Dear Dr. Battin,

Please, find below a point-by-point response (blue) to the reviewers comments (black). We thoroughly revised our manuscript according to the suggestions of the four referees.

Major changes include

- to shift the focus from individual biochemical processes towards in-stream vs. transport control on diel nitrate patterns
- restructuring the manuscript so that we now use the identified clusters as a starting point for further analysis
- substantial shortening of the discussion and reduction of speculations.

We hope that we addressed all comments satisfactorily and that our revised manuscript now meets the requirements for publication.

Best regards,

Jan Greiwe

Author's response to comments by anonymous referee #1

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: In concordance with the suggestions by referee #1, we shifted the focus of the paper towards in-stream vs. transport control on diel nitrate patterns and accordingly changed the title to *"Diel patterns in NO_3^- concentration produced by in-stream processes".*
>
> We also revised the conclusions accordingly (l. 318-331):
>
> *"In a 5.1 km stream reach of the river Elz in Southwest Germany we identified diel patterns in stream NO_3^- concentration, differentiated between in-stream and transport control, and analyzed how patterns were related to environmental conditions and potential drivers. We found a set of six clusters representing different characteristic diel NO_3^- patterns. Relatively small temporal shifts between adjacent monitoring sites indicated that NO_3^- concentration patterns were predominantly formed by in-stream processes and not by a transport of upstream NO_3^- inputs. Most patterns were characterized by a pre-dawn maximum and an afternoon minimum of varying intensity, and mostly the change rate of NO_3^- concentration was negatively correlated with global irradiance. We therefore conclude that these patterns were primarily produced by photoautotrophic NO_3^- uptake. However, we also found indications that other biochemical processes like nitrification and denitrification contributed to the formation of NO_3^- patterns. In depth interpretation and eventually quantification of process rates would require spatially distributed high frequency information on stream metabolism, e.g. dissolved oxygen concentrations, and on different N species, most importantly NH_4^+. Nevertheless, our analysis suggests that particular combinations of different in-stream processes may generate distinct diel NO_3^- patterns. A seasonal shift in patterns may then indicate shifts in the relative importance of the underlying processes. The clustering method used in this study proved useful for making the data set accessible for this kind of analysis and may be used as a blueprint for the analysis of other stream solutes."*

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: We added a section in the discussion that explicitly deals with lateral inputs (l. 254-273):
>
> *"Diel NO_3^- patterns may also be influenced by lateral inputs, including tributaries and groundwater interaction. The only surface tributary within the studied stream reach was between S1 and S2. It was initially considered negligible and therefore not accounted for. However, snap shot sampling on a hot day during low flow conditions revealed nitrate concentration to be twice as high as in the main stream. It is also possible that groundwater influx influenced NO_3^- concentration at the monitoring sites. In fact, NO_3^- levels in groundwater were higher than in stream water in the proximity of the upper reach and lower than in stream water along the lower reach (Fig. S3). Although the overall flow direction of groundwater was parallel to the stream, groundwater inputs might explain the increase in average NO_3^- concentration from S1 to S2 and subsequent decrease from S2 to S3 (Fig. S2). Previous research identified diffuse groundwater inputs as a considerable challenge for determining mass balances using paired high-frequency probes (Kunz et al., 2017). We were unable to separate the effects of groundwater inputs from a potential effect of increased NO_3^- removal in the lower reach due the revitalization measures.*
> *Although lateral inputs may have affected average NO_3^- levels, their influence on diel NO_3^- patterns was only marginal. In the upper reach, which received the tributary, diel NO_3^- patterns were mostly longitudinally stable, except for the deployment in September (Fig. 3). We therefore consider the influence of the tributary to be limited. Riparian groundwater interaction induced by evapotranspiration was suggested by Aubert and Breuer (2016) to explain a seasonal shift in diel NO_3^- patterns. Flewelling et al. (2014) showed that diel fluctuations in groundwater level and stream flow induced by evapotranspiration may be sufficient to produce measurable diel patterns in stream NO_3^- concentration. Groundwater inputs may not only directly affect NO_3^- concentrations but also alter stream chemistry, e.g., by introducing labile organic carbon which promotes heterotrophic processes (Lupon et al., 2020). In the present study, however, diel water level fluctuations were usually minimal so that we generally have little evidence for diel variability in groundwater influx."*

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: It is true that the mass balances have many uncertainties. We therefore removed them from the manuscript but, for the interested reader, show the distribution of concentrations at the monitoring sites in the supplementary material (Fig. S2). We addressed the suggestions made by referee #1 as follows.
>
> (i) Seasonal patterns at monitoring sites are compared in the new Fig. 3 (see below) and in the text (l. 177-180): *"In terms of cluster occurrence, a largely similar seasonal pattern was apparent at all monitoring sites, despite different numbers of recorded days. Cluster A dominated in May and again in October and was replaced by cluster B during the summer months from June to September. Both clusters usually formed continuous blocks of several days. Cluster C occurred occasionally throughout the*

season but preferentially in early summer, while cluster D and E mainly occurred in fall."

(ii) We tested if longitudinal propagation differed among clusters by including clusters in the revised Fig. 2 (Fig. 4 in the revised manuscript, see below). This is described in l. 196-199: *"In the lower reach, lags formed an evenly distributed point cloud. Within this cloud, Cluster D, E, and F only appear at above median flows. In the upper reach, time lags were concentrated towards the extremes, i.e. either close to zero or close to travel time estimates. Days with below median stream flow were mainly assigned to cluster B and those above median stream flow to cluster A."*

(iii) Time lags between potential drivers and nitrate concentration is apparent in the different clusters as timing of drivers was more or less constant throughout the year. We think that this topic is sufficiently addressed by the corresponding correlations and Fig. 6 (see below) (l. 215-220): *"In addition to different environmental conditions, we identified different relationships with potential drivers of diel cycles among clusters (Fig. 6). The correlation of δC_{diel} and S was positive in cluster D, negative in clusters A and C, and strongly negative in cluster B. Moderate correlations of δC_{diel} and T were found in cluster C (negative) and cluster E (positive). Correlations of δC_{diel} with h were weak and difference among clusters were less pronounced than with S and T. The relationship of C_{obs} and h was very variable and included both strongly positive and negative correlations. However, strong overlapping of boxplots in Fig. 6c and Fig. 6d indicated that variability within clusters was higher than among cluster."*

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: The discussion has been shortened and is now devided into 5 sections: "General patterns" (l. 232-241), "In-stream vs. transport control" (l. 242-253), "Lateral inputs" (l. 254-273), "Interpretation of diel patterns" (l. 274-316), "Conclusions" (l. 317-331). In the "Interpretation of diel patterns" section, we substantially reduced speculations and implemented the suggestions made above. It now reads (l. 274-316):

"Diel NO_3^- patterns with a maximum in the early morning and a minimum in the afternoon are usually explained by photoautotrophic NO_3^- uptake by primary producers (Nimick et al., 2011). This was also the largest group of diel patterns in our study including cluster A and B, jointly accounting for about 70 % of the data. In our study, the idea that such diel patterns reflect photoautotrophic uptake is supported by a strongly (cluster B) and moderately (cluster A) negative correlation between δC_{diel} and global irradiance. The higher amplitude of cluster B (Fig.2) suggests a stronger photoautotrophic NO_3^- uptake compared to cluster A. Consequently, the seasonality in cluster occurrence suggests that photoautotrophic NO_3^- uptake was strongest from June to early September when cluster B prevailed. In May and October the dominance of cluster A suggests reduced photoautotrophic NO_3^- uptake which may be due to reduced light availability in autumn or due to lower water temperatures and higher flow during both periods. The latter may have influenced photoautotrophic NO_3^- uptake via reduced light penetration through a higher water layer, via an increased volume of water on which the same uptake in terms of mass would have a smaller impact in terms of concentration, and via disruption of stream metabolism due to destruction of vegetation by flood events (Burns et al., 2019).

Patterns with a midday maximum such as those observed in cluster C have also been explained by photoautotrophic uptake in streams where timing of light availability changed seasonally with canopy development (Rusjan and Mikoš, 2010; Roberts and Mulholland, 2007; Rode et al., 2016). Although global radiation was comparatively intense during occurrence of cluster C and δC_{diel} was weakly correlated with global irradiance, this explanation seems unlikely in our river reaches, since banks are unforested and the seasonal occurrence of cluster C did not correspond to canopy development. Despite being most obvious, diel variability is not exclusively caused by photoautotrophic uptake and has been observed in other biochemical processes of the nitrogen cycle (Hensley and Cohen, 2020), such as nitrification (Warwick, 1986; Laursen and Seitzinger, 2004; Dunn et al., 2012) and denitrification (Christensen et al., 1990; Harrison et al., 2005; Cohen et al., 2012). The interplay of these processes can be regulated by oxygen availability (Rysgaard et al., 1994), i.e. nitrification and denitrification are expected to be most intense during oxygen maxima and minima, respectively. In addition, microbial processes may vary with water temperature fluctuations that propagate into the hyporheic zone and influence the rate of microbial processes (Zheng and Bayani Cardenas, 2018). Timing of nitrification and denitrification may also be shifted relative to photosynthesis and photoautotrophic uptake due to oxygen-dependency of nitrification and denitrification and due to travel time to reactive zones in stream sediments.

Considering that denitrification was found to be the dominant pathway of NO_3^- removal in some streams (Preiner et al., 2020; Heffernan et al., 2010), it seems possible that varying diel NO_3^- patterns are caused by variability in denitrification or nitrification rather than in photoautotrophic uptake. Following this line of thought, negative (cluster C) and positive (cluster E) correlations of δC_{diel} with stream water temperature suggest that nitrification and denitrification, respectively, may be the underlying processes. In that case higher light inputs during cluster C compared to cluster E (Fig. 5) may have caused higher photosynthetic oxygen availability and thus a dominance of aerobic nitrification over anaerobic denitrification. Diel patterns with peaks in the afternoon or evening such as those in cluster D have been observed by Hensley and Cohen (2020) during NO_3^- limitation, which was obviously not the case in the present study. Similar patterns to cluster D were also found by Aubert and Breuer (2016) and Flewelling et al. (2014) in streams subject to intense evapotranspiration which has been shown to influence hydrologic retention of NO_3^- (Lupon et al., 2016). Although diel water level fluctuations were usually minimal, this may have been the case during the persistent occurrence of cluster D at S2 after a prolonged dry period in September (Fig. 3).

These findings suggest that, despite a dominance of photoautotrophic assimilation, other processes contribute to the formation of diel NO_3^- patterns in the river Elz. These may be contrary processes like nitrification and denitrification and possibly also physical processes like diel variability in lateral inputs induced by evapotranspiration. The relative importance of these processes varies seasonally and is reflected in shifts of diel NO_3^- patterns. Although the distinct clusters identified in our analysis invite for speculation, in-stream NO_3^- processing is complex and processes may overlap and interact which makes unambiguous interpretation solely based on NO_3^- recordings challenging."

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 have been revised and an additional figure (Fig. 3) showing the raw data was added.

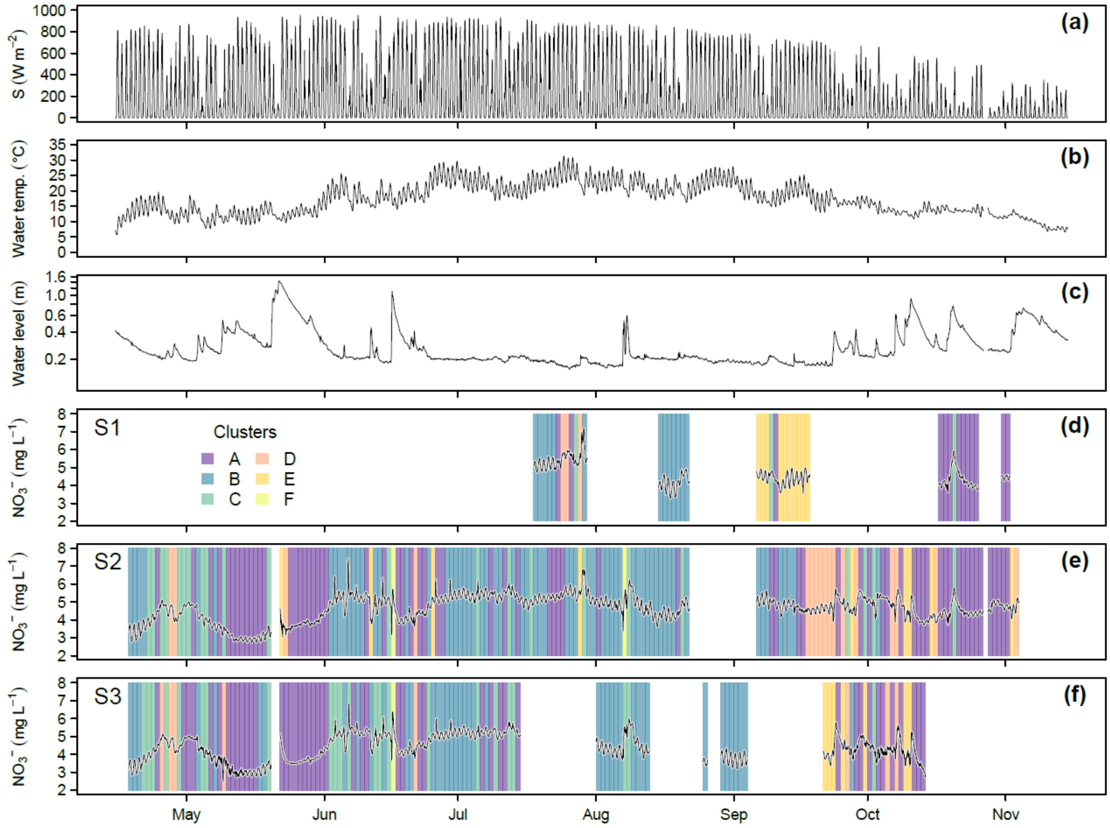

Figure 3: Global irradiance (a), water temperature (b) and water level (c) at S3 as well as NO_3^- concentration and cluster occurrence at the monitoring sites S1 (d), S2 (e), and S3 (f). Background colors in panels d to f indicate to which cluster the corresponding day was assigned.

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: The title was changed 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 was 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: This section was shortened and merged with the following and now reads (l. 30-42):

"Similar to other solutes, e.g. dissolved oxygen (DO) or carbon dioxide (CO_2), NO_3^- concentrations can exhibit diel (i.e. 24 h) cycles. However, the increasing body of high frequency NO_3^- monitoring data from optical in-situ probes shows that such diel cycles are not ubiquitous. Some streams consistently exhibit strong diel patterns (Heffernan and Cohen, 2010), while others do so only during certain seasons (Rusjan and Mikoš, 2010; Aubert and Breuer, 2016; Schwab, 2017; Rode et al., 2016), and still others do not show diel patterns at all (Duan et al., 2014). Biochemical processes influencing NO_3^- concentration include NO_3^- depletion via denitrification and photoautotrophic uptake, as well as production via

mineralization and subsequent nitrification. Previous studies have suggested that diel variation in stream NO_3^- concentration are mainly related to in-stream photoautotrophic uptake (Nimick et al., 2011; Burns et al., 2019). Due to photosynthetic light requirements, photoautotrophs take up NO_3^- mostly during the day (Mulholland et al., 2006), which causes minimum and maximum NO_3^- concentrations to typically occur in the late afternoon and in the early morning (prior to sunrise), respectively. However, there is evidence that diel variation may not be influenced by photoautotrophic uptake alone. In many systems, diel variability has also been found in rates of nitrification and denitrification (Laursen and Seitzinger, 2004; Dunn et al., 2012; Scholefield et al., 2005), e.g. due to changing oxygen levels in sediments (Christensen et al., 1990)."

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: See above.

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 revised our research questions in accordance with the suggestions by referee #1. We restructured our research questions and provided information how we addressed them. The section now reads as follows (l. 51-57):

"Here we analyze high-frequency NO_3^- data observed at three monitoring sites delimiting two reaches in the lower course of the river Elz in Southwest Germany. We aim to investigate, (1) if there are diel patterns in NO_3^- concentration, (2) if these patterns are subject to in-stream or transport control, and (3) how they are related to environmental conditions and potential drivers. In order to address these questions, we performed a cluster analysis on high-frequency NO_3^- recordings. We further differentiated between in-stream and transport control by comparing travel time estimates to time lags between concentration signals at adjacent monitoring sites. Finally, we compared environmental conditions among clusters and determined correlations between the concentration rates of change and potential 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: We added a sentence about this to the discussion of lateral inputs (l. 263-264): *"We were unable to separate the effects of groundwater inputs from a potential effect of increased NO_3^- removal in the lower reach due the revitalization measures."*

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 sentence was changed to (l. 84-86): *"In addition, biweekly grab samples were collected along the studied stream reach, including the probe locations, to provide a local calibration for probe measurements (Fig. S1) and to assess longitudinal concentration evolution between monitoring sites."*

Information on the uncertainty is presented in the supplementary material (Fig. S1).

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

Reply: As we dropped the mass balances, discharge measurement is no longer a central source of uncertainties in our study. Our field rating curve certainly reflects reality much better than discharge data from an 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: We used two independent measures as τ_a is more accurate but can only be determined using tracer data. τ_n is subject to more uncertainties but could be estimated continuously. We added an explanation for this in the methods section (l. 126-135):

"Time lags were compared to two independent estimates of solute travel time: mean tracer travel time (τ_a) and nominal water residence time (τ_n) according to Kadlec (1994). While τ_a is the first moment of the tracer residence time distribution and was determined from the breakthrough curves of the salt dilution measurements, τ_n is the ratio of reach volume and discharge. In contrast to τ_a, which requires tracer data as an input and could only be determined for our own dilution measurement (raw data of low flow measurements was not available from the regional water authority), τ_n was calculated continuously from water level recordings and channel width. As discharge, water depth, and channel width vary along the stream reach, we decided to account for variability in channel geometry and flow conditions by estimating a range of likely travel times based on channel width. Channel widths were estimated from aerial imagery and ranged from 20 to 25 m in the lower sub-reach and from 15 to 20 m in the upper sub-reach. Time lags obtained from cross-correlation were tested for difference from zero using t-tests and for difference from travel time estimates using paired t-tests."

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: Water level recordings were mainly used to generally charaterize flow conditions so that we consider the uncertainty of extrapolating the measurements at S3 acceptable. We revised the description of the measurement setup to make clear what parameters were measured where and when (l. 85-87): "*Stream temperature (T) and water levels (h) were continuously recorded at site S3 (TD-Diver, Van Essen Instruments, Netherlands) at 15 minute intervals.*" Also see the explanation of travel time estimation above.

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 restructured the manuscript accordingly. Now we first identify the different clusters and then investigate their formation.

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: We added this information to the manuscript (l. 175-176): *"Daily NO_3^- amplitudes were neither correlated with water level (ρ=-0.03, p=0.76), water temperature (ρ=0.11, p=0.22), nor with global irradiance (ρ=-0.07, p=0.21)."*

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 provided a reasoning for using the change rate of nitrate concentration for assessment of correlation with potential drivers (l. 136-150):

"In order to characterize the clusters, we compared environmental parameters during the occurrence of the respective clusters. We particularly assessed daily means of NO_3^- concentration, water levels (h_{mean}), and water temperature (T_{mean}) as well as the daily

maximum solar irradiance (S_{max}). The relationships between clusters and potential drivers were investigated by calculating daily Spearman rank correlations between C_{diel} and the diel course of the drivers. As potential drivers we considered global irradiance (S), water temperature (T) and discharge, the latter represented by water level (h). These environmental parameters are usually considered to influence the rate of biogeochemical processes, i.e. the rate of change of NO_3^- concentration rather than instantaneous NO_3^- concentration. Laboratory experiments have shown such behavior for the effect of light on NO_3^- uptake rates of algae (Grant, 1967) or the effect of temperature on denitrification rate (Pfenning and McMahon, 1997). We therefore assessed correlations between drivers and the first derivative (δC_{diel}) of the diel concentration signal C_{diel}. This corresponds to the way biochemical processes are implemented in some recent solute models (Hensley and Cohen, 2016; Grace et al., 2015). However, changes in water level may affect NO_3^- concentrations both indirectly, e.g. by influencing hyporheic exchange and biochemical processes therein (Trauth and Fleckenstein, 2017), and directly, since additional flow components may be enriched or depleted in NO_3^- compared to pre-event water. In the case of water level, we therefore calculated correlations with both C_{diel} and δC_{diel}."

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: Fig. 2 (Fig. 4 in the revised manuscript) was revised accordingly.

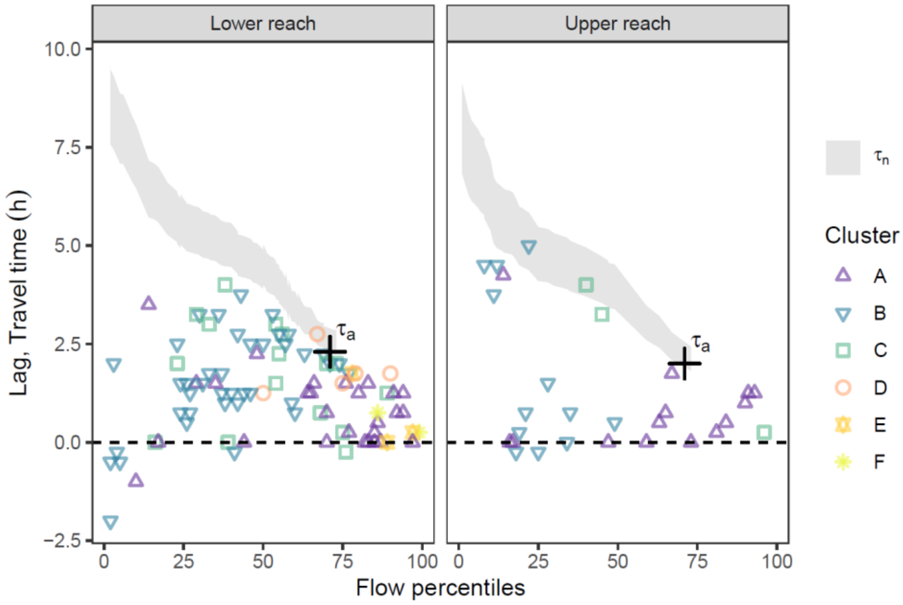

Figure 4: Travel time between diel NO_3^- signals at adjacent monitoring points compared to the tracer travel time (τ_a, black cross) and the range of nominal travel time estimates (τ_n, shaded area). No travel times were estimated when discharge exceeded the validity range of the rating curve. The figure only shows lags determined from signals with a corresponding cross-correlation coefficient above 0.75 (84.0% of the days).

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 included clusters in Fig. 4 to illustrate differences in lags among clusters as suggested above and improved our description of the findings of Hensley and Cohen (2016) which can be understood as a combination of in-stream and transport processes and which we think partially explain our findings (l. 243-253):

"The comparison of time lags between monitoring sites with travel time revealed that lags were usually too small to be produced by transport alone, but higher than expected for the case of pure in-stream control (Fig. 4). The existence of lags may thus be caused by an interaction of transport and in-stream processes. Simulating the longitudinal evolution of NO_3^- concentration downstream of a constant source, Hensley and Cohen (2016) found that timing of NO_3^- extremes was variable in the proximity of the source, but with increasing travel distance, NO_3^- concentration converged into a stable signal solely defined by in-stream processing. Depending on the position of observation points along such a stream reach, one may find time lags like those observed at our river Elz. Although boundary conditions at our study site are far less constrained than in the simulation of Hensley and Cohen (2016), their results might principally explain our observed time lags. Non-zero lags would then indicate that at the study site NO_3^- concentration had not yet fully converged and was still partially influenced by transport. Nevertheless, observed time lags were clearly smaller than estimated travel times. We therefore conclude that the observed diel NO_3^- patterns were not primarily produced by transport processes."

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 added information about seasonality and longitudinal stability of clusters and included information on cluster occurrence at the individual sites in Fig. 3 (l. 177-184):

"In terms of cluster occurrence, a largely similar seasonal pattern was apparent at all monitoring sites, despite different numbers of recorded days. Cluster A dominated in May and again in October and was replaced by cluster B during the summer months from June to September. Both clusters usually formed continuous blocks of several days. Cluster C occurred occasionally throughout the season but preferentially in early summer, while cluster D and E mainly occurred in fall. On most days (62.0%), diel NO_3^- recordings at the upstream and downstream monitoring sites were attributed to the same cluster. However, longitudinal stability was different in the stream reaches (50.0% in the upper and 66.1% in the lower reach) and among clusters. Cluster A was most stable (84.2%, n=57), while cluster B (62.3%, n=53) and C (61.9%, n=21) were close to the average. Cluster D (28.6%, n=14) and cluster E (12.5%, n=16) turned out to be comparatively unstable."

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: Figure 4 was split into two figures to make it more easily digestible. Fig. 5 deals with environmental conditions during clusters and Fig. 6 deals with correlations of nitrate concentration and potential drivers.

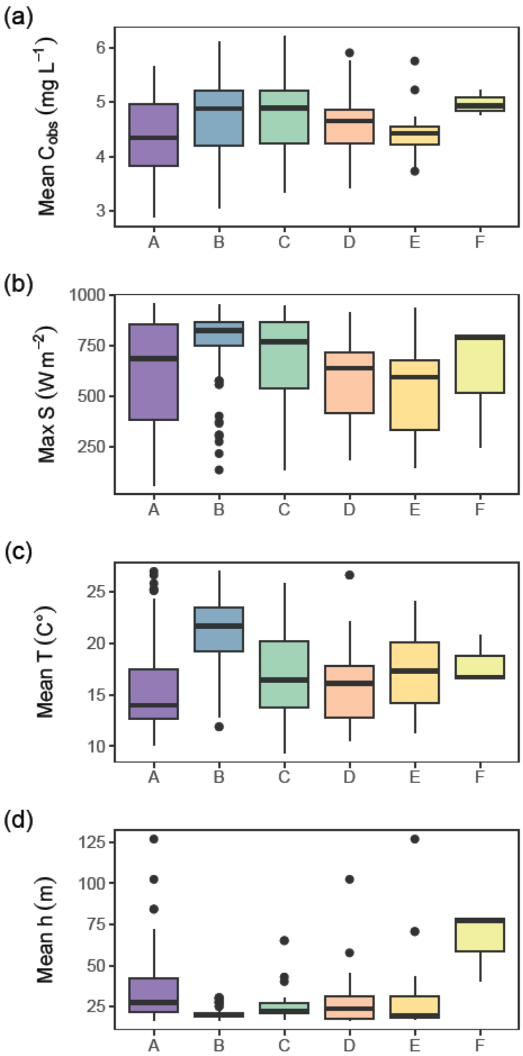

Figure 5: Environmental conditions during occurrence of clusters. The panels show daily average NO_3^- concentration (a), daily maximum of global irradiance (b), daily average water temperature (c), and daily average water level (d).

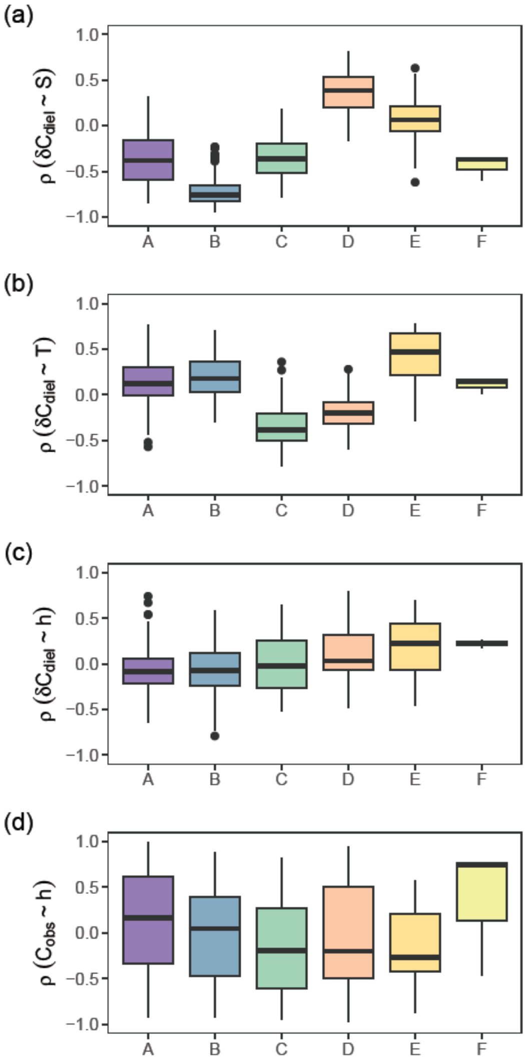

Figure 6: Daily Spearman correlations of the NO_3^- signal with potential drivers by cluster. The panels show correlation strength of diel concentration change rate with global irradiance (a), diel concentration change rate with water temperature (b), diel concentration change rate with water level (c), and observed concentration with water level (d).

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 decided to drop Fig. 5 to avoid redundancy with Fig. 3.

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 we were a bit quick here and revised our interpretation of clusters A-E as follows (l. 274-316). See above.

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: As mentioned above, seasonal variability in timing of drivers was minimal and shifts in nitrate concentration relative to drivers are reflected in the different shapes of the clusters. We therefore do not consider such an analysis to provide additional information.

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: Seasonal variation in timing of sunrise and sunset is in the order of 3 hours (between June and November) at the study site and timing of maximum irradiance only varies marginally. These variation is too small to explain the observed differences between patterns (about 6 hours). As referees generally recommended to shorten the discussion, we decided not to go into detail about this aspect.

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: The corresponding section was removed during the shortening process.

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: The corresponding section was removed during the shortening process.

Technical notes

Ln 11. "sites" instead of "locations"

Reply: We replaced "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: The sentence was changed to (l. 20): *"In-stream processing of nutrients can significantly influence loads and concentrations transported to receiving ecosystems"*

Ln 27: nitrogen (N)

Reply: We revised 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 checked and revised terminology throughout the manuscript.

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

Reply: We deleted the corresponding sentence.

Ln 55. Same here. “Such diel variability in these other in-stream processes would cause: : :”
Reply: We deleted the corresponding sentence.

Ln 72. Technically, you are studying a stream section that is divided in two reaches.
Reply: We used 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 was revised and now shows the catchment contributing to S3. The longitudinal profile was dropped but can be seen in the supplementary material (Fig. S2).

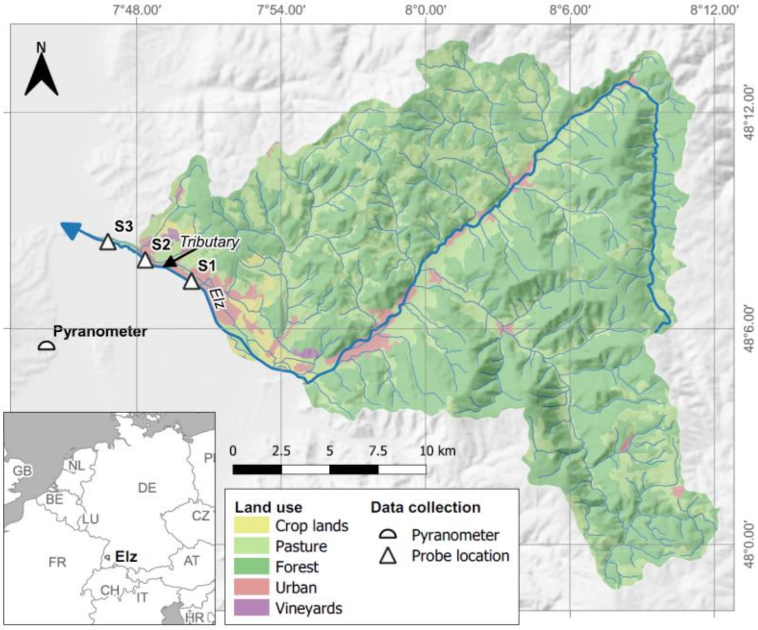

Figure 1: Location of monitoring points along the stream reach and land use in the contributing catchment.

Ln 79-82. I would divide this sentence into two: one for each reach.
Reply: The sentence was divided (l. 65-67): *“The upper reach (2.7 km) is characterized by a uniform gravel bed which is straightened and protected against erosion by regularly spaced groundsills. The lower reach (2.4 km) was subject to extensive revitalization including flood dam relocation and installation of a near-natural meandering river course.”*

Ln. 80. Delete “and in this sense it (: : :) southwest Germany”
Reply: We deleted the corresponding sentence.

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 was added (l. 68-73): *“Both reaches are characterized by largely open canopies and shallow (usually below 0.4 m) water depths, which allows light to reach the stream bed. However, in the downstream reach water depths are more variable, exceeding 1.5 m at some locations. As a consequence, also flow velocities are more variable in the downstream reach. Both reaches are scarcely colonized by macrophytes and filamentous algae and a visible biofilm develops on the gravel bed, particularly in the second half of the growing season.”*

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

Reply: A similar sentence was added to the introduction (l. 54-56): *"We further differentiated between in-stream and transport control by comparing travel time estimates to time lags between concentration signals at adjacent monitoring sites."*

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

Reply: Done (l. 115-116): *"In order to differentiate between in-stream and transport control on diel NO_3^- patterns, we determined time lags between adjacent monitoring sites by cross-correlation analysis and compared these to estimated solute travel time."*

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: We decided to refer to the diel portion of the concentration signal as C_{diel} throughout the manuscript.

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

Reply: As part of restructuring the manuscript this paragraph was revised and is now part of the "characterization of cluster" section (l. 136-150) quoted above.

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: In the revised manuscript we also account for the possibility that discharge alters the rate of biochemical processes by relating water level to both C_{obs} and C_{diel} (l. 147-150): *"However, changes in water level may affect NO_3^- concentrations both indirectly, e.g. by influencing hyporheic exchange and biochemical processes therein (Trauth and Fleckenstein, 2017), and directly, since additional flow components may be enriched or depleted in NO_3^- compared to pre-event water. In the case of water level, we therefore calculated correlations with both C_{diel} and δC_{diel}."*

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

Reply: In the revised manuscript the corresponding heading reads "In-stream vs. transport control on diel patterns" (l. 189).

Ln 152. Move this sentence to the methods section.

Reply: The sentence was removed from the results section.

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

Reply: This was deleted.

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

Reply: The sentence was deleted. See above.

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

Reply: Everything related to drivers was moved to the "characterization of cluster" section (l. 215-220):

"In addition to different environmental conditions, we identified different relationships with potential drivers of diel cycles among clusters (Fig. 6). The correlation of δC_{diel} and S was positive in cluster D, negative in clusters A and C, and strongly negative in cluster B. Moderate correlations of δC_{diel} and T were found in cluster C (negative) and cluster E (positive). Correlations of δC_{diel} with h were weak and difference among clusters were less pronounced than with S and T. The relationship of C_{obs} and h was very variable and included both strongly positive and negative correlations. However, strong overlapping of boxplots in Fig. 6c and Fig. 6d indicated that variability within clusters was higher than among cluster. "

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

Reply: This information was added to the figure (Fig. 2 in the revised manuscript).

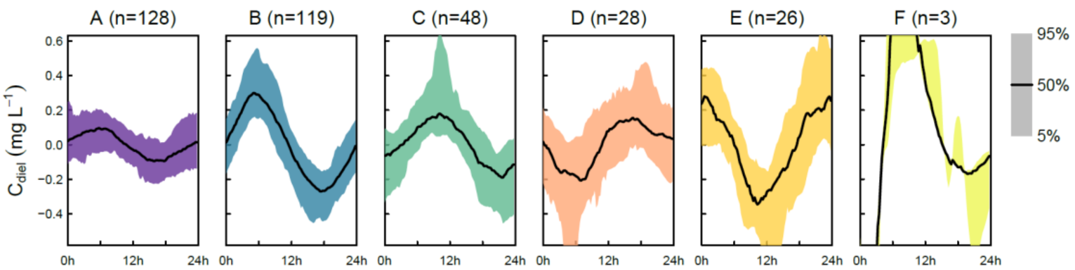

Figure 2: Clusters found in diel residuals of NO_3^- concentration (C_{diel}). Capital letters above panels are cluster names ordered alphabetically according to cluster size. Black lines indicate median diel patterns, shaded areas indicate the 5th to 95th percentile. Note that C_{diel} reflects deviations from the 24 h floating average so that negative values do not imply that negative concentration were observed.

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

Reply: This sentence was removed.

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: The paragraph was revised to make clear how the findings by Hensley and Cohen (2016) may explain the observed lags (l. 242-253), see above.

Ln 241. "in-stream processes"

Reply: The corresponding paragraph has been deleted.

Ln 306. Clusters A and B, right?

Reply: In the revised manuscript, we refer to both cluster A and B as to be caused by photoautotrophic uptake (l. 275-277): *"Diel NO_3^- patterns with a maximum in the early morning and a minimum in the afternoon are usually explained by photoautotrophic NO_3^- uptake by primary producers (Nimick et al., 2011). This was also the largest group of diel patterns in our study including cluster A and B, jointly accounting for about 70 % of the data."*

Author's response to comments by anonymous referee #2

Summary

Greiwe et al. collected diel nitrate data from three locations in a stream over multiple months to determine the controls of diel nitrate signals. They used cross correlation to show that diel signals were controlled by local in-stream processes rather than from upstream. Next, they used cluster analyses to identify consistent patterns in the diel signals. This is a novel and interesting approach. Finally, they relate the clusters with light and discharge to tease apart what is controlling each cluster. I think this is an interesting and worthwhile paper. I particularly like the use of cluster analyses on the diel data to identify common trends in the diel cycle. However, I believe major revisions are necessary before publication.

The biggest issue I have is the attempt to explain diel patterns based on unmeasured microbial processes. This is especially complicated given that many of these processes can cancel each other out (e.g., nitrification and denitrification) and we do not have easily measured proxies (like we have light for photosynthesis). Thus, I suggest that the authors tone down much of the speculation about microbial pathways, and instead focus on what they can show with data.

> Reply: We toned down our speculations on the relative importance of microbial processes and substantially shortened the discussion in this regard. In the revised manuscript the section on biochemical interpretation of the observed clusters reads as follows (l. 274-316), see above.

I also have some concerns with the methods. The cross-correlation approach could be described in more detail. Most importantly, there should be more detail about how the cluster analysis was performed. I am not an expert on cluster analyses and found it confusing how diel curves with multiple data points were put into a cluster analysis. As I mentioned above, I really liked this novel approach and I think it could be used for other constituents (DO, CO2, etc.). A better description of the methods would make it easier for others to replicate the analysis.

> Reply: We added some background information about the cluster analysis in the methods section (l. 93-99):
>
> *"We used k-means cluster analysis to identify and classify diel patterns in stream NO_3^- concentrations as done previously by Aubert and Breuer (2016). This method partitions a data set into a pre-defined number of k clusters by iteratively minimizing the within cluster sum of squares. We used the algorithm by Hartigan and Wong (1979) that is implemented in the 'stats' R-package (R Core Team, 2019). The input to this algorithm is a matrix whose rows represent elements to be partitioned (days in the present case) and whose columns represent the dimensions according to which the elements are compared. In the present case, these dimensions correspond to the time of day of the measurements (n=96 at a measurement interval of 15 minutes). More information about the method can be found in e.g. Tan et al. (2019). "*

Title: I would remove the reference to microbial pathways. This paper has no data to back up the suggested trends in microbial processes.

> Reply: The title was changed to *"Diel patterns in nitrate concentration produced by in-stream processes".*

Line 15: What is plug-flow?

> Reply: We decided that the term "plug-flow" was not required here and deleted it to avoid excessive explanation in the abstract.

Line 25: A key part of the spiral is that the nutrients are then mineralized to the water column to be taken up again downstream. This should be added here.

> Reply: We rephrased this sentence to also account for the mineralization step (l. 21-22): *"Nutrients are repeatedly taken up and released again by organisms during downstream transport, a concept known as "nutrient spiraling"*

Line 31: Can you better describe the link between climate change and nutrient retention? What role does drought play?

> Reply: We provided some additional references to illustrate the link between nutrient processing and climate change (l. 27-29): *"This may particularly be relevant in light of a changing climate and a predicted reduction of summer flow (Austin and Strauss, 2011; Mosley, 2015; Hellwig et al., 2017) ."*

Line 46: Denitrification is a heterotrophic process. This line implies that denit could occur via autotrophic processes. Please revise.

> Reply: The sentence was moved forward and now reads (l. 34-35): *"Biochemical processes influencing NO_3^- concentration include NO_3^- depletion via denitrification and photoautotrophic uptake, as well as production via mineralization and subsequent nitrification."*

Line 93: Please provide more information about the periodical movement of the sensors.

Were the sensors moved at equal intervals? Is the data available from each sub-reach stratified across the sample period?

Reply: We introduced a new figure (Fig. 3) in order to make clear what parameters were measured where and when.

Lines 106-108: Could you provide more info on interpreting the cross-correlation data? What does a low and high correlation mean? How does this help better elucidate N transported from upstream vs. from in stream processes? A few lines here will help the reader going forward, especially to understand figure 2.

Reply: We revised the description of cross-correlation method and provided some additional background information. The section now reads (l. 114-135):

"In order to differentiate between in-stream and transport control on diel NO_3^- patterns, we determined time lags between adjacent monitoring sites by cross-correlation analysis and compared these to estimated solute travel time. If diel NO_3^- variation originated from some upstream source and subsequent downstream transport, time lags between sites should correspond to solute travel times. In contrast, if diel patterns were produced by in-stream processes simultaneously at all points along the flow path, we expected the time lag to be zero in most instances. Cross-correlation analysis is a standard method to determine time lags between signals (Derrick and Thomas, 2004). It is based on the idea that the strength of a correlation between two signals changes according to a temporal shift. The shift that maximizes the strength of the correlation is considered the time lag between the signals. This method works best, if the two signals have a similar shape, i.e. they are strongly correlated at an optimal lag. We therefore determined time lags only for days when the correlation coefficient r between up and downstream sites exceeded 0.75. This was true for 121 out of 144 days with complete measurements at both the upstream and the downstream monitoring site.

Time lags were compared to two independent estimates of solute travel time: mean tracer travel time (τ_a) and nominal water residence time (τ_n) according to Kadlec (1994). While τ_a is the first moment of the tracer residence time distribution and was determined from the breakthrough curves of the salt dilution measurements, τ_n is the ratio of reach volume and discharge. In contrast to τ_a, which requires tracer data as an input and could only be determined for our own dilution measurement (raw data of low flow measurements was not available from the regional water authority), τ_n was calculated continuously from water level recordings and channel width. As both water depth and channel width vary along the stream reach, we decided to account for variability in channel geometry and flow conditions by estimating a range of likely travel times based on channel width. Channel widths were estimated from aerial imagery and ranged from 20 to 25 m in the lower sub-reach and from 15 to 20 m in the upper sub-reach. Time lags obtained from cross-correlation were tested for difference from zero using t-tests and for difference from travel time estimates using paired t-tests."

Line 110: What travel time distribution is this referring to? You only conducted one tracer release (I think).

Reply: We improved the description of the cross-correlation method to avoid misunderstanding as mentioned. See above.

Line 120: I believe that residual should be added earlier in the sentence. ": : :was done on the residuals of the diel solute concentration signal."

Reply: The sentence was reordered (l. 100-101): *"The analysis was done on the diel portion of the solute concentration signal, hereafter referred to as diel concentration (C_{diel}), to ensure that the resulting clusters represented variability in diel cycles and not in NO_3^- background concentrations."*

Lines 115-127: I am having troubles understanding how the clusters were determined, or in other words, how the k-means approach turned diel data into clusters. Could that be described more? I am used to clusters being used with single values (i.e., animal abundance data), so how can multiple points be used (i.e., from a diel curve). I do not have much experience with clustering, but that will be true for many readers as well. More detail would be helpful.

Reply: We revised the description of the clustering method and added some background information (l. 93-99), see above.

Line 160/Figure 2: This took some time to determine what I am looking at. Is the main point that points with a high cross-correlation are typically between 0 and the nominal travel time (the shaded area)? Either way I would add a line or two describing the main result out of this figure. Also, how is it possible that a travel time is negative?

Reply: The corresponding figure (Fig. 4 in the revised manuscript) was simplified. All points with r<0.75 were removed. The description was also revised and now reads (l. 190-199):

"The time lags between diel NO3 signals at adjacent monitoring sites were usually shorter than the solute travel times between the stations. The salt dilution measurement resulted in a discharge of 2.0 $m^3 s^{-2}$ resulted in travel time (τ_a) estimates of 2.0 h in the upper and 2.3 h in the lower reach (Fig. 4). Estimates of nominal residence time (τ_n) resulted in a range of plausible values and displayed increasing travel times with decreasing stream flows. The fact that the independently determined τ_a was included in the range of τ_n, showed that the estimated travel times were plausible. In both reaches the time lags between the concentration signals roughly ranged between zero and the travel time estimates, but were significantly different from both zero ($p<0.001$, both reaches) and minimum travel time ($p<0.001$, both reaches). In the lower reach, lags formed an evenly distributed point cloud. Within this cloud, Cluster D, E, and F only appear at above median flows. In the upper reach, time lags were concentrated towards the extremes, i.e. either close to zero or close to travel time estimates. Days with below median stream flow were mainly assigned to cluster B and those above median stream flow to cluster A."

Line 175/Figure 3: Do the shaded areas represent a confidence interval? And what calculations were used to calculate the shaded area?

Reply: Shaded areas in figure 3 represent the range between the 5th and 95th percentile, i.e. 90% of the data and black dots represent medians. This information has been added both to the figure and its caption (Fig. 2 in the revised manuscript). See above.

Line 200/Figure 5: Would it be logical to make the y-axis a proportion? The ups and downs are distracting. Making them a proportion would better show the seasonal trends.

Reply: Figure 5 was removed to avoid redundancy with the newly introduced Figure 3 showing the raw data, which was requested by referee #1.

Line 201: Something is missing here. Maybe, "Relation of nitrate clusters and reach balance"

Reply: Reach balances were dropped from the revised manuscript. But see (Fig. S2) in the supplement.

Line 220: Please define or further explain short-circuiting.

Reply: This phrasing was dropped when revising the discussion of the cross-correlation (l. 242-253). See above.

Line 223: "stated"? Maybe observed?

Reply: The corresponding sentence was deleted.

Line 226: This explanation of the Hensley and Cohen paper is confusing and hard to follow. Could you describe the point of the paper without getting into the details?

Reply: The explanation of the Hensley & Cohen pater was revised (l. 242-253). See above.

Line 240: I don't believe the description of clusters E and F being influenced by discharge is in the results section. How did you come to this conclusion?

Reply: We dropped this paragraph as we shortened the discussion. In the revised manuscript we interpret cluster A to E but not F the low number of data points. See l. 274-316 quoted above.

Line 260: What is the relevance of the 0.5 mg/L SRP? What does this threshold indicate?

Reply: We dropped this threshold from the revised manuscript.

Line 287: This is also true for estimates of stream metabolism.

Reply: We dropped this paragraph from the revised manuscript.

Line 247-300: There is a lot of speculation on the drivers of diel patterns in here. It would be much more convincing to use a statistical analyses/models to make conclusions about what is controlling the diel trends rather than relying on the literature and instinct. The correlations with light are somewhat compelling for the first two clusters but it is still hard to disentangle the different microbial pathways relative to the autotrophic. For the other clusters it gets much more complicated and interpretation is pure speculation. That being said, I still think these data are useful and novel. But tying each cluster to a specific driver is for another paper in my opinion. I suggest that this part of the discussion be substantially shortened. I like how you first describe the strong evidence that in-stream, not upstream, processes are driving diel trends. Then go through the clusters or sets of clusters and do some light speculation on the drivers of the signals in relation to the literature. This is done quite well in lines 303-331.

Reply: As also suggested by referee #1, we substantially shortened the discussion and reduced speculation. See l. 274-316 quoted above.

Line 353: Is there a citation for these data?

Reply: The data are publicly available and presented in the supplementary material (Fig. S3) along with the data source.

Line 355: The topic of groundwater should be introduced and described much earlier in the methods section. Also, please address how groundwater might affect the diel curves? Groundwater is likely an important factor for diel curves during summer low flows.

Reply: We added a section to the discussion that deals with how lateral inputs may affect in-stream concentrations including groundwater (l. 254-273). See above.

Line 371: We know this already–In my opinion, this is not the strength of this paper. I would end here with a line noting how you were able to separate diel trends in NO3 concentrations into clear clusters with distinct diel patterns and probably different drivers. These clusters can be used a blueprint for future efforts to model drivers of N cycling. Likewise, using the cluster analyses on diel data is a novel approach and could be used for other measurements (e.g., DO, CO2, SRP, etc).

Reply: We changed the end of our conclusions accordingly. It now reads (l. 327-332):

"In depth interpretation and eventually quantification of process rates would require spatially distributed high frequency information on stream metabolism, e.g. dissolved oxygen concentrations, and on different N species, most importantly NH_4^+. Nevertheless, our analysis suggests that a particular combination of different in-stream processes may generate distinct diel NO_3^- patterns. A seasonal shift in patterns may then indicate shifts in the relative importance of the underlying processes. The clustering method used in this study proved useful for making the data set accessible for this kind of analysis and may be used as a blueprint for the analysis of other stream solutes."

Author’s response to comments by anonymous referee #3

This novel approach of analyzing and visualizing diel nutrient data is an important contribution to stream ecosystem science. It fits the scope of this journal well. Overall, I found this manuscript to be interesting and advancing the use of diel cycles of nutrients to interpret ecological functions in streams. However, the lack of simultaneously measured process rates (such as metabolism, nitrification or denitrification) makes parts of the discussion and conclusions very speculative and I strongly recommend to shorten and nuance that section.

> Reply: We toned down our speculations on the relative importance of microbial processes and substantially shortened the discussion in this regard. In the revised manuscript the section on biochemical interpretation of the observed clusters reads as follows (l. 274-316). See above.

Specific comments:

1. In figure 1 I wonder why the evaluated stream reach is mapped outside of the land use map? In particular, information on urban areas including pasture between the measuring points are of interest to the interpretation of this data set.

> Reply: The map was revised and now shows the catchment contributing to site S3 (see above). The longitudinal profile was dropped from the manuscript but can be seen in the supplementary material (Fig. S2).

2. Line 220. “Downstream transport of solute signals therefore fails to explain most of our data. We therefore interpret our data to indicate primarily in-stream origin of diel nitrate cycles.” What about signals from land, i.e. soil water signals. Especially during low flow. I realize this comes later in the manuscript but I would move some of that discussion here and clarify it also in the methods.

> Reply: We added a section in the discussion that deals with lateral inputs (l. 254-273). See above

3. Line 368-60 “In the remaining clusters temporal shifts were evident that could be explained by temporal shifts in microbial nitrate processing but not by photosynthesisdriven uptake.” This line makes it sound like you measured microbial processing or photosynthesis, please re-phrase.

> Reply: The conclusions were revised. The corresponding sentence now reads (l. 326-327): “However, we also found indications that other biochemical processes like nitrification and denitrification contributed to the formation of NO_3^- patterns.”

4. Line 250-256. My experience of dissolved oxygen signals is that they can often match cluster C, with maximum %O2 in the afternoon. I would not be so quick to discard cluster C from being driven by photoautotrophs without evidence. Especially since there was a negative correlation between solar radiation and cluster C (line 183), which is what you use to argue for photoautotrophic dominance in driving cluster A and B.

> Reply: We revised the interpretation of cluster C accordingly and now also consider photoautotrophic uptake as a possible explanation for cluster C (l. 287-311). See above.

5. Could spring photoautotrophs be light inhibited during mid-day and therefore cluster C peaks in the afternoon? Cluster C was most prominent in spring when harmful UV is the highest. Which were the light levels in this study? Was light ever measured under water?

> Reply: Light levels are provided in terms of global irradiance at the surface in Fig. 3 and Fig. 5.

6. No statistics are presented in the results section on page 8, please include that.

> Reply: We are not entirely sure what referee #3 is referring to. We added a description of the shaded areas in Fig. 2. We are a bit hesitant to mention all the statistical measures of the boxplots in Fig. 4 (Fig. 5 and Fig. 6 in the revised manuscript) as most of this information is apparent in the figures and all the numbers may be overwhelming for the reader.

Author's response to comments by anonymous referee #4

Summary The manuscript by Greiwe et al. describes a spatially-repeated sampling of diel variation in nitrate export along a reach in an intermediate watershed. The authors collected high-frequency diel nitrate concentrations from three stream stations, and quantified the magnitude of diel amplitude and estimated the travel times between stations. The authors used a cross-correlation approach to conclude that instream processes controlled emergent diel signals, and were minimally driven by upstream inputs. Overall, I enjoyed the paper, as it presents a means to interpret an essential ecohydrological question: which is more important, the physical or biological context, and when do these abiotic/biotic controls matter most? It is also an interesting way to use spatially-explicit data, especially that which is emerging from the application of highfrequency sensors. I found the topic highly relevant, especially as high-frequency hydrochemistry paired with discharge is becoming more widely available, and questions about source pathways and mixing have become a topic of interest of the research community.

However, there were some points of confusion that I hope the authors can clarify in a revision. I have several main comments, and some minor ones mainly focusing on improving clarity of the manuscript, that I hope the authors find insightful.

Major Comments

(1) While I am intrigued by the paper, one issue is that the authors overplayed the role of microbial processing. While this is generally assumed to be the case, this is still a "black box" situation with no microbial processing measured directly. I encourage the authors to take greater care in describing their findings and the assumptions of their interpretations, which as written are overly speculative.

> Reply: We shifted the focus of the manuscript towards our substantial results about in-stream vs. transport control on diel nitrate patterns and changed the title accordingly. We also reduced speculations and shortened the discussion of biochemical processes (l. 274-316).

(2) How were tributary inputs accounted for in the authors' approach (based on Figure 1 there were some small inputs in between monitoring stations)? Part of the difficulty in parsing apart nitrate removal/production processes is the fact that there is mixing happening from multiple landscape units, which are hydrologically mixed as tributaries meet, and it was not clear how this variability in inputs was accounted for in the authors approach.

> Reply: We added a section in the discussion that addresses the issue of lateral inputs (l. 254-273).

(3) While the approach of using a time lag is compelling, I am curious if the authors had thought about the distributions of travel and reaction times in this study? The assumption of a mean travel time or reaction rate is to capture 'average' behavior and likely represents what is generally happening, but the use of a single value assumes that either transport or removal processes influencing what water/solutes make it to a point in the watershed network are occurring at a single rate. I am not encouraging the authors to use this approach, but it should likely be discussed as a potential limitation of the study. Somewhat relatedly, why are there negative travel times in Figure 2, do you mean this to be the time lag?

> Reply: In order to address the issue of 'average' behavior we calculated a range of likely travel times which we described in more detail in the method section (l. 131-133): *"As discharge, water depth, and channel width vary along the stream reach, we decided to account for variability in channel geometry and flow conditions by estimating a range of likely travel times based on channel width."* In addition, the Figure 2 (Fig. 4 in the revised manuscript) was revised according to suggestions by referee #1, i.e. we only show points with r>0.75 and indicated cluster attribution of data points. See above.

(4) The authors could significantly shorten the discussion, as many of the processes mentioned were not directly measured and so the discussion does not need to be as nuanced as it is. Instead, presenting this as an open "call for the community" might be a more appropriate approach. Alternatively, one suggestion would be for the authors to develop a conceptual diagram of diel patterns in their watershed, indicating the open questions on the processes that the authors did not directly measure but infer as important instream drivers. Not only would this figure be useful for the community to visualize nitrate processing/transport in this system, but also likely hone the discussion around what is "known" and what is yet "unknown".

Reply: We substantially shortened the discussion (l. 274-316) and decided not to present a definitive statement on which processes produced the clusters.

Minor Comments and Line-by-Line Suggestions

P1, Line 10: Change to "allow calculation"

Reply: Done (l.10).

P1, Line 15: Omit "suggested"

Reply: Done (l.14).

P2, Line 50: Please define insolation

Reply: This sentence was deleted. We added a sentence on light measurements in the description of the monitoring setup (l. 89-90): *"We used global irradiance (S) data from a nearby climate station (< 10 km, Figure 1) as a measure of sunlight intensity."*

P4, Section 2.2: Please describe in further detail how the s::can data were calibrated and turbidity-corrected.

Reply: Local calibrations are provided in the supplementary material (Fig. S1)

P5, Section 2.3.1: Were the time lags / mean travel times estimated at the same intervals as the s::can data (i.e., did they also account for high/low Q, or are they averaged for a day)? Did you measure Q continuously at all three stations? Some additional clarity is needed here on time-scale and context for when travel times were estimated.

Reply: We introduced Fig. 3 showing the raw data and probe placement to provide a better idea of our data set and also revised the description of the lag calculation procedure (l. 115-135). See above.

P11, Line 223: This sentence seems to come out of nowhere, I'd delete or expand on this idea before describing the Hensley & Cohen paper.

Reply: The sentence was deleted and the description of the Hensley & Cohen paper was improved (l. 242-253). See above.

Figures & Tables

Generally, I thought the figure legends needed to have much greater detail. For example, in the caption for Figure 2, r should be more clearly defined. I also wouldn't put the shading for the nominal travel time on the figure, as this looks like a regression or confidence interval. In Figure 3, the letters should be defined in the figure legend and the confidence interval should be described. Additionally, in the spirit of inclusivity, I encourage the authors to check that their figures are color-blind friendly.

Reply: These points have been addressed (see above). The original color scheme was already based on the viridis color scheme designed for color-blindness. We modified colors slightly to improve contrasts (particularly in Fig. 3) and ensured that our color selection was still color-blind friendly using color blindness simulation software.