would coincide with maximum rate of change (i.e. its first derivative) of the nitrate concentration. The idea that drivers influence the change of concentrations is also common in solute models, e.g. consider Hensley and Cohen (2016) for the case of nitrate or Grace et al. (2015) for the case of dissolved oxygen.

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).

Reply: Good idea. We will revise Figure 2 accordingly.

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.

Reply: We will include a comparison of time lags across clusters – which we did already but which was not included in the original manuscript. The interpretation of this finding is a bit challenging. It is clear that both processes (biochemical processing and transport) occur simultaneously as water travels downstream but it is not clear how exactly this produces the lags. If the signal was simply advected, lags should be at least as long as nominal travel time, probably longer (due to dispersion). Values clearly below travel time cannot be explained by advection. Lags may be influences by sudden changes in discharge conditions, e.g. a sudden flood wave may cause the downstream advection of a longitudinal pattern previously created by biochemical processes during a low flow period. However, our data do not show a clear water level threshold that separates in-stream control from transport control of diel nitrate patterns. We will add this aspect to the discussion.

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.

Reply: We thank referee #1 for the suggestion to also assess longitudinal stability/transformation of the clusters and will include this aspect in a revised manuscript.

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).

Reply: We agree that figure 4 contains much information. However, we are convinced that this information is more easily accessible to the reader when presented as figures rather than as tables. In order to make this information more digestible we suggest to split figure 4 into two figures, one showing daily values of environmental parameters and the other one showing daily correlations.

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.

Reply: We agree with the bars but not with the relative values. Relative values may suggest information were we actually have missing data. It is transparent for the reader if we keep showing absolute values. We will add an explanation to the caption that columns with less than 30 or 31 days are due to missing data.

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.

Reply: We agree that discharge during cluster E was not sufficiently different from clusters A,C and D to justify discarding in-stream processes. We will revise this section and also consider in-stream processes.

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.

Reply: To be precise, Heffernan and Cohen discuss the lag between photosynthesis (not instantaneous light availability) and nitrate assimilation. However, as mentioned above, a lag between light and assimilation is expected because light as a driver of assimilation influences the rate of change (i.e. the derivative) of both nitrate and dissolved oxygen concentration. We thank referee #1 for the suggestion to use cross-correlation to analyse this effect. The lag between variable diel patterns in nitrate concentration and comparatively stable diel patterns in drivers is also reflected in the results of the cluster analysis. We, therefore, do not see a real advantage in using a different methodology to show the same effect. However, we will refer to the lag between drivers and concentrations more explicitly in a revised manuscript.

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).

Reply: True, this information should will be added to the discussion.

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.

Reply: We agree and will revise this section accordingly.

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.

Reply: We agree and will revise this section to make it less confusing.

Technical notes

Ln 11. “sites” instead of “locations”

Reply: We will replace “locations” by “sites”.

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.

Reply: We will revise this sentence and add the recommended reference.

Ln 27: nitrogen (N)

Reply: We will revise the use of abbreviations throughout the manuscript.

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

Reply: s. above

Ln 32. “Carbon dioxide”

Reply: s. above

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.

Reply: We will check and revise terminology throughout the manuscript.

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

Reply: We will replace “microbial net depletion” by “other in-stream processes”.

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

Reply: s. above

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

Reply: We will use the terms “stream section” and “upper/lower reach” in the revised manuscript.

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.

Reply: The map will be revised accordingly.

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

Reply: The sentence will be divided.

Ln. 80. Delete “and in this sense it (: : :) southwest Germany”

Reply: This will be corrected.

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.

Reply: Information about biotic compartments will be added.

Ln. 105. I would move this whole sentence to the introduction, when you state your expectations.
Reply: This will be corrected.

Ln 107. "patters, we determined (: : :) cross-correlation, which is (: : :)"
Reply: This sentence will be simplified.

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.
Reply: Not sure, if 'corrected' better describes what we did. We suggest to call it "C diel".

Ln 129-141. Move this paragraph to the "Assessing the origin of diel nitrate variation" section.
Reply: This will be done as part of our restructuring of the manuscript.

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.
Reply: We agree that the phrasing may be misunderstood. We did not mean to exclude the impact of water level and discharge on biochemical process and will revise this section accordingly.

Ln 149. Nitpicking, but this heading does not seem right for the results. What about "Sources of diel patterns "?
Reply: The heading will be revised accordingly.

Ln 152. Move this sentence to the methods section.
Reply: The sentence will be moved to the methods section.

Ln. 168. Delete "a quarter of a period (0.5 travel time)"
Reply: This will be deleted.

Ln.169. Delete the whole sentence "Note that (: : :)."
Reply: The sentence will be deleted.

Ln 171. Move everything related to drivers to another section and keep this one strictly to diel patterns characteristics.
Reply: We are happy to do so, particularly as figure 4, to which these lines refer, will be split into two (s. above).

Figure 3. Please, describe what the black dots and the shaded area represent (mean and standard deviation?).
Reply: This information will be added to the figure.

Ln 214. Delete "However, (: : :) lag estimation."
Reply: This sentence will become obsolete anyway as the information necessary for the interpretation of figure 2 will be provided in the methods section.

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?
Reply: Here we attempt to interpret our findings regarding in-stream vs. transport control of diel nitrate cycles. The paragraph will be revised to make it more understandable.

Ln 241. "in-stream processes"
Reply: This will be corrected.

Ln 306. Clusters A and B, right?

Reply: Yes, this will be revised accordingly.

References

Grace, M. R., Giling, D. P., Hladyz, S., Caron, V., Thompson, R. M., and Mac Nally, R.: Fast processing of diel oxygen curves: Estimating stream metabolism with BASE (BAyesian Single-station Estimation), Limnol. Oceanogr. Methods, 13, e10011, doi:10.1002/lom3.10011, 2015.

Hensley, R. T. and Cohen, M. J.: On the emergence of diel solute signals in flowing waters, Water Resour. Res., 52, 759–772, doi:10.1002/2015WR017895, 2016.