Biogeosciences
Discussions

Diel patterns in nitrate concentration suggest importance of microbial pathways for in-stream processing

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Abstract. Diel cycles in stream nitrate concentration represent the sum of all processes affecting nitrate concentration along the flow path. Being able to partition diel nitrate signals into portions related to different biochemical processes would allow

10   to calculate daily rates of such processes that are urgently needed for water quality predictions. In this study we analyzed diel nitrate patterns at three locations in a 5.1 km long stream reach draining a 430 km², mainly forested but anthropogenically influenced catchment during one growing season. We tested if the observed diel variability in nitrate concentration resulted from upstream sources and subsequent downstream transport or emerged simultaneously along the stream. We determined time lags between monitoring sites by cross-correlation. We found that time lags were closer to zero than travel time estimation

15   assuming plug-flow suggested and concluded that ubiquitous in-stream processes prevailed in the creation of diel variability. To further analyze the diel nitrate signals we used k-means clustering to identify patterns in the diel portion of nitrate concentrations and interpreted the resulting clusters with regard to potential drivers and the calculated nitrate balance of sub-reaches. We found that 70% of all diel patterns were attributed to clusters negatively related to the diel course of insolation with highest nitrate amplitudes on warm and sunny days and low water levels. We argue that temporal shifts towards the

20   remaining clusters are rather due to shifts in microbial nitrate processing than in photosynthesis-driven plant uptake. These results suggest that the magnitude of microbial nitrate processing may be large compared to plant uptake.

1   Introduction

In-stream processing of nutrients can significantly influence loads and concentrations transported to receiving ecosystems (Roberts and Mulholland, 2007). Nutrients are temporally taken up and immobilized by organisms during downstream

25   transport, a concept known as "nutrient spiraling" (Ensign and Doyle, 2006). Thus, in-stream nutrient processing may reduce the risk of harmful eutrophication (Birgand et al., 2007). As a result of human activity, many streams nowadays exhibit increased levels of nitrogen (Dodds and Smith, 2016). Among the different N-species, NO_3^- (nitrate) is of special interest since it usually represents the largest fraction in dissolved inorganic nitrate (DIN) and it is nowadays easily detectable using in-situ spectrometer probes. At the same time, water quality management requires knowledge of nitrate processing rates to predict

30   how rapidly nitrate inputs will be transformed and attenuated. This is particularly important in light of changing climatic conditions and possible associated drought periods (Austin and Strauss, 2011).

Similar to other solutes, e.g. dissolved oxygen (DO) or CO_2, nitrate concentrations exhibit diel (i.e. with a period of 24 h) cycles. The increasing body of high frequency nitrate monitoring data resulting from availability of optical in-situ probes

35   shows that such diel cycles are not ubiquitous. Some streams consistently exhibit strong diel patterns (Heffernan and Cohen, 2010), others do so only during certain seasons (Rusjan and Mikoš, 2010; Aubert and Breuer, 2016; Schwab, 2017), still others do not present diel patterns at all (Duan et al., 2014). Complementary to other approaches such as stable isotope studies (Pellerin et al., 2009; Mulholland et al., 2009; Cohen et al., 2012; Gammons et al., 2010), nutrient addition experiments (Covino et al., 2010), and benthic chambers (Hensley and Cohen, 2020), analysis of diel nitrate cycles can be used to quantify processing

40   rates (Heffernan and Cohen, 2010; Rode et al., 2016). Diel nitrate cycles can be described as the convolution of upstream boundary conditions and processes occurring along spatially heterogeneous flow paths (Hensley and Cohen, 2016). Correspondingly, this method aims at estimating rates of individual processes by deconvolving the nitrate concentration time series. This requires prior knowledge on the timing of the involved processes, including biochemistry, transport and external inputs.

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Biochemical processes influencing nitrate concentration include nitrate depletion via denitrification and uptake by both autotrophs (U_A) and heterotrophs, as well as production via mineralization and subsequent nitrification. These processes depend on environmental conditions and are interrelated with stream metabolism on a fine temporal scale (Burns et al., 2019). Due to photosynthetic light requirements, U_A can be considered zero at night (Heffernan and Cohen, 2010) and conceptualized

50   as a function of insolation (Hensley and Cohen, 2016). This restriction has been used to partition the difference between observed nitrate concentrations and an interpolated baseline into U_A and microbial net depletion (Heffernan and Cohen, 2010), assuming all other processes to be constant. However, there is evidence that diel variation may not only be influenced by U_A. 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). Such

55   diel variability in microbial nitrate processing would cause the above partitioning approach to fail (Kunz et al., 2017).

In flowing waters, biochemical processes are superposed with downstream transport. The solute signal measured at any location integrates over all conditions and events that water parcels passing that location were previously exposed to. Correspondingly, the benthic footprint, i.e. the upstream area influencing concentrations at the measurement point, depends

60   on the flow velocity and the solute turnover rate. While gaseous solutes like DO may quickly equilibrate with the atmosphere, upstream discontinuities in nitrate (e.g. tributary confluxes, lakes or reservoirs, groundwater or waste water inputs) may persist further downstream (Hensley and Cohen, 2016). In open systems with unknown upstream boundary condition, it is therefore

a priori unclear whether observed diel patterns are produced by conditions in the associated stream reach or at some upstream origin from which they are advected downstream.

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In the present study we analyze diel nitrate patterns observed at three connected monitoring sites along the lower course of the river Elz in Southwest Germany. We hypothesize that (1) diel nitrate cycles in the investigated system are predominantly produced by in-stream conditions and (2) are indicative of daily net nitrate processing in the stream sub-reaches. We test our first hypothesis by comparing time lags between adjacent monitoring sites to benchmark travel time determined by a
70   conservative tracer injection. In order to test the second hypothesis, we analyze the different diel patterns present in the monitoring data, under which conditions they emerge, and how they are related to nitrate balances of the sub-reaches.

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
75   Emmendingen and Riegel (Figure 1). At our study site the river Elz drains an area of approximately 430 km² of which 66% are forested and 21% are grassland. The fraction of cropland is below 2%. The catchment contains man-made structures including several weirs and one wastewater treatment plant about 25 km upstream of the study site. Most wastewater, however, is collected in a larger treatment plant further downstream. The monitored stream reach spans a distance of 5.1 km and is subdivided into two sub-reaches with different morphology. While the upstream reach (2.7 km) is characterized by a uniform
80   gravel bed which is straightened and protected against erosion by regularly spaced groundsills and in this sense is representative of many rivers in Southwest Germany, the downstream sub-reach (2.4 km) was subject to extensive revitalization measures including flood dam relocation and installation of a near-natural meandering river course. The constructions were completed in 2016 and since then natural dynamics are controlling its morphology. Both sub-reaches are characterized by a largely open canopy and shallow water depth usually below 0.4 m, allowing light to reach the stream bed. However, in the downstream
85   sub-reach water depth is more variable, so that depths exceed 1.5 m at some locations. This pattern corresponds to an increased variability in flow velocities in the downstream sub-reach. A minor tributary enters in the upstream sub-reach, its influence on the results of this study is discussed later.
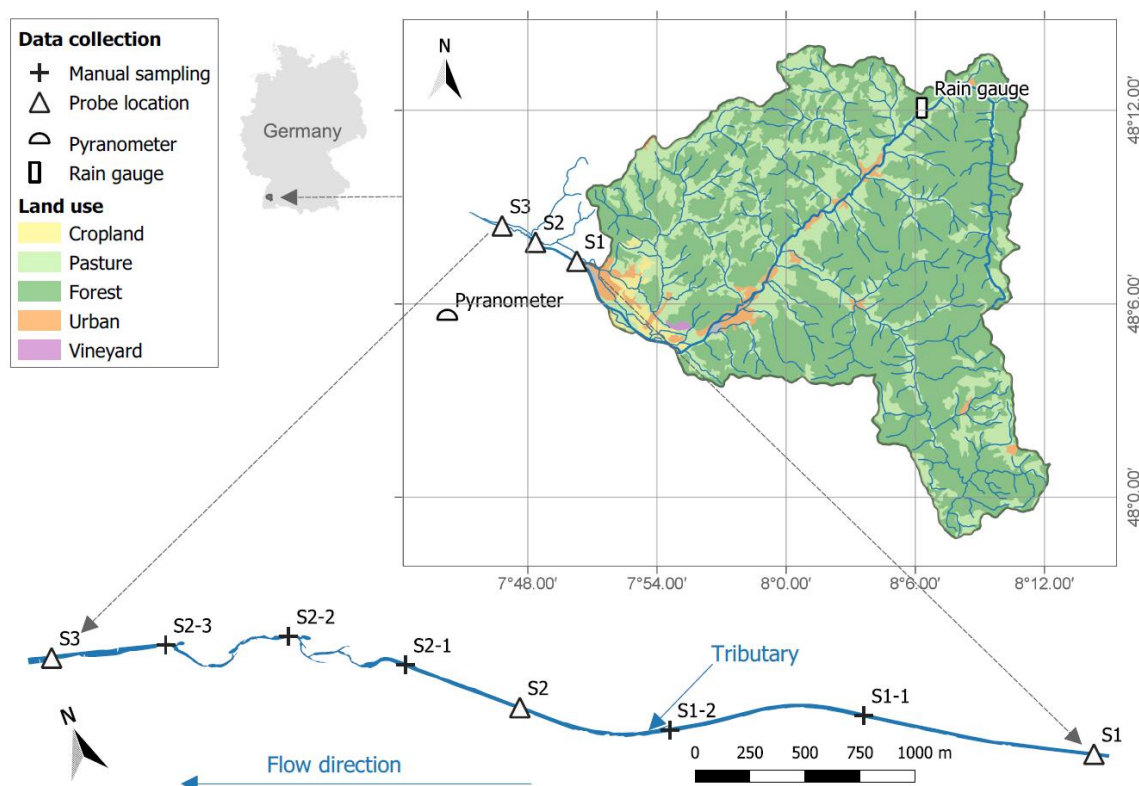
Figure 1: Location of monitoring points in the stream reach and contributing catchment.

2.2 Data collection

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Nitrate concentration was measured at 15 minute intervals at the three monitoring sites using UV-Vis spectrometer probes (spectro::lyser, s::can Messtechnik GmbH, Vienna) 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 to the in-situ measurements, biweekly grab samples were collected at eight locations

95 along the studied stream reach, including the probe locations, to assess longitudinal concentration profiles and validate probe measurements. Samples were analyzed using ion chromatography (Dionex ICS-1100, Thermo Fisher Scientific Inc., USA). Stream temperature and water levels were continuously recorded at S3 (TD-Diver, Van Essen Instruments, Netherlands) at 15 minute intervals. Discharge was calculated using a rating-curve based on two local NaCl tracer injections during stationary flow conditions, data of which was provided by the local water agency, and one additional tracer test during elevated water

100 levels on November 15th 2019 (> 70 % of recorded water levels). In the latter tracer test we injected 33 kg of NaCl at S1 so that both sub-reaches were covered. Solar radiation data was obtained from a climate station at the nearby (< 10 km) Loechernbach experimental site in Eichstetten.

2.3 Data analysis

2.3.1 Assessing the origin of diel nitrate variation

105 If diel nitrate variation originated from some upstream source and subsequent downstream transport, time lags should be detectable between nitrate signals at adjacent monitoring points that correspond to solute travel times between these points. In order to exclude an external source for diel nitrate patterns we determined the time lags between nitrate signals by means of cross-correlation which is a standard method for determining temporal shifts between signals (Derrick and Thomas, 2004) and compared them to the tracer travel time (τ_a) and the nominal water residence time (τ) according to Kadlec (1994). While τ_a is

110 the first moment of the tracer residence time distribution, τ is the ratio of reach volume and discharge. We determined reach volume from water level recordings at S3 and observations of channel width. In order to account for variability in channel geometry, we estimated minimum and maximum values of τ using rough estimates of channel widths between 20 and 25 m in the lower sub-reach and 15 to 20 m in the upper sub-reach. Time lags obtained from cross-correlation were tested for difference from zero using two-sided t-tests.

2.3.2 Identification and characterization of diel patterns

We used k-means cluster analysis to identify and classify diel patterns in stream nitrate 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 algorithms by Hartigan and Wong (1979) that is implemented in the 'stats' R-package (R Core Team, 2019). In order to ensure that the resulting clusters represented variability in diel cycles and not in

120 nitrate background concentrations the analysis was done on the diel portion of the solute concentration signal, hereafter referred to as residual concentration (C_{res}). 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 introduces some degree of subjectivity is the determination of number of clusters k. We therefore tested cluster numbers ranging between 2 and 20 and determined the best partition by both assessment of explanatory benefit per additional

125 cluster, also known as 'elbow method', and visual inspection of clusters. The elbow method was not clearly indicative, however, we opted for six clusters as higher values of k did not produce new clusters in terms of timing but rather caused further splitting of existing clusters by amplitude.

In order to further characterize the identified diel patterns, we assessed environmental conditions during the occurrence of the

130 respective clusters. Particularly, we compared daily average and amplitude of nitrate concentration, average of water levels (h_{mean}), solar radiation (S_{mean}) and water temperature (T_{mean}). We further investigated the relationship of diel patterns to diel signals of potential drivers, i.e. insolation and water temperature. Diel nitrate cycles reflect the time-varying balance between nitrate inputs and producing and depleting processes, e.g. nitrate concentration increases the quickest when nitrate production

is most dominant. This means that potential drivers should be correlated to the rate of change of C_{res}, i.e. to its first derivative

135 δC_{res}. Correspondingly, we related δC_{res} to the observed diel signals of insolation (S) and water temperature (T) by calculating daily Spearman rank correlation coefficients. Another potential driver of diel solute cycles is discharge variation. In contrast to the above drivers of biochemical processes, discharge would directly affect solute concentration. For example, if stream water was diluted by rainfall, maximum discharge and minimum concentration would occur simultaneously and not be mediated by the rate of a process. We therefore expected a potential relationship of discharge with C_{obs} rather than with δC_{res}.

140 Correspondingly, we assessed the potential effect of diel variation in discharge by correlating C_{obs} to water level recordings (h), avoiding uncertainties from rating-curve extrapolation.

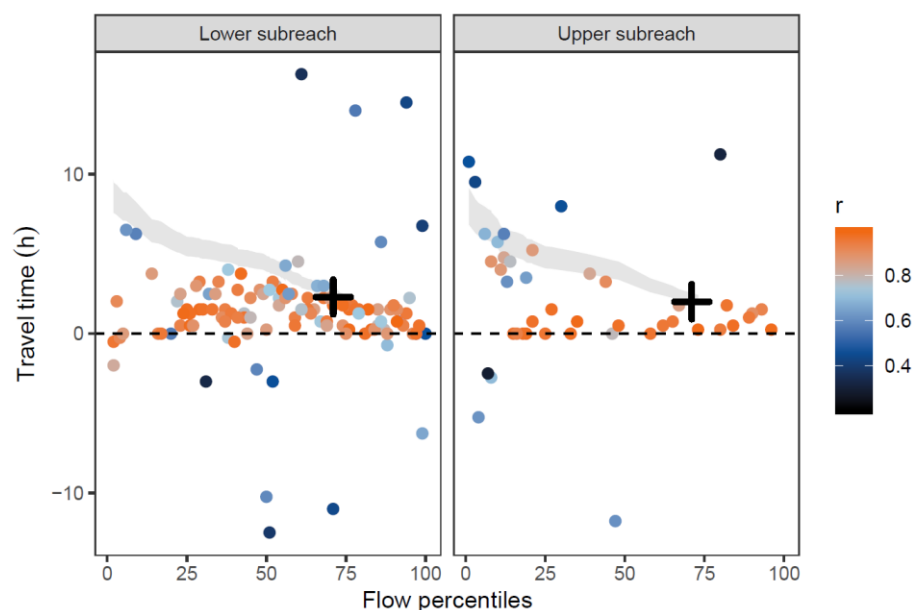### 2.3.3   Assessing the relation of diel patterns to the nitrate balance

The influence of diel patterns on the stream nitrate budget was assessed by calculating both temporal and spatial net change. As sub-reach balance we understand the difference between daily means of nitrate concentration at an upstream (C_{up}) and

145 downstream (C_{down}) monitoring site, thus exclusively representing changes that happen in the stream reaches between these points:

$$\Delta C = C_{down} - C_{up} . \tag{1}$$

3   Results

3.1   Prevalence of in-stream processes in creation of diel patterns

150 The benchmark tracer injection at a water level of 15.4 cm and a corresponding discharge of 2.0 m^3 s^{-2} resulted in travel time estimates of 2.0 h in the upper and 2.3 h in the lower sub-reach, while time lags of nitrate determined by cross-correlation were very variable. We considered lags between signals with strong cross-correlation more reliable than for signals with a weak or no correlation. Lags resulting from strong cross-correlation (r > 0.75) ranged between zero and travel time estimates from both the tracer injection and nominal water residence time (Figure 2), indicating that time lags were shorter than solute travel time.

155 Thus, time lags were usually too small to be considered the result of advective downstream transport of the concentrations signal. At the same time, lags were statistically different from zero (both sub-reaches p<0.001 in two-sided t-tests).
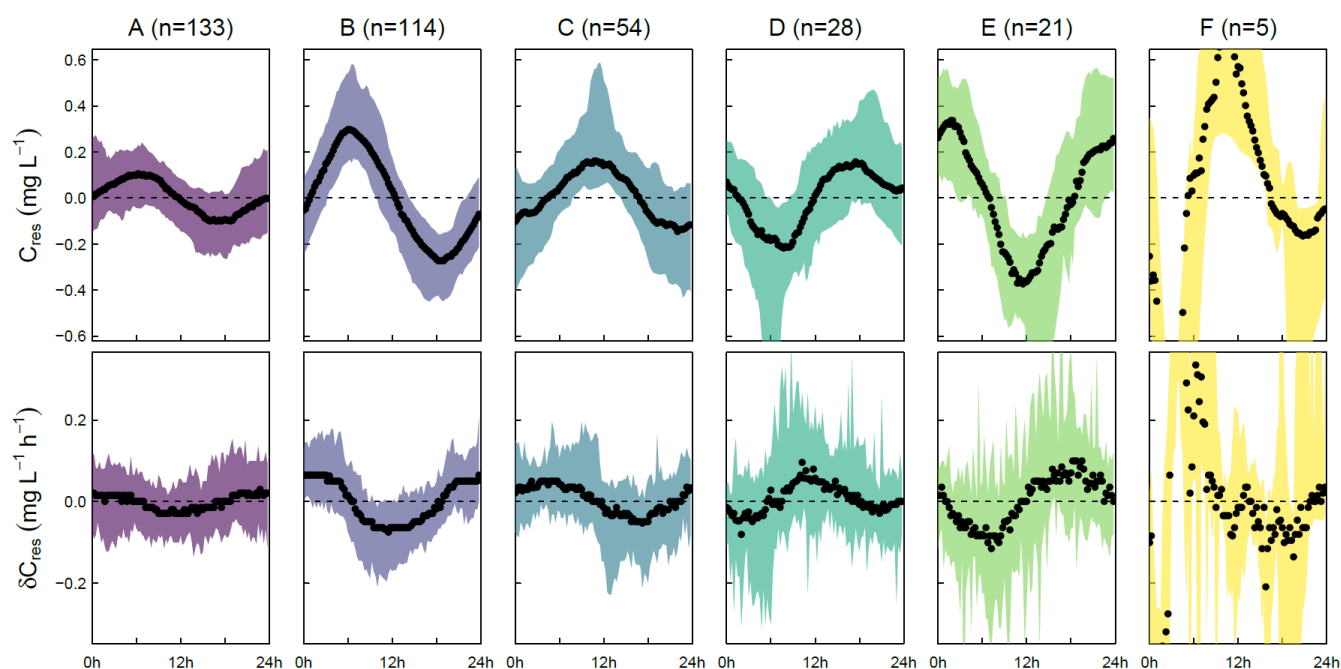
Figure 2:Travel time between diel nitrate signals at adjacent monitoring points compared to the tracer travel time (black crosses)
160   **and the range of estimated nominal travel time (shaded area). Color of the dots indicates strength of cross-correlation.**

3.2   Diversity in diel patterns

Data collection at the three monitoring sites resulted in 355 complete diel nitrate signals, almost all of which showed a diel pattern (Figure 3). The cluster analysis resulted in 6 clusters that clearly differed in terms of amplitude and timing of minimum and maximum concentrations. 70% of the days were attributed to the clusters A (n=132) and B (n=115) which both reached

165   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=54), in the afternoon (cluster D, n=28) and around midnight (cluster E, n=21). The last cluster F did not include enough days (n=5) for a proper characterization. The medians of clusters A to E roughly represented sine waves shifted in phase by a quarter of a period (0.5 π) or 6 hours in units of time. Correspondingly, the derivatives of the clusters preceded the residuals by another quarter of a period. Note that

170   δC_{res} was the rate of concentration change of the diel portion of the concentration signal (C_{res}) and not of observed concentrations (C_{obs}). The change rate of diel residuals resembled the signal shape of potential drivers of diel patterns but may differ in absolute terms as it was determined from the detrended data. Particularly the fact that its sign switches between positive and negative does not imply that the direction of associated processes would also do so in reality.
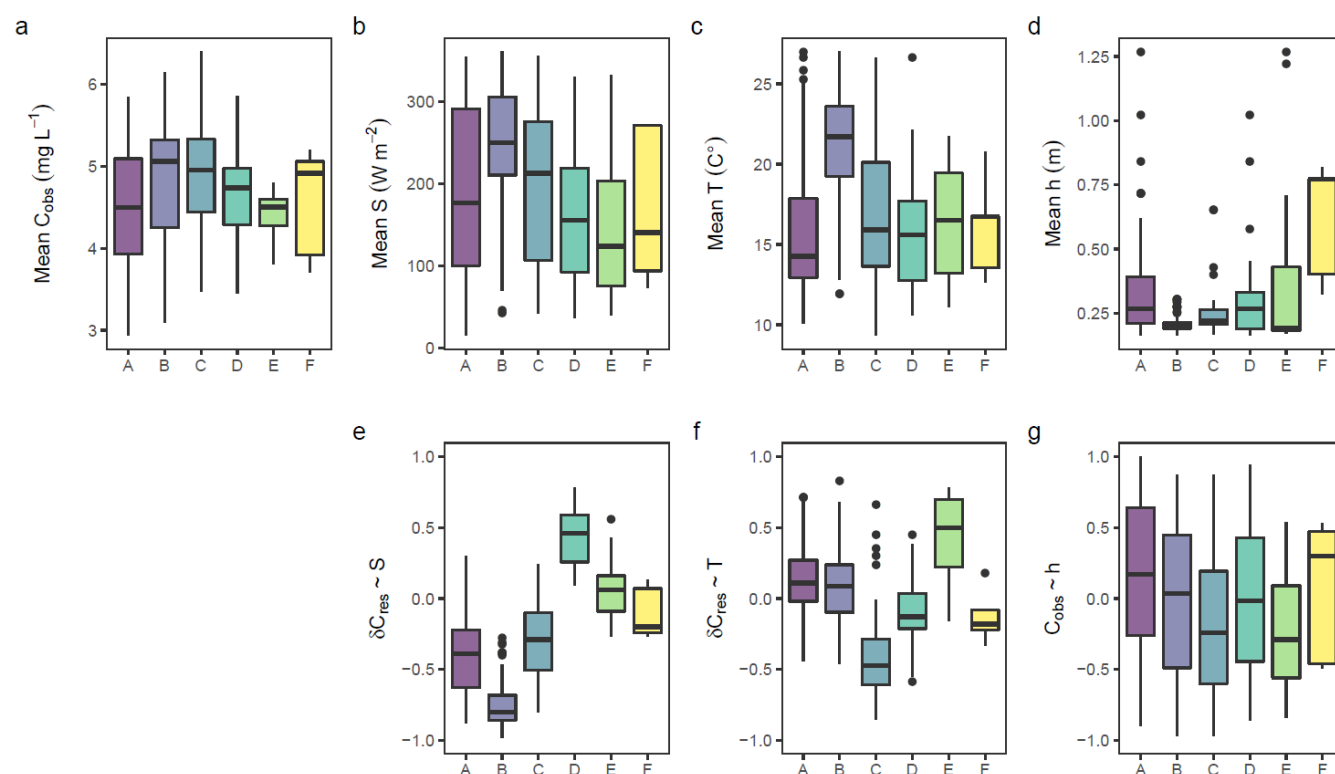
Figure 3: Clusters found in diel residuals of nitrate concentration (C_{res}) and its derivative (δC_{res}).
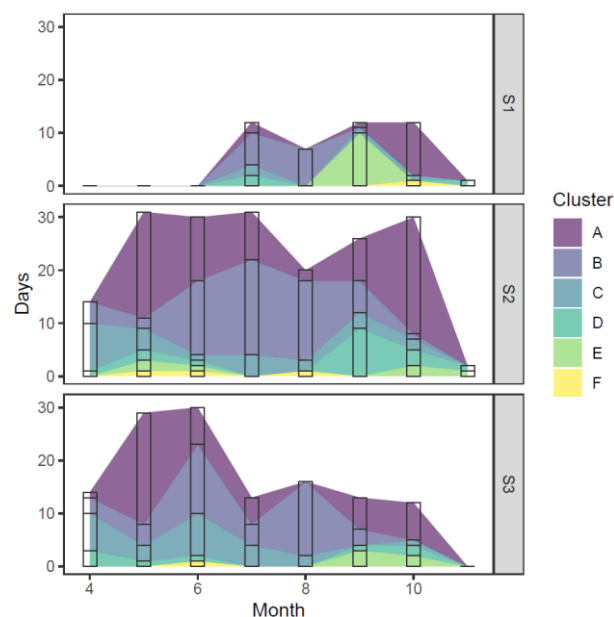
While the diel patterns are not clearly related to different concentration levels of nitrate, they are characterized by different environmental conditions (Fig. 4). The most distinct cluster B shows the highest solar radiation (median: 250.0 W/m²) and highest water temperature (median: 21.7 °C). The other clusters emerged during lower water temperatures (median: 15.2 °C) and variable solar irradiation. Daily average water levels were lowest and strongest confined in cluster B (median: 20.1 cm) and highest in cluster F (median: 77.2 cm) with the remaining clusters representing intermediate flow conditions. Further assessment of the diel dynamics of potential drivers revealed that δC_{res} was positively correlated with solar radiation in cluster D (Fig. 4e), and negatively in clusters A, C, and especially strongly in cluster B. Correlations of δC_{res} with temperature appear in cluster C (negative) and cluster E (positive) (Fig. 4f). Correlations of nitrate concentrations with diel water level fluctuations (Fig. 4g), despite being high in some cases, are not very systematic and show a high variability in all clusters.

Figure 4: Properties of clusters and associated environmental conditions. The upper panels show daily means of measured nitrate concentration (a), irradiance (b), water temperature (c), and water level (d). The lower panels show daily Spearman rank correlation of the change rate of diel residual concentrations (δC_{res}) with irradiance (e) and water temperature (f) as well as daily correlations of measured concentration with water level (g).
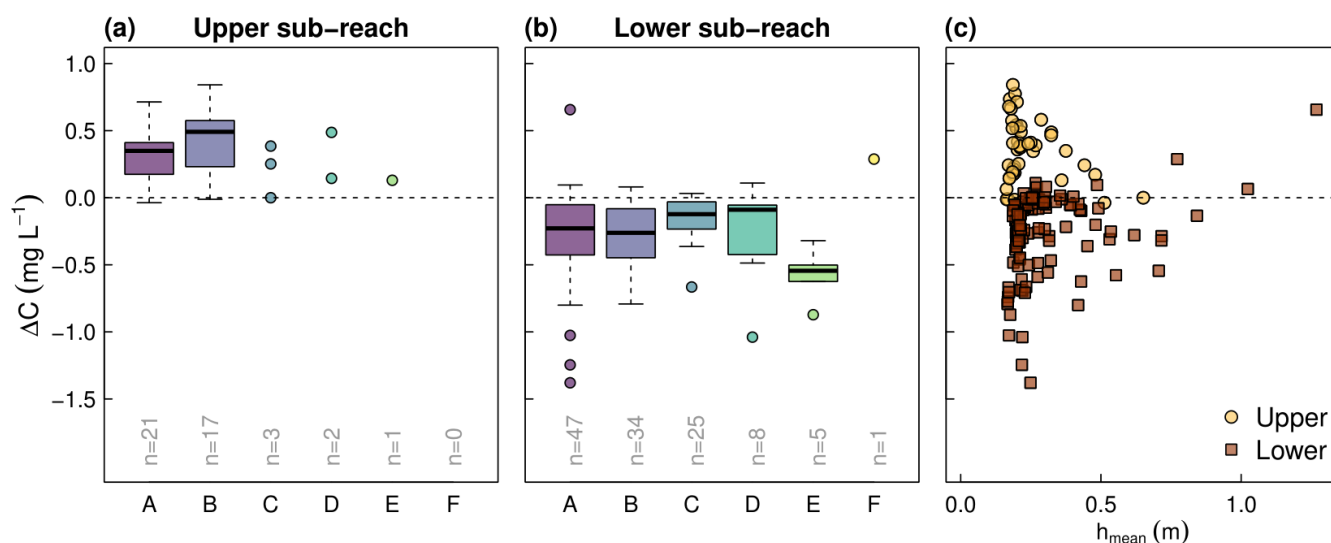
The relationships of the clusters with the different environmental variables are reflected in their seasonal occurrence (Fig. 5). The general pattern is similar at all monitoring sites, but most clearly visible at the intermediate site S2 where the data set was most complete. From June to August the radiation-related cluster B dominates. In colder months in spring and fall the diel nitrate amplitude decreased and cluster A replaces cluster B. Cluster C appeared most often in early spring but continued to play a minor role throughout the entire season. Cluster D, E and F are marginally present throughout the season with a short continuous block of cluster D and E at sites S1 and S2, respectively, in September.

200   **Figure 5: Seasonality of cluster occurrence**

3.3   Relation of nitrate clusters reach balance

No relation was found between clusters and ΔC. In the upper sub-reach (Fig. 6a), nitrate concentrations increased in almost all cases. Median nitrate surplus was 0.35 mg L^{-1} in cluster A and 0.49 mg L^{-1} in cluster B. In the lower sub-reach a deficit was observed for most days (Fig. 6b). Decreases in median concentration ranged between -0.09 (cluster C) and -0.54 mg L^{-1}

205   (cluster E). In both sub-reaches, imbalance is most pronounced during low water levels (Figure 6c), i.e. low flow conditions, and decreased for higher water levels.

Figure 6: Sub-reach balance of nitrate concentrations by cluster attributed to the corresponding downstream monitoring point and compared to water level (h_{mean}) as well as daily net change in nitrate by cluster.

4   Discussion

4.1   Prevalence of in-stream processes in creation of diel patterns

We found that travel times calculated from the diel nitrate signals at the monitoring points were usually between zero and the estimated water travel time (Fig 2). However, lags only exceeded our estimate of minimum travel time in a few cases. These data points were often associated with a low cross-correlation and hence less reliable for time lag estimation. On the one hand, spatial heterogeneity in environmental conditions and stream properties may cause some transformation of solute signals despite spatial synchrony in biochemical processes (e.g. start of photosynthesis may be delayed in temporary shaded areas), explaining lags scattering around zero. On the other hand, solutes cannot travel faster than water and travel time will increase with decreasing flow. Our estimates of minimum nominal water travel time can be considered conservative in the sense that plug-flow (maximum flow velocity) was assumed and no factors were included that may further delay solute transport when water levels decrease, e.g. reduced short-circuiting. 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.

In-stream biotic control on nutrient biogeochemistry was also stated by Roberts and Mulholland (2007) in a forest stream in Tennessee, US. A simulation of longitudinal evolution of diel nitrate patterns by Hensley and Cohen (2016) showed that a distance of tens of kilometers can be required for nitrate concentration from a constant source to converge to a stable diel pattern. During convergence, they observed that the timing of the daily nitrate minimum oscillated with a longitudinal period

of about 11 km, corresponding to the mean distance travelled in 24 h, i.e. distance between extremes was 5.5 km. This distance is comparable to the distance between monitoring sites in our study (5.7 km from S1 to S3) and flow length from the main source area of the river Elz to the monitored stretch ($\approx$ 45 km) was also comparable to the convergence distance observed by

230 Hensley and Cohen (2016). It therefore seems plausible that longitudinal stability of diel nitrate patterns was not yet fully reached at our monitoring sites and the observed differences in timing (time lags > 0 in Fig. 2) were due to longitudinal variability in the diel nitrate signal. However, convergence distance in such a model may depend on transport parameters and residence time in the simulated stream reach. Hence, further research is needed to investigate the influence of river hydraulics on the diel solute patterns.

235 **4.2   Which processes may cause the observed diel nitrate patterns?**

According to Nimick et al. (2011), diel nitrate concentrations reported in literature are usually characterized by a minimum in the early evening and a maximum just before dawn as represented by cluster A and B in our study, accounting for 69.6 % of all measured days (n=355). Such patterns are often attributed to nitrate uptake by primary producers. In our study the strong negative correlation between δC_{res} and global radiation, which was observed in cluster B (and to a minor extend also in cluster

240 A), points in the same direction. The interpretation of the remaining clusters is more complex. While cluster E and F are strongly influenced by unstable discharge conditions and should not be interpreted in regard to instream processes due to non-stationary conditions, flow was stable in clusters C and D. These clusters were characterized by midday and evening concentration peaks, respectively. Possible explanations for the deviation of these clusters from the general pattern of morning peaks and afternoon minima are that either our assumptions on the diel course of plant uptake were violated (i.e. assimilatory

245 uptake was not a function of insolation) or that variability in timing observed in these clusters was driven by microbial processes rather than plant uptake.

4.2.1   Assimilatory uptake likely varies in intensity but not in timing

If plant uptake was the only control on diel nitrate patterns, variability in clusters would indicate temporal shifts in plant uptake that could either evolve from shifts in drivers of photosynthesis or from rate limitations caused by factors other than

250 temperature or light availability. Drivers of photosynthesis did not undergo, despite a minor seasonal effect, systematic time shifts in this study. Neither did canopy development reduce light availability as observed in other studies (Rusjan and Mikoš, 2010; Roberts and Mulholland, 2007) due to absence of a forest or large trees near the stream, nor did certain clusters represent a typical weather condition such as e.g. days with cloudy morning and sunny afternoon. If that would be the case, correlations of δC_{res} with drivers of photosynthesis should have been more or less equal for all clusters. We found that such correlations

255 were clearly different among the clusters (Fig. 4), indicating that photosynthesis-related plant uptake was not the only control on nitrate processes and thus the formation of diel nitrate patterns.

Plant uptake, however, may be temporarily limited by factors other than light availability, e.g. by limitation of other nutrients such as phosphorous. Phosphorus was not continuously measured, however, we did not observe phosphate concentrations >

260   0.5 mg L^{-1} in longitudinal grab samples (as measured with IC). The interplay between diel nitrate and phosphorus cycles is not fully investigated. Cohen et al. (2013) found temporal decoupling of phosphorus and nitrate uptake in the Ichetucknee River (Florida, USA). Yet, phosphorus was not considered a limiting factor in their ecosystem. Mesocosm experiments by Chamberlin et al. (2019) on the effect of nutrient limitation on nitrate uptake showed that P limitation caused reduction of primary production but the influence on diel nitrate uptake remained unclear. We assume that if phosphorus became limiting

265   at some point of time and its uptake less efficient, this would rather cause nitrate uptake to slowly fade out but not to stop abruptly. Then phosphorus limitation would influence the overall intensity of nitrate uptake rather than its diel pattern. We therefore conclude that timing of assimilatory nitrate uptake, in contrast to intensity, most likely did not undergo substantial changes throughout the season.

4.2.2   Variability in microbial nitrate pathways likely cause variability in timing

270   An alternative explanation for the variability in diel cycles could be a dominance of microbial nitrate production or depletion. This would require diel variability in timing of microbial processing. In fact, diel variability was reported for both nitrification and denitrification. Denitrification rate was reported to be reduced during daytime when oxygen levels are high (Christensen et al., 1990; Harrison et al., 2005). Nitrification rates, in contrast, were found to increase with oxygen availability (Dunn et al., 2012; Laursen and Seitzinger, 2004) and depend on pH and temperature (Warwick, 1986). Nitrification can also enhance

275   denitrification in freshwater (Lorenzen et al., 1998) or marine sediments (Marchant et al., 2016) so that denitrification resulting from coupled nitrification-denitrification at an aerobic-anaerobic boundary may, despite unfavorable water column oxygen levels, be stronger during day than during night. Recently, Hensley and Cohen (2020) observed evidence for diel variation in non-assimilatory pathways such as denitrification and nitrification in stream chamber experiments. Lupon et al. (2020) observed spatial variability in nitrification in boreal streams in response to inputs of labile organic carbon. As microbial nitrate

280   processing may happen via these different pathways, the likelihood that changes in environmental conditions cause variability in the dominance among these processes and thereby variability in timing of net processing rate is considered greater than in the case of assimilatory plant uptake.

4.2.3   Dominance of microbial nitrate processing

The above suggests that at least in the formation of clusters C and D, microbial processes dominate over primary production

285   and therefore can imprint their diel course on the nitrate concentrations during these days. However, this is only true, if no plant uptake occurs at night, i.e. all plant uptake happens at daytime and contributes to the formation of corresponding diel patterns. Zero plant uptake at night is a common assumption in studies that aim to estimate nitrate processing rates from high frequency data by interpolating between night-time maxima of nitrate concentration (Heffernan and Cohen, 2010). However,

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Mulholland et al. (2006) determined nitrate uptake in two streams by tracer ^{15}N-NO$_3$ addition and found that uptake at night

290   was smaller than during the day but not zero. This finding was attributed to continued uptake by algae until photosynthate reserves accumulated during daytime photosynthesis were depleted. If such reserves are indeed depleted during the night, the assumption of zero plant uptake at night remains valid. If, however, baseline plant uptake occurs at night, the relative importance of plant uptake would be underestimated.

295   In contrast to plant uptake, rates of opposed microbial processes (e.g. of nitrification and denitrification) may partially cancel each other out. However, if microbial processing generally dominates over plant uptake, minor relative changes in microbial processing might strongly influence diel nitrate patterns and thus increase the likelihood to observe variability in such patterns. In fact, dominance of denitrification over plant uptake has been found in other studies. Heffernan et al. (2010) found that assimilatory uptake was responsible for about 20% of total nitrate removal in the Ichetucknee River (Florida, USA) and

300   attributed the remainder mainly to denitrification. Recently, a similar ratio was found by Preiner et al. (2020) in three reaches with different macrophythe density in the river Fischa (Austria).

4.2.4   Interpretation of individual clusters

The suggested dominance of microbial processes in in-stream nitrate processing has implications for the interpretation of the diel nitrate patterns observed in this study. The diel course of microbial nitrate processing rates has to adopt a shape

305   complementary to plant uptake so that the combination of both rates (δC_{res} in Fig. 3) can reproduce the observed concentration patterns. Diel patterns such as those observed in cluster A are often attributed to primary production and associated plant uptake. The fact that diel change rate in concentrations closely reflected patterns in insolation as observed in this study and also in stream chamber experiments by Hensley and Cohen (2020) invites to this interpretation. However, the effects of plant uptake and denitrification cannot be separated, if both processes work synchronously. Increasing amplitudes from cluster A to

310   B suggest a superposition of both nitrate depleting processes particularly during times of reduced flow velocity at low water levels associated with cluster B. Then a larger fraction of water interacts with stream sediments where denitrification occurs. Although similar diel patterns are often simply attributed to assimilatory uptake, Heffernan et al. (2010) argued that denitrification may be promoted by algal exudates which are rich in labile organic matter and are released during photosynthesis (Wyatt et al., 2012). If such exudates are released by benthic algae, labile organic matter might relatively

315   quickly diffuse into anoxic zones of river sediments and promote denitrification.

Depending on how quickly such zones in the sediment (hyporheic zone) are reached, the resulting peak in denitrification will lag behind the peak of primary production and produce a nitrate concentration pattern like that observed in cluster C. Shifts in diel nitrate patterns similar to those between cluster A and B on the one hand and cluster C on the other hand were observed

320   at a seasonal scale by Rusjan and Mikoš (2010). They attributed this finding to inhibition of photosynthesis and associated

plant uptake by low morning water temperatures in spring and fall. This, however, was not the case in our study, since water temperatures did not regularly fall below the threshold of 10 °C referred to by Rusjan and Mikoš (2010). Furthermore, the temperature range of cluster C did not differ much from the remaining clusters (Fig. 4), except for cluster B. The exact circumstances that caused the formation of cluster C in our study therefore remain unclear.

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The opposite behavior than in cluster A and B was observed in cluster D where a perfect synchrony of nitrification and plant uptake may have caused partial extinction so that a net nitrate production was observed. Similar patterns were found by Hensley and Cohen (2020) during nitrate limitation. Although in our study the reason for low plant uptake is certainly not nitrate limitation, this shows that microbial nitrate processing, i.e. nitrification in this case, may become dominant and imprint its diel

330 variability on nitrate concentrations, if plant uptake is low. Nitrate maxima during the second half of the day were also found by Aubert and Breuer (2016) during summer in two different years.

4.2.5   Implications for seasonality of nitrate processing

The above interpretation of the clusters has implications for the seasonal dominance of certain nitrate processing pathways (Fig. 5). Moderate daytime dominance of nitrate depleting processes occurred mainly in spring and fall (cluster A). Daytime

335 dominance of nitrate depleting processes increased in the summer months (cluster B) when both solar radiation and temperature were highest. During this period synchrony in plant uptake and (possibly coupled by photosynthetic exudates) denitrification may have caused the highest daily concentration amplitudes, except for the disturbed clusters E and F. Cluster C occurred less frequently than cluster A and B but throughout the season and preferentially in spring and early summer. Patterns suggesting daytime dominance of nitrate production, i.e. nitrification, occurred several days in early and late summer (cluster D). A

340 seasonal pattern in nitrate cycles was also found by Roberts and Mulholland (2007) who attributed highest amplitudes to gross primary production, i.e. plant uptake. However, due to dense forest coverage of the investigated stream, maximum amplitudes occurred earlier in the year than at our exposed study site. Aubert and Breuer (2016) associated seasonal shifts in diel nitrate cycles with diel fluctuations in discharge induced by evapotranspiration. We consider this explanation highly unlikely in the investigated system as no consistent diel cycles in discharge were observed.

345 ### 4.3   Reach balance dominated by external processes

No relationship was found among clusters and ΔC but ΔC was clearly different in both sub-reaches across all clusters. This suggests that nitrate mass balances of the sub-reaches were overridden by processes that affected nitrate concentrations regardless of cluster occurrence. These processes might be lateral inflows or groundwater exfiltration, not considered so far in our interpretation. In the upper sub-reach we identified a tributary as a potential nitrate source in which snapshot sampling on

350 a hot day during low flow conditions revealed nitrate concentration to be twice as high as in the main stream. The negative nitrate balance between S2 and S3 may be the result of biological net nitrate removal. Moreover, the increase between as S1

and S2 and subsequent decrease towards S3 reflects the regional pattern of nitrate concentration in the groundwater monitoring wells measured by the state agencies. Regional groundwater wells upstream of S2 typically show higher concentrations than in stream water, while nitrate concentrations in wells downstream of S2 are mainly lower than stream water concentrations.

355 Groundwater influence was originally considered minimal in the study reach due to the presence of drainage ditches along the outside of the levees on both sides of the stream, collecting lateral groundwater flowing to the stream. However, longitudinal sampling with high spatial resolution and infrared imagery suggested the presence of groundwater inflow zones in the lower sub-reach. The explanation of nitrate reach imbalances by unconsidered inflows is in line with the observation that in both reaches nitrate imbalance was greatest during phases of low flow when inflows of limited volume but strongly deviating

360 concentrations cause maximum effects (Fig. 6c).

4.4 Conclusions

Our study shows that diel nitrate patterns recorded at three locations in a 5.1 km long stream reach largely resulted from in-reach processes. Downstream advection of upstream concentration perturbations was ruled out as an explanation for diel patterns since time lags between monitoring sites determined by cross correlation were predominantly smaller than water travel

365 time estimates. Analysis of diel patterns revealed that approximately 70% of all diel patterns were attributed to two clusters that were negatively related to the diel course of insolation with highest nitrate amplitudes on warm and sunny days and low water levels. We suggest that these patterns were caused by synchronous denitrification and autotrophic nitrate uptake, relative importance of which is unclear and may vary according to environmental conditions. In the remaining clusters temporal shifts were evident that could be explained by temporal shifts in microbial nitrate processing but not by photosynthesis-driven uptake.

370 In these cases, microbial processing rates need to be higher than assimilation rates in order to reproduce the observed patterns. In summary, our study suggests that varying dominance and synchronicity of autotrophic assimilation and microbial processes may cause different diel nitrate patterns in stream systems.

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