Diel patterns in ~~NO₃⁻~~ stream nitrate concentration produced by in-stream processes

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Abstract. Diel ~~cycles~~ variability in stream NO_3^- concentration represents the sum of all processes affecting NO_3^- concentration along the flow path. Being able to partition diel NO_3^- signals into portions related to different biochemical processes would

10   allow calculation of daily rates of such processes that ~~are needed~~ would be useful for water quality predictions. In this study, we aimed to identify distinct diel patterns in high-frequency NO_3^- monitoring data and investigated the origin of these patterns. Monitoring was performed at three locations in a 5.1 km long stream reach draining a 430 km² catchment. Monitoring ~~and~~ resulted in 355 complete daily recordings on which we performed a k-means cluster analysis. We compared travel time estimates to time lags between ~~observations at the~~ monitoring sites to differentiate between in-stream and transport control on

15   diel NO_3^- patterns. We found that travel time failed to explain the observed lags and concluded that in-stream processes ~~or lateral inputs~~ prevailed in the creation of diel variability. ~~At~~ Results from the cluster analysis showed that at least 70% of all diel patterns reflected shapes typically associated with photoautotrophic NO_3^- assimilation. The remaining patterns suggested that other ~~biochemical~~ processes (e.g. nitrification, ~~and~~ denitrification and heterotrophic assimilation) ~~or physical processes (lateral inputs)~~ contributed to the formation of diel NO_3^- patterns. Seasonal trends in diel patterns suggest that the relative

20   importances of the contributing processes varied throughout the year. These findings highlight the potential in high-frequency water quality monitoring data for a better understanding of the seasonally in biochemical processes.

1   Introduction

In-stream processing of nutrients can significantly influence loads and concentrations transported to receiving ecosystems (Peterson et al., 2001; Roberts and Mulholland, 2007). Nutrients are repeatedly taken up and released again by organisms

25   during downstream transport, a concept known as "nutrient spiraling" (Ensign and Doyle, 2006). Depending on the rates of

nutrient uptake and release, in-stream nutrient processing may reduce the risk of harmful eutrophication (Birgand et al., 2007). ~~As a result of human activity, many streams exhibit increased levels of nitrogen~~ (Dodds and Smith, 2016). Among the different ~~N species~~nutrients, nitrate (NO_3^-) is of special interest since it usually represents the largest fraction in dissolved inorganic nitrogen ~~(DIN)~~ and is nowadays easily detectable using in-situ optical spectrometer probes. At the same time, water quality

30 management requires knowledge of NO_3^- processing rates to predict how rapidly NO_3^- inputs are transformed and attenuated. This is particularly relevant in light of a changing climate and a predicted reduction of summer flow (Austin and Strauss, 2011; Mosley, 2015; Hellwig et al., 2017).

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~~patterns. However, the increasing body of high frequency NO_3^- monitoring data from optical in-situ probes shows that

35 such diel ~~cycles~~ patterns 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 (Mulholland et al., 2009) and ~~photoautotrophic uptake~~both autotrophic (Lupon et al., 2016b) and heterotrophic (Middelburg and Nieuwenhuize, 2000; Luque-Almagro et al., 2011) assimilation, as

40 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

45 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).

In flowing waters, biochemical processes are superposed by downstream transport. Therefore, the solute signal measured at a specific location integrates over all conditions and events that water parcels were previously exposed to. As a result, the benthic footprint, i.e. the upstream area influencing concentrations at the measurement point, depends on flow velocity and solute

50 turnover rate. While gaseous solutes like DO may quickly equilibrate with the atmosphere, upstream discontinuities in non-gaseous solutes like NO_3^- (e.g. tributary confluxes, lakes or reservoirs, ~~groundwater or~~ waste water inputs from point sources) may persist further downstream (Hensley and Cohen, 2016). In open systems with unknown input signals, it is therefore unclear whether diel concentration patterns are produced by conditions in the investigated stream ~~reach (in-stream control)~~ or stem from upstream sources ~~(Pellerin et al., 2009)~~ ~~and~~ from which they are transported downstream ~~(transport control)~~ (Pellerin et

55 al., 2009). We will refer to the first case as in-stream control and to the second case as transport control. In this sense, we use

the term 'in-stream' to refer to average properties of a reasonably long stream reach and its immediate surroundings including biochemical conditions in the stream and in the hyporheic zone as well as diffuse groundwater interaction.

Here In the present study, 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.

2 Methods

2.1 Study site

The studied stream reach is located in the lower course of the river Elz in Southwest Germany between the municipalities of Emmendingen and Riegel (Figure 1Figure 1). At our study site, the river Elz drains an area of approximately 430 km² of which 66% are forest and 21% are grassland. The fraction of cropland is below 2%. The river contains several weirs and receives inflows from a small wastewater treatment plant approximately 25 km upstream of the study site. Yet, most wastewater of the upstream catchment is transferred to a large treatment plant located further downstream. The monitored stream section spans a distance of 5.1 km and is divided into two reaches with different morphology. 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. Revitalization measures were completed in 2016 and since then natural dynamics have controlled river morphology. 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 aA visible biofilm develops was observed on the gravel bed, particularly in the second half of the growing season. There are no obvious influxes along the two stream reaches, except for a minor tributary in the upstream reach (Fig. 1).
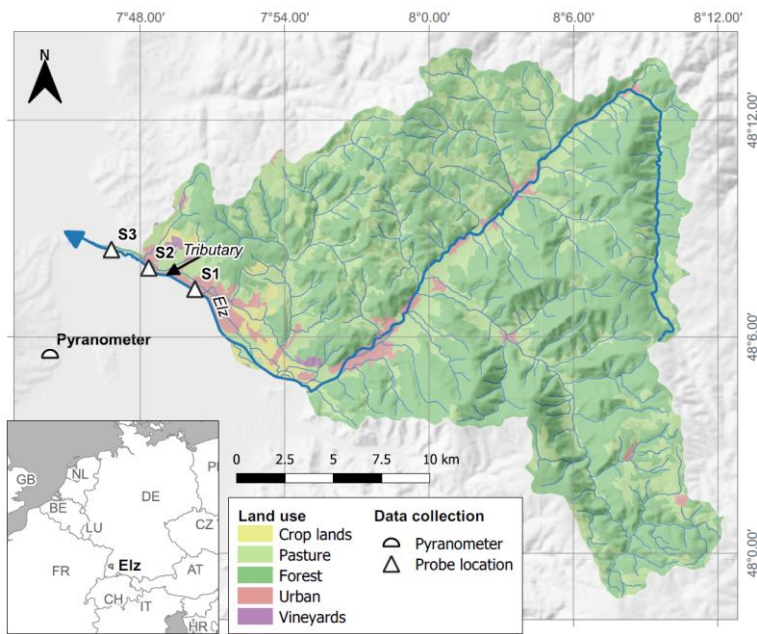
Figure 1: Location of monitoring points along the stream reach and land use in the contributing catchment.

 2.2   Data collection

Concentration of NO_3^- was measured at 15 min intervals at the three monitoring sites (S1, S2, S3) using UV-Vis spectrometer probes (spectro::lyser, s::can Messtechnik GmbH, Vienna, Austria) from April to November in 2019. As only two spectrometer probes were available, one probe was periodically repositioned so that input and output concentrations of either the upper or the lower stream reach were measured. In addition, biweekly grab samples were collected at eight locations along the studied

90   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. Samples were analyzed using ion chromatography (Dionex ICS-1100, Thermo Fisher Scientific Inc., USA). Stream temperature (T) and water levels (h) were continuously recorded at the downstream site S3 (TD-Diver, Van Essen Instruments, Netherlands) at 15 minute intervals. Discharge was calculated using a rating-curve based on two salt dilution measurements by the regional water authority, and one additional salt dilution

95   measurement during higher water levels on 15 November 2019 (> 70 % of recorded water levels). In the latter dilution measurement, we injected 33 kg of NaCl at site S1 to cover both ~~sub~~ reaches. We used global irradiance (S) data from a nearby climate station (< 10 km, Fig. 1) as a measure of sunlight intensity.

2.3 Data analysis

2.3.1 Identification of ~~diel~~ patterns in **stream** NO_3^- concentration

100 ~~Besides the diel scale, p~~Patterns in NO_3^- concentration may also vary ~~seasonally and longitudinally~~ on the seasonal, daily, and the spatial scale. We ~~therefore~~ assessed seasonal ~~the~~ relationships of absolute NO_3^- concentrations and ~~intensity of~~ diel NO_3^- variability ~~patterns~~ to environmental conditions by determining Spearman rank correlations of daily means of C_{obs} and daily NO_3^- amplitudes with global irradiance (S), water temperature (T), and, water level (h). 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

105 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

110 be found in ~~e.g.~~ Tan et al. (2019).

The **k-means** 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. ~~Residual~~ Diel (residual) concentrations were obtained by subtracting a 24 hour centered moving average from the measured concentrations (C_{obs}) and smoothed by applying a moving average of 2 hours. One feature of the k-means method that

115 introduces some degree of subjectivity is the determination of the number of clusters k. We therefore tested k values between 2 and 20 and determined the best partition by both an assessment of explanatory benefit per additional cluster, also known as 'elbow method', and by visual inspection of clusters. The elbow method was not clearly indicative as no sharp bent was observed. Instead, we visually found an optimum number of six clusters, since higher values of k did not produce new clusters in terms of timing but rather caused further splitting of existing clusters by amplitude.

120 ~~Besides the diel scale, patterns in NO_3^- concentration may also vary seasonally and longitudinally. We therefore assessed the relationship of absolute NO_3^- concentrations and intensity of diel patterns to environmental conditions by determining Spearman rank correlations of daily means of C_{obs} and daily NO_3^- amplitudes with global irradiance (S), water temperature (T), and, water level (h).~~ As a measure for longitudinal stability of diel patterns, we calculating the fraction of days on which diel patterns at the upstream and downstream monitoring sites of each reach were assigned to the same cluster.

2.3.2 In-stream vs. transport control on diel NO$_3^-$ patterns

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 more or less 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 upstream and downstream sites exceeded 0.75. This was true for 29 121 out of 42 144 completely recorded days in the upper and 92 out of 121 complete days at the lower reach 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 our 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.

2.3.3 Characterization of clusters

In order to characterize the clusters, we compared environmental parameters during the occurrence of the respective clusters. We pParticularly, we 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}). Differences between clusters were statistically assessed using analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) tests as implemented in the 'stats' R-package (R Core Team, 2019).

The relationships between clusters and potential drivers were investigated by calculating daily Spearman rank correlations
155   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
160   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}.

165   ## 3   Results

3.1   Variability of diel patterns in space and time

Data collection at the three monitoring sites resulted in 352 complete diel NO_3^- ~~signals~~recordings. Throughout the season,
stream NO_3^- concentrations ranged between 2.47 mg L^{-1} and 7.44 mg L^{-1} (Fig. 3). Mean values and standard deviations at the
three monitoring sites were 4.64 ± 0.66 mg L^{-1} (S1), 4.63 ± 0.73 mg L^{-1} (S2), and 4.36 ± 0.75 mg L^{-1} (S3). Considering only
170   days with complete upstream and downstream observations, i.e. comparing averages of the same day, NO_3^- concentration
significantly increased between S1 and S2 (from 4.61 to 4.86 mg L^{-1}, p<0.001, n=42) and significantly decreased between S2
and S3 (from 4.54 to 4.40 mg L^{-1}, p<0.001, n=121) (Fig. S2). Daily averages of NO_3^- were negatively correlated with water
level (ρ=-0.34, p<0.001), positively with water temperature (ρ=0.53, p<0.001), and uncorrelated with global irradiance
(ρ=0.01, p=0.93). The overall negative correlation between NO_3^- and water level was dominated by large floods in May and
175   June. Particularly in the second half of the study period, e.g., in early August (S2 and S3) and late October (S1 and S2), NO_3^-
concentrations increased in response to floods (Fig. 3). 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).

~~, almost all of which showed a diel pattern.~~ The cluster analysis resulted in 6 clusters that clearly differed in terms of amplitude
and timing of minimum and maximum concentrations (Fig. 2). 69.6% of the days were attributed to the clusters A (n=128)
180   and B (n=119), which both reached peak concentration in the early morning and minimum concentration in the late afternoon,
but the daily amplitude was higher in cluster B. The remaining clusters were characterized by peaks around midday (cluster

C, n=48), in the afternoon (cluster D, n=28) and around midnight (cluster E, n=26). The last cluster (cluster F, n=3) did not include enough days for a proper characterization. Average time of the daily concentration maxima in clusters A to E were 4:33 h, 5:32 h, 10:18 h, 16:44 h, and 23:33 h, respectively. The respective average times of daily minima were 17:46 h, 17:36 h, 21:39 h, 6:06 h, and 11:58 h.
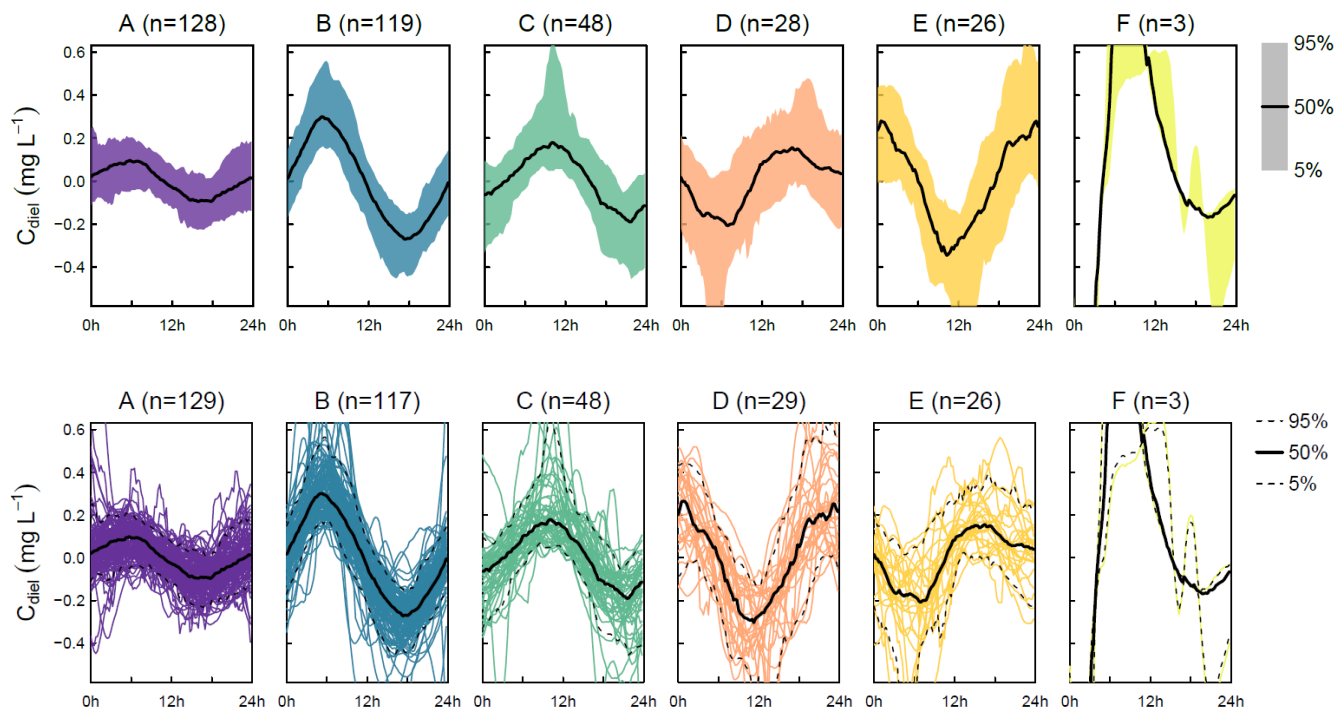
Figure 2: Clusters found in diel residuals of NO₃⁻ concentration (C_{diel}). Capital letters above panels are cluster names ordered alphabetically according to cluster size. Black lines indicate median diel patterns, ~~shaded areas~~dashed lines indicate the 5th ~~and~~ 95th percentile. Note that C_{diel} reflects deviations from the 24 h floating average so that negative values do not imply that negative concentration were observed.

~~Throughout the season, stream NO₃⁻ concentrations ranged between 2.47 mg L⁻¹ and 7.44 mg L⁻¹ (Fig. 3). Mean values and standard deviations at the three monitoring sites were 4.64 ± 0.66 mg L⁻¹ (S1), 4.63 ± 0.73 mg L⁻¹ (S2), and 4.36 ± 0.75 mg L⁻¹ (S3). Considering only days with complete upstream and downstream observations, i.e. comparing averages of the same day, NO₃⁻ concentration significantly increased between S1 and S2 (from 4.61 to 4.86 mg L⁻¹, p<0.001, n=42) and significantly decreased between S2 and S3 (from 4.54 to 4.40 mg L⁻¹, p<0.001, n=121) (Fig. S2). Daily averages of NO₃⁻ were negatively correlated with water level (ρ= 0.34, p<0.001), positively with water temperature (ρ= 0.53, p<0.001), and uncorrelated with global irradiance (ρ=0.01, p=0.93). The overall negative correlation betweeen NO₃⁻ and water level was dominated by large~~

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In terms of cluster occurrence, a largely similar seasonal pattern was apparent at all monitoring sites, despite different numbers of recorded days (Fig. 3). 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
205 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, i.e. these patterns were longitudinally stable. However, longitudinal stability ~~was different in~~differed ~~between 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
210 out to be comparatively unstable.
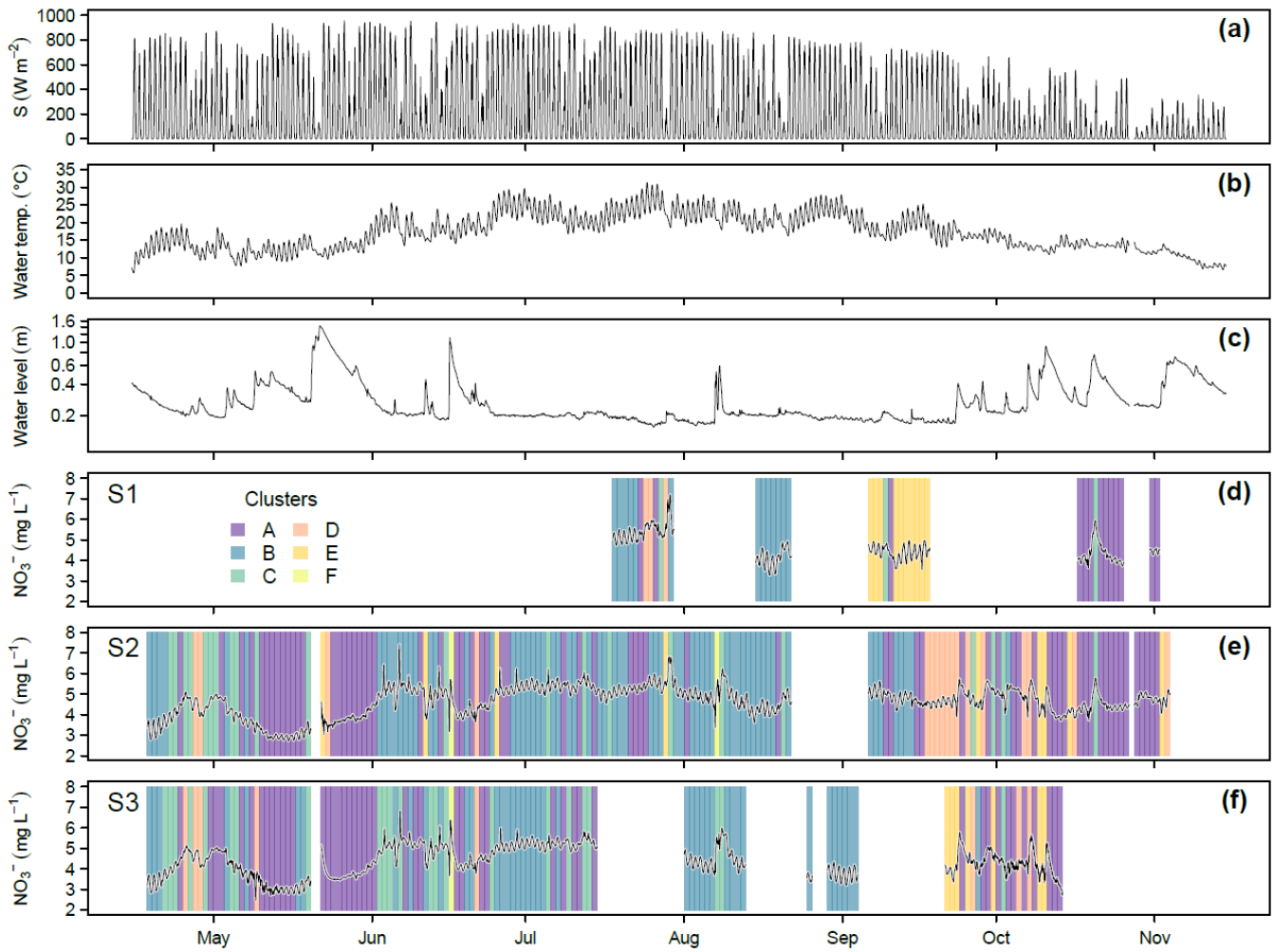
Figure 3: Global irradiance (a), water temperature (b) and water level (c) at S3 as well as NO$_3^-$ concentration and cluster occurrence at the monitoring sites S1 (d), S2 (e), and S3 (f). Background colors in panels d to f indicate to which cluster the corresponding day was assigned.

215 **3.2   In-stream vs. transport control on diel patterns**

The time lags between diel NO$_3^-$ 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^{-1} ~~resulted in~~and 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~~increased with decreasing stream flows. The fact that the independently determined

220 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

225 stream flow were mainly assigned to cluster B and those above median stream flow to cluster A.
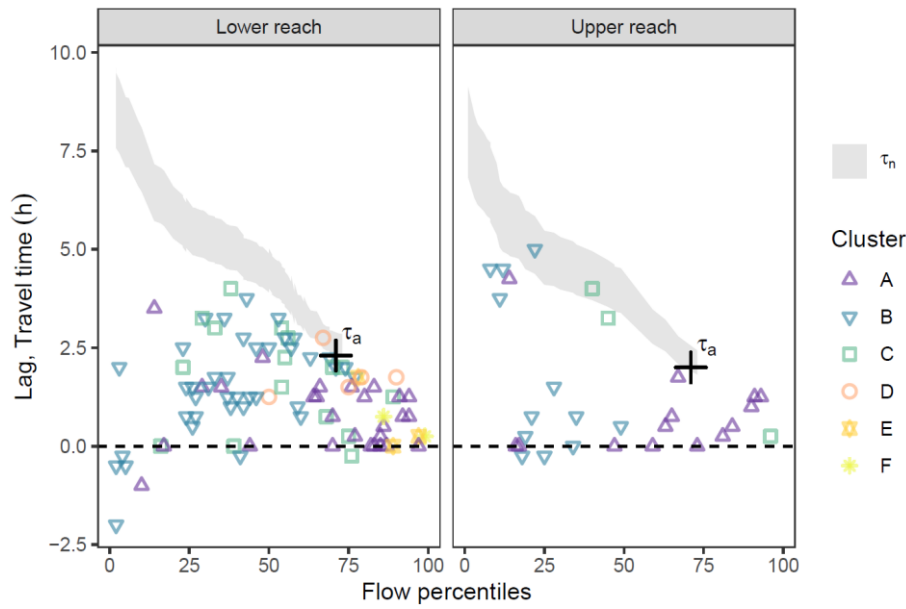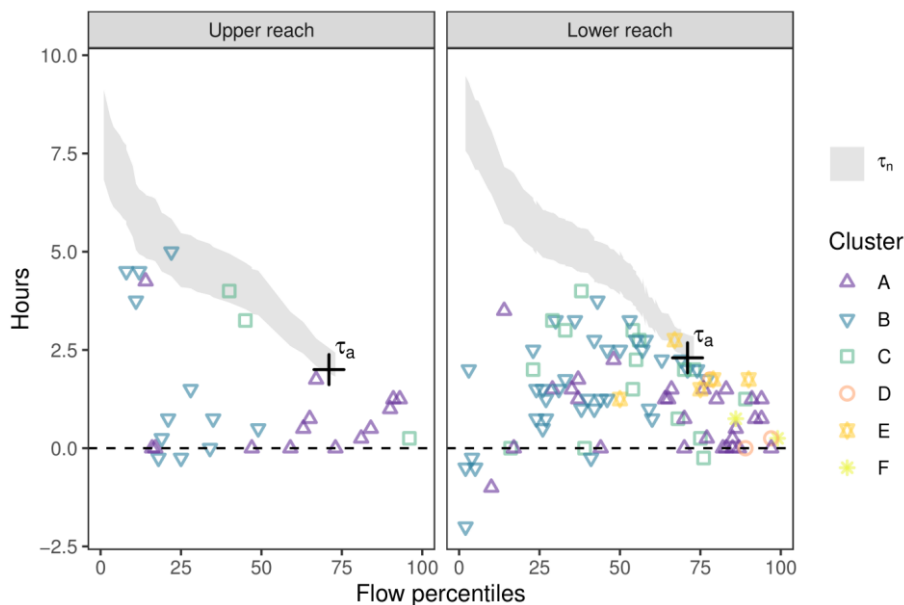
Figure 4: ~~Travel time~~Time lag **between diel NO₃⁻ signals at adjacent monitoring points (colored symbols) compared to the tracer travel time (τ_a, black cross) and the range of nominal travel time estimates (τ_n, shaded area). No travel times were estimated when discharge exceeded the validity range 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). Points falling below the shaded areas indicate in-stream control on diel NO₃⁻ patterns, whereas points within the range of travel time estimates suggest transport control.**~~.~~

3.3 Characterization of clusters

We found clear differences in the distribution of daily means of environmental parameters among clusters (Fig. 5). The following characterization of the clusters refers to significant differences ($p<0.05$) according to Tukey HSD test applied to an ANOVA on the cluster data. Cluster A presented overall lowest NO₃⁻ concentrations (median 4.36 mg L^{-1}) which differed from those during cluster B (median 4.87 mg L^{-1}) and C (median 4.88 mg L^{-1}). Cluster A also showed the lowest water temperature (median 14.1 °C) and elevated water levels (median 41.9 cm) compared to cluster B and C. Cluster B was characterized by the highest global irradiance (median 825.0 W/m²), highest water temperature (21.7 °C) and lowest water levels (median 21.2 cm). Disregarding cluster F with only 3 data points, the difference in water temperature was significant for all remaining clusters. Global irradiance in cluster B differed from all clusters but C. Water level differed with all clusters but C and E. Cluster C occurred during very similar conditions as cluster B and only differed from cluster B in terms of water temperature (median 16.4 °C). The two clusters D and E were characterized by lower global irradiance than cluster B and C and did not differ from one another. ~~Despite minor variability in NO₃⁻ concentration among clusters, cluster assignments were~~

 Cluster F consisted of only 3 days, but all of these represented water levels (median 77.2 cm) hardly ever observed in the remaining clusters.

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 the differences 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.
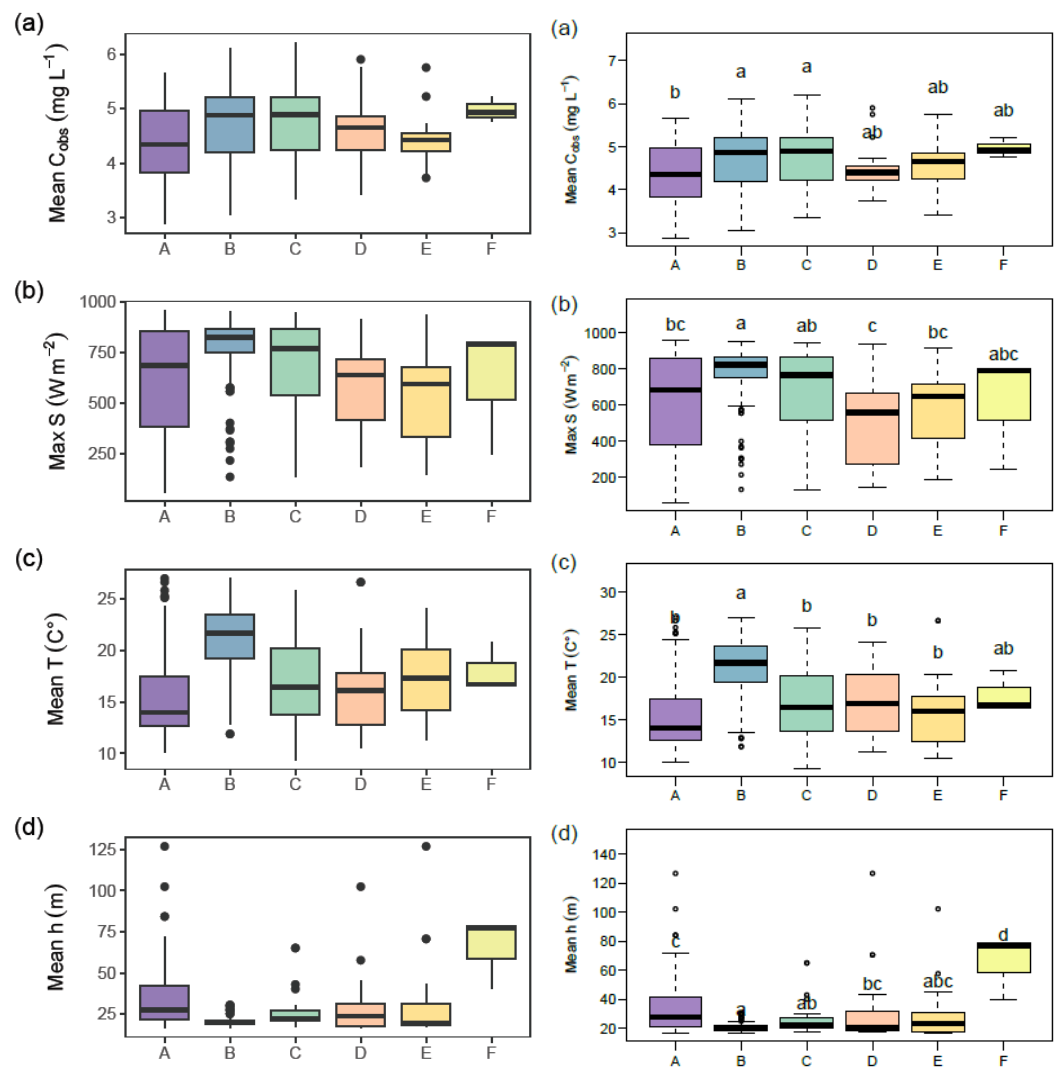
Figure 5: Environmental conditions during occurrence of clusters. The panels show daily average NO₃⁻ concentration (a), daily maximum of global irradiance (b), daily average water temperature (c), and daily average water level (d). Lowercase letters above boxplots were assigned to groups that do not differ significantly according to analyses of variance (ANOVA) and Tukey tests.

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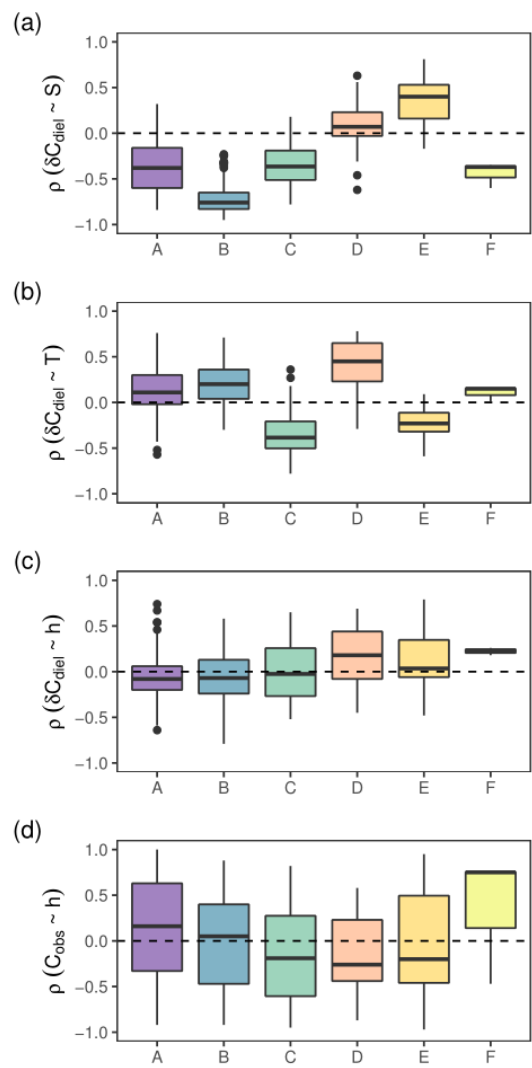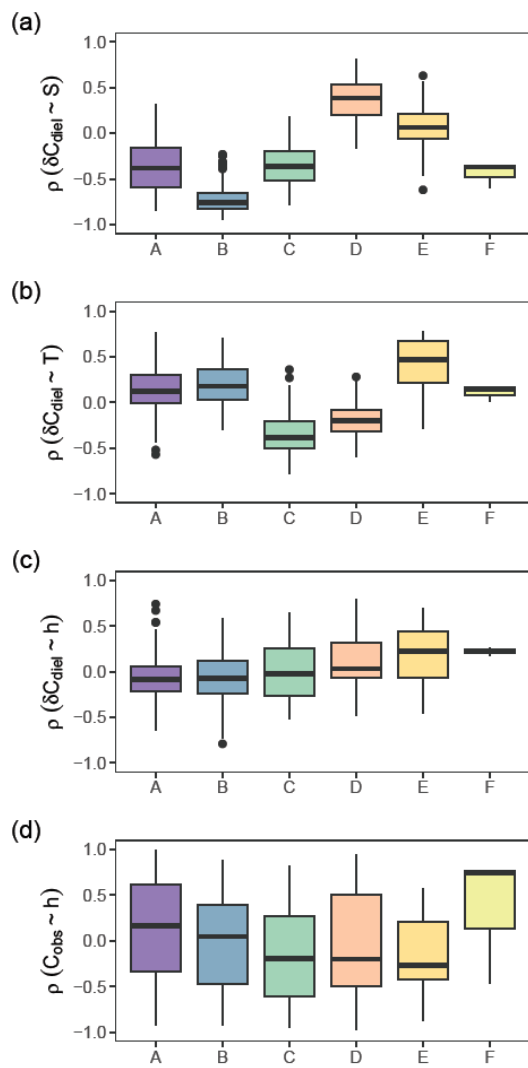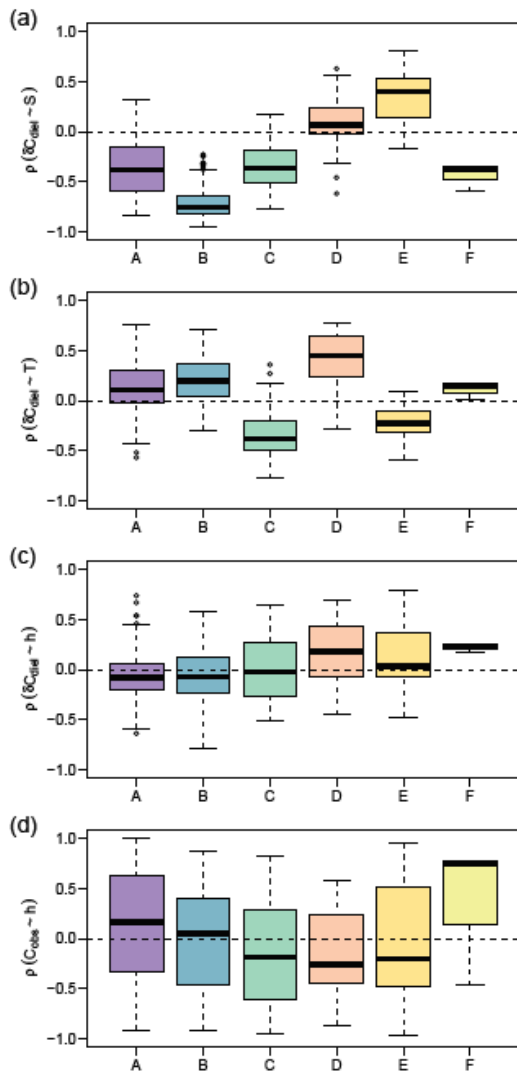
Figure 6: Daily Spearman correlations of the NO₃⁻ signal with potential drivers by cluster. The panels show correlation strength of diel concentration change rate with global irradiance (a), diel concentration change rate with water temperature (b), diel concentration change rate with water level (c), and observed concentration with water level (d).

4 Discussion

4.1 General patterns

In our data, we found patterns in NO_3^- concentration both on the diel and on the seasonal scale. On the seasonal scale, a weak negative correlation of NO_3^- and water level indicated that flow events tended to dilute NO_3^- concentrations in our river. However, particularly after the low flow period in summer, NO_3^- increased during discharge events, an observation that is often explained by the mobilization of previously accumulated NO_3^- in soils (Burns et al., 2019; Lange and Haensler, 2012). The fact that NO_3^- was correlated with stream temperature but not with global irradiance may be a consequence of a more intense seasonal pattern in water temperature than in irradiance, since we started our monitoring campaign in late spring when daily irradiance peaks were already close to their seasonal maximum. On the diel scale, we identified six different NO_3^- patterns that varied seasonally. Interestingly Although daily NO_3^- amplitudes would usually be expected to be highest on days with high metabolic activity (Rode et al., 2016; Heffernan and Cohen, 2010), daily amplitudes of these patterns did not show correlations with daily averages of light intensity, water temperature or water level. The fact that longitudinal stability varied among cluster suggests that less stable clusters (e.g. D and E) either indicated a shift in in-stream conditions between monitoring sites or external controls on diel patterns, e.g. transport.

Variability in Diel NO_3^- patterns concentration 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. Consequently, it is possible that the tributary influenced average NO_3^- levels. It may also be subject to its own diel NO_3^- fluctuations. Yet, the influence on patterns in the main stream must have been small as diel patterns were usually longitudinally stable, i.e. the same upstream (S1) and downstream (S2) of the tributary.

It is also possible that diffuse groundwater influx influenced average NO_3^- concentration at the monitoring sites. In fact, NO_3^- levels in regional groundwater wells 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). Due to these difficulties, Wwe were unable to separate the effects of diffuse groundwater inputs from a potential effect of increased NO_3^- removal in the lower reach due the revitalization measures.

4.2 Interpretation of diel patterns

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~~in the case of pure in-stream control (Fig. 4). The ~~existence~~presence of time 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. W~~, but w~~ith increasing travel distance, however, NO_3^- concentration converged into a stable signal solely defined by in-stream ~~processing~~processes. Depending on the position of observation points along such a stream reach, one may find time lags like those observed ~~at our river Elz~~in our study. Although in our study boundary conditions ~~at our study site are~~were 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~~.

4.3 Lateral inputs

~~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 NO₃⁻ concentration. Groundwater inputs may not only directly affect NO₃⁻ 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.

4.41.1 Interpretation of diel patterns

In the following, we therefore aim to interpret our findings based on in-stream processes. In terms of biochemical processes, diel NO_3^- variability depends on the time-varying balance of NO_3^- removal (via assimilation by both heterotrophs and autotrophs as well as denitrification) and NO_3^- production (via mineralization and subsequent nitrification). We do not regard our interpretations on the controls of the observed patterns complete but as hypotheses for further research on diel dolute patterns to build upon. Considering the idea of multiple superposed biochemical processes as a starting point, some assumptions can be made on the diel course of the processes mentioned above. Photoautotrophic assimilation depends on the light availability and can be conceptualized as a function of solar irradiance. In contrast, the degree of diel variability in nitrification, denitrification, and heterotrophic assimilation is less clear. However, we generally assume that the rate of microbial metabolism (besides other influences) increases with temperature. Figure 7 schematically illustrates the diel concentration signals resulting from overlaying the diel courses of light-dependent photoautotrophic (r_{aa}) assimilation and a complementary temperature-dependent processing rate (r_{comp}). The latter represents the combined net effect of heterotrophic assimilation, nitrification and denitrification. Particularly, we consider different levels of light intensity (columns in Fig. 7) and different types of relationship with temperature (rows in Fig. 7), including positive and negative correlation with temperature as well as constant r_{comp}. The shapes of r_{aa} and r_{comp} reflect means over all measurements of global irradiance and water temperature, respectively, during the course of the present study.

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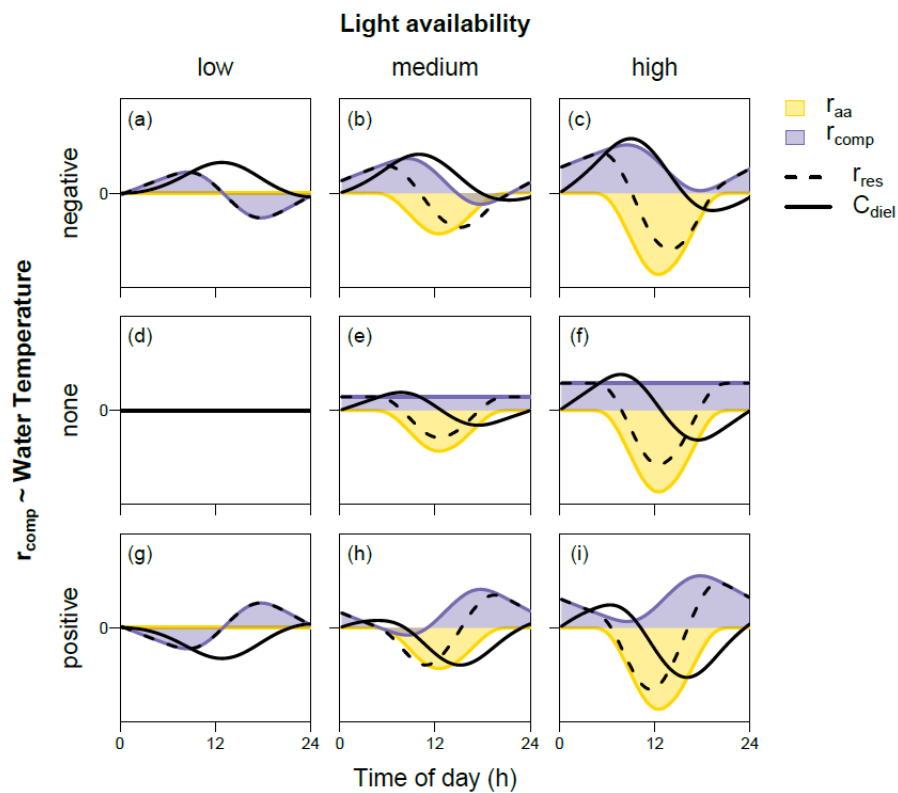
Figure 7: Schematic representation of diel courses of assimilation rate (r_{aa}.) and a complementary processing rate (r_{comp}) required to produce equilibrium conditions. Black lines show the resulting change rate (r_{res}) and concentration (C). While r_{aa} is considered a function of global irradiance (columns represent different levels of irradiation intensity), $r_{comp.}$ is conceptualized as a function of stream temperature (rows represent a negative (a-c), no relationship (d-f) and a positive relationship (g-i)).

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; Heffernan and Cohen, 2010). 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~~ may result from either~~a~~ stronger photoautotrophic NO_3^- uptake compared to cluster A (Fig. 7e and f) or added effects of intense assimilation and diel variability in the interplay of other NO_3^- depleting or producing processes (Fig. 7c and i). 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 are hard to explain by photoautotrophic assimilation alone in systems without intense seasonal shading by riparian vegetation (as opposed to e.g. Rusjan and Mikoš (2010)).~~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.~~ Figure 7 (a, b, c, f) shows that NO_3^- peak time gradually shifts towards midday when photoautotrophic assimilation decreases and the relative importance of a NO_3^- depleting process negatively related with temperature increases. This suggests that either denitrification or heterotrophic assimilation or both are promoted by stream temperature and drive the shape of the signal. Diel variability has been observed both in denitrification (Christensen et al., 1990; Harrison et al., 2005; Cohen et al., 2012) and heterotrophic respiration (Hotchkiss and Hall, JR., 2014) which is closely linked to heterotrophic NO_3^- assimilation. However, peak NO_3^- depletion occurs in the afternoon, when oxygen levels are expected to be elevated and unfavorable for anaerobe denitrification (Rysgaard et al., 1994). In addition, it is not clear how denitrification in the lower anoxic sediments could be promoted by temperature without simultaneously increasing nitrification in the upper sediment layers. However, it seems possible that the driving force of cluster C was not temperature but another process with similar diel

pattern. Exudation of algal photosynthate rich in labile organic carbon (Kaplan and Bott, 1982) may have a similar diel course and stimulate assimilation and denitrification by heterotrophs but not nitrification by autotrophs. Under such conditions, heterotrophic assimilation or denitrification or both may drive diel NO_3^- fluctuation in cluster C.

In literature, diel patterns with a NO_3^- peak in the afternoon (cluster D) have been attributed to intense evapotranspiration (Aubert and Breuer, 2016; Flewelling et al., 2014; Lupon et al., 2016a). In the present study, evapotranspiration was not measured, however, it did not produce systematic diel fluctuations in water level and the latter were not correlated with diel NO_3^- signals. Such patterns could also not be reproduced by overlaying rates of light-dependent and temperature-dependent processes (Fig. 7). A satisfactory explanation for cluster D is therefore still needed.

Diel patterns with a midday low (cluster E) could be the result of low photoautotrophic assimilation and a temperature-dependent NO_3^- producing processes like nitrification (Fig. 7g). ~~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~~ Diel variability in nitrification is well documented (Warwick, 1986; Laursen and Seitzinger, 2004; Dunn et al., 2012) and it seems principally plausible that temperature promotes nitrification without influencing denitrification in deeper anoxic sediment layers. Another reason for independence of nitrification and denitrification may be limitation of heterotrophic denitrification in absence of an organic carbon source.

~~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~~
415 ~~influence hydrologic retention of NO₃⁻ (Lupon et al., 2016a). 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~~ adverse processes like nitrification on the one hand and~~and~~ denitrification and heterotrophic assimilation on the other hand ~~and possibly also physical processes like diel variability in~~
420 ~~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.

~~4.5~~4.3 Conclusions

425 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
430 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~~ heterotrophic respiration 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,
435 and on different N species~~, most importantly NH₄⁺~~. 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.

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