Collocation of hydrological and biological attenuation of nitrate in an urban stream

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ABSTRACT

The hydrologic and biogeochemical processes that control nutrient export in urban streams are not well understood. Attenuation can occur by tributary dilution, groundwater discharge, and biological processing both in the water column and the hyporheic zone. A wastewater treatment plant (WTP) on Pennypack Creek, an urban stream near Philadelphia, PA, provided high nitrate concentrations for analysis of downstream attenuation processes. Longitudinal sampling for an 8 km reach revealed decreases in nitrate concentration of 2 mg-l^{-1} at high flow and 4.5 mg-l^{-1} during low flow. During high flow δ^{15}N-NO$_3$ increased from 9.5 to 10.5‰, and during low flow increased from 10.1 to 11.1‰. Two reaches were sampled at fine spatial intervals (approximately 200 m) to better identify attenuation processes. Mixing analysis indicated that groundwater discharge and biological processing both control nitrate concentration and isotope signatures. However, fine scaled sampling did not reveal spatially discrete zones; instead these processes were occurring simultaneously. While both processes attenuate nitrate, they have opposite isotope signatures, which may have muted changes in δ^{15}N-NO$_3$. At high flow, a decrease in Cl/NO$_3$ ratios helped distinguish groundwater discharge occurring along both finely sampled reaches. At low flow, biological processing seemed to be occurring more extensively, but the δ^{15}N-NO$_3$ signature was not consistent with either a single process or a sequential combination of groundwater dilution and biological nitrate attenuation. The collocation of processes makes it more difficult to assess biological processing hot spots and predict how urbanization and subsequent stream restoration influence nitrate attenuation.

INTRODUCTION

Nitrogen retention in streams is influenced by a number of complex and interrelated biological and physical processes. The relative proportions of retention occurring in the water column versus the hyporheic zone (i.e., the sediments beneath and adjacent to the stream saturated with stream water) can differ considerably from stream to stream. The role of dilution by groundwater and even tributaries has often been avoided by selecting study reaches where neither is presumed significant, and there is no unified understanding of how processing rates vary with nitrogen concentration and flow conditions. Instead, the data indicate that the relationship between these controlling factors and in-stream nitrate dynamics vary considerably with stream- and region- specific characteristics.

For example, measurements of nitrate biological processing rate are influenced by spatial and temporal scale. Bohlke et al (2009) attempted to quantify the effects of N concentration on denitrification rates, finding a direct correlation between these variables at the reach scale despite a high degree of local variability. Denitrification rates were also influenced by temporal scale as a result of changes in other factors, such as discharge and weather conditions. Spatial and

temporal variability in rates were also identified as a complicating factor in studies modeling nitrate loss as a function of discharge (Alexander et al., 2007) and those using tracer tests to evaluate the role of headwater streams (Claessens et al, 2010). These variations are sometimes referred to as hot spots and hot moments (Groffman et al., 2009; McClain et al., 2003) and can make modeling predictions highly uncertain (Tague, 2009).

One attempt to focus research on spatial and temporal variation is to try to determine the relative importance of biological processing in the water column versus the hyporheic zone. In a study of 72 small urban, agricultural, and forested streams, Mulholland et al. (2008) found a significant correlation between primary productivity and nitrate uptake efficiency, indicating that autotrophic assimilation is an important nitrate removal process. They estimated that denitrification accounted for a mean of 16% of total nitrate removal, although denitrification exceeded 43% of total nitrate removal in a quarter of these cases. Several studies have also illustrated a relationship between hyporheic zone residence time and nitrogen processing; short residence times promote nitrate production via aerobic nitrification of ammonium, while longer residence times over which all available oxygen is consumed promote denitrification (Briggs et al., 2013; Zarnetske et al., 2011). The relative contributions of these processes to nitrate removal have been observed to vary among streams of different sizes, in different locations, and in varying states of degradation (Mulholland et al., 2008).

The impacts of urbanization on stream biogeochemistry contribute additional complexity (Kaushal and Belt, 2012). Higher concentrations might be expected to increase retention rates; instead, however, increased nitrate export often results from the loss of vegetated areas, where much nitrate is normally processed (Walsh et al., 2005; Marti et al., 2004). In addition, nitrogen processing may be decreased by shorter hyporheic zone residence time resulting from increased flow velocity and volume, changes in the quantity and grain size of sediment supply, and channelization (Lawrence et al., 2013; Hancock, 2002). Nevertheless, higher denitrification rates have been observed in some urban stream sediments, possibly as a result of higher nitrate or dissolved organic carbon concentrations (e.g., Groffman et al., 2005; Inwood et al., 2005). Similarly, Mayer et al. (2010) showed that denitrification in shallow groundwater under an urban stream in Baltimore, MD was higher where organic carbon availability was high, though denitrification rates were lower during high flow conditions partially associated with urbanization.

Despite the complexity of multiple scales and competing processes, the higher nitrogen loads in urban streams means understanding retention is especially important. Furthermore, attempts to remediate urban streams could alter retention mechanisms. Many techniques that could reduce nitrogen loading in degraded streams involve alterations of the hyporheic zone, such as increasing carbon concentrations in streambed sediments and increasing hyporheic exchange and residence time through additions of woody debris, riffle-pool sequences, and meander bends (Lawrence et al., 2013; Crispell and Endreny, 2009; Craig et al., 2008). A study comparing natural and constructed riffles suggested that nitrate removal was low relative to total loads in both cases (Kasahara and Hill, 2006). Restoration of an urban stream in Baltimore County, MD associated with stormwater management significantly decreased in-stream nitrate concentrations relative to an unrestored reach of the same stream (Kaushal et al., 2008). However, Klocker et al. (2009) also reported no significant differences in total nitrate uptake rate among restored and degraded streams in the same study area and measured the highest denitrification rates at an unrestored site. These studies indicate that attempts to use stream restoration to reduce nitrate loads have had mixed results. Further research on nitrate dynamics in

these systems is clearly necessary to better predict outcomes from both stream degradation and restoration.

Nitrogen isotope geochemistry can be used both to trace nitrate sources and to identify transformation processes. Common nitrate sources are associated with distinct, though occasionally overlapping, nitrogen isotopic signatures; for example, atmospheric deposition yields nitrate with average δ^{15}N-NO$_3$ ranging from -15‰ to 15‰, while δ^{15}N-NO$_3$ values in nitrate derived from organic nitrogen in animal waste, such as nitrate in manure and wastewater, are usually between 10‰ and 20‰ (Kendall et al., 2007). These source-related differences in nitrate isotopic composition have led to the use of nitrate nitrogen isotopes to study sources of nitrate at the watershed scale (e.g., Barnes and Raymond, 2010; Chang et al., 2003; Kaushal et al., 2011).

The isotopic composition of nitrogen species may also change as a result of biological processes, such as nitrification, denitrification, and assimilation. Processes that consume nitrogen result in enrichment of residual substrate nitrogen (e.g., NO$_3^-$ in denitrification and assimilation, NH$_4^+$ in nitrification) in the heavy nitrogen isotope, ^{15}N, due to the preferential use by organisms of isotopically light nitrogen molecules. Such fractionation is commonly described using the per mille enrichment factor ε, which represents the slope of the line for the relationship between the natural logarithm of the remaining fraction of substrate nitrogen and the substrate isotopic composition. For example, denitrification causes an exponential increase in stream δ^{15}N-NO$_3$ with decreasing nitrate concentration with an enrichment factor of -40‰ to -5‰, where the negative sign indicates that substrate nitrogen is isotopically heavier than product nitrogen (Mariotti et al., 1981). In a similar manner, nitrogen assimilation in aquatic systems yields enrichment factors ranging from -27‰ to 0‰ (Fogel and Cifuentes, 1993).

The relationship between δ^{15}N-NO$_3$ and nitrate concentrations often provides evidence of biological processing of nitrate at the reach scale. Kellman and Hillaire-Marcel (1998) determined that denitrification was often an important source of nitrate attenuation in an agricultural stream in Quebec, measuring an increase in δ^{15}N-NO$_3$ from 16‰ to 25‰ and a nearly 50% decrease in nitrate concentration over a 600 m reach during one sampling event. Hinkle et al. (2001) measured an increase in δ^{15}N-NO$_3$ from 6.74‰ upstream to 24.75‰ in a downstream well installed in the hyporheic zone of the Willamette River in Oregon. This change occurred concomitantly with an order of magnitude decrease in nitrate concentration, providing evidence for denitrification. More recently, Lofton et al. (2007) identified denitrification as an important process along a 1 km reach of an urban stream in North Carolina from the observed inverse relationship between nitrate concentration and δ^{15}N-NO$_3$.

Mixing analysis can be used where multiple processes and sources modify nitrogen isotope signatures. The relationship between δ^{15}N-NO$_3$ and nitrate concentration is hyperbolic where mixing is occurring between two different nitrate sources (Mariotti et al., 1988). In contrast, the Rayleigh fractionation equation predicts a negative exponential relationship between nitrate concentration and δ^{15}N-NO$_3$ for biological processing via either denitrification or assimilation. Thus, samples falling along the hyperbolic curve provide evidence in support of mixing as a controlling factor, while those plotting along the exponential curve suggest biological processing (Mayer et al; 2002; Kendall et al, 2007).

Although longitudinal studies and isotope analyses have added to our understanding of nitrogen processing in urban streams, questions remain about the relative influence of different physical and biological processes. Moreover, factors like changes in flow regime may change the relative importance of these processes. The objective of this study was to identify the relative

influences of tributary dilution, groundwater discharge, and biological processing on nitrate concentrations in Pennypack Creek, an urban stream near Philadelphia, PA. Sampling was conducted during high and low flow periods to examine the effects of flow regime on these processes. This research is part of an effort to understand baseline water quality in Pennypack Creek.

SITE DESCRIPTION

Pennypack Creek is a tributary to the Delaware River and flows through suburbs northwest of Philadelphia before entering the city (Figure 1). The creek is 40.2 km in length and drains an area of 144.5 km^2 (Philadelphia Water Department, 2009). Almost 50% of land in the watershed is developed, while about 29% is preserved as open space and 18% is naturally vegetated, with less than 2% each of wetlands and cultivated land. The Pennypack is underlain by sandstone in the upper third of the watershed, with carbonate units and Precambrian metamorphic rocks in the lower two thirds. Discharge measured at the USGS Pine Rd gauging station (approximately 4 km downstream of the study area) for the 2014 water year indicates an average baseflow of about 1.9 m^3-s^{-1} during high flow, extending from December through late July, and a baseflow of about 0.7 m^3-s^{-1} during low flow, which occurs from August through November. The stream has been incised by urban flows, and is considered moderately to severely impaired (Philadelphia Water Department, 2009).

A wastewater treatment plant (WTP) on Pennypack Creek releases high nitrate wastewater to the stream, providing a source term for studying attenuation processes. Average discharge during summer 2014 ranged from about 0.16 to 0.24 m^3-s^{-1}, with higher discharge at the beginning of the summer. Nitrate decreased from 8.5 to 3.5 mg-l^{-1} over 15 km downstream of the WTP in a 2007 study (Philadelphia Water Department, 2009).

To better understand the relative influence of tributary dilution, groundwater dilution, and biological processing on nitrate attenuation, study reaches were identified along which each process was observed. One reach had a meander bend with potential hyporheic flow and some small tributary inputs (discharge from each < 0.001 m^3-s^{-1}). A second study reach began just upstream of Terwood Run, a larger tributary, and flowed past a woody longitudinal bar, a feature typically associated with enhanced hyporheic flow and biological processing. Terwood Run discharge measured approximately 0.03 m^3-s^{-1} in August and September 2014. The end of this reach was underlain by carbonate rocks (Ledger Formation) and had observable spring discharge along the banks. Groundwater discharge, estimated from measured head differences and reported hydraulic conductivity values, was 0.03 m^3-s^{-1} in September 2014. Each study reach was about 1 km in length (Figure 1). While most sampling points were located between 150 and 400 m apart, some locations were separated by less than 100 m to target the features of interest. Sampling at small scales provides insights into the effects that may be associated with these features which is critical for the design of effective restoration strategies aimed at increasing nitrogen retention.

METHODS
Field and Laboratory Methods

Longitudinal sampling for anion concentrations and δ^{15}N-NO$_3$ was conducted during high flow (June) and low flow (September) conditions. Samples were collected along two study reaches located between about 5.4 and 6.5 km and between 7.0 and 8.0 km downstream of the WTP, where groundwater discharge, tributary input, or hyporheic zones had been identified (Figure 1). The sampling was conducted at a fine scale, between 100 and 300 m intervals. High

flow samples were collected June 24-25, 2014 at 12 sample sites along the mainstem of Pennypack Creek with additional samples collected from Terwood Run Tributary, one groundwater spring, and the WTP discharge. Low flow samples were collected on September 11, 2014 at 16 sample sites along the mainstem with additional samples collected from Terwood Run, one groundwater spring, upstream of the WTP, and the WTP discharge. Four mainstem sites were added in September to increase spatial resolution around the longitudinal bar, and one sample near a small tributary was moved to another tributary about 160 m upstream because the original tributary was no longer flowing.

Two temporary wells were also installed during the low flow sampling event: one was used to sample water from the hyporheic zone in the woody longitudinal bar, while the second was used to sample water from the streambed in the groundwater discharge area. To install the wells, a hollow metal pipe with a plastic dowel in its center was first hammered to a depth of about 50 cm. The plastic dowel was then removed, holding the pipe in place, and plastic tubing, open at the bottom, was inserted into the hole. The pipe was then carefully removed allowing sediment to surround the tubing, and the water in the tubing was sampled using a syringe.

Unfiltered samples for anion concentrations were analyzed using ion chromatography (Dionex ICS-1000). Samples collected for δ^{15}N-NO$_3$ were filtered within 24 hours in the laboratory using a glass filtration system equipped with 0.45 μm filters. Frozen δ^{15}N-NO$_3$ samples were shipped overnight to the University of California at Davis Stable Isotope Facility (SIF). SIF employs the denitrifier method, in which samples are exposed to cultures of denitrifying bacteria to produce N$_2$O (Sigman et al., 2001). Duplicate samples were collected for 10% of the analyses, which showed a nitrate sensitivity of 0.3 mg/L and a nitrogen isotope sensitivity of 0.1‰ excepting one outlier, for which the difference between duplicate samples was 0.6‰. This range in isotope uncertainty is similar to typical values reported of 0.3 to 0.5‰.

Nitrogen isotope mixing lines

Measured nitrate concentrations and δ^{15}N-NO$_3$ were compared to their theoretical relationship for either conservative mixing or biological processing. This comparison was used to describe the processes controlling nitrate attenuation along the study reaches. Several samples were selected as end members for mixing analysis (Table 1). A sample just downstream of the WTP was selected as the initial concentration end member. Terwood Run was selected as a type sample for tributary mixing. A spring in the Ledger Formation was selected as the groundwater end member, and its concentration was confirmed as representative of subsurface water with the streambed well sampled at low flow. These end members are used to represent processes occurring all along the study area rather than in the specific locations associated with each sample. As such, this assessment shows likely trends, but may not be able to quantify mixing portions.

Hypothetical mixing lines were calculated using the hyperbolic mixing equation presented by Mariotti et al. (1988). The isotope mass balance used to predict the δ^{15}N-NO$_3^-$ for a mixture of two nitrate sources is:

$$\delta_m = \frac{Q_a(\delta_a - \delta_b)}{Q_m} + \delta_b \quad \textbf{eq. 1}$$

δ_m δ^{15}N-NO$_3^-$ of mixture (‰)
Q_a Nitrate load of end-member A (mg)
δ_a δ^{15}N-NO$_3^-$ of end-member A (‰)
δ_b δ^{15}N-NO$_3^-$ of end-member B (‰)

Q_m Nitrate load of mixture (mg) or $Q_a + Q_b$
Q_b Nitrate load of end-member B (mg)

In equation 1, nitrate load is the product of the measured nitrate concentration and the volume of water in which the nitrate is dissolved. If Q_a is arbitrarily assigned values ranging from 0 to 1, the load of end-members A and B can be determined using the end member nitrate concentrations (C_a and C_b, respectively). Once the load of each end-member is determined, the resulting nitrate concentration of the mixture, C_m, can be calculated using standard end-member mixing:

$$C_m = \frac{1}{(\frac{Q_a}{C_a} + \frac{Q_b}{C_b})} \quad \textbf{eq. 2}$$

To better distinguish tributary and groundwater end members, the ratio of nitrate to chloride concentration was used instead of nitrate concentration alone in the mixing calculations such that $C = \frac{Cl}{NO_3}$. The tributary and groundwater had similar nitrate concentrations, but the tributary had higher chloride due to urbanization (Table 1). At high flow, the groundwater also had a distinct ratio from the upstream water, but there was overlap at low flow. The use of chloride to nitrate ratios also implicitly corrects for changes in discharge, which could not be measured accurately at all sample locations.

In addition to conservative mixing lines between sources, a line for biological processing of the initial concentration end member was calculated for both high and low flow. Predicted residual nitrate concentrations and δ^{15}N-NO$_3$ due to biological processing assumed Rayleigh fractionation of the nitrate according to the equation (Mariotti et al., 1988):

$$\delta_s = \delta_0 + \epsilon \ln(\frac{C}{C_0}) \quad \textbf{eq. 3}$$

δ_s δ^{15}N-NO$_3^-$ of substrate as processing progresses
δ_0 Initial δ^{15}N-NO$_3^-$ of substrate (WTP-affected stream water)
ϵ Per mille enrichment factor
C Nitrate concentration as processing progresses
C_0 Initial nitrate concentration of substrate (WTP-affected stream water)

This equation strictly applies only to unidirectional, single-step reactions; however, it has been used as a good approximation for denitrification, nitrification, and photosynthesis despite the complexities of these processes (Fogel and Cifuentes, 1993; Mariotti et al., 1981).

An enrichment factor of -5.0‰ was assumed, which is the minimum of the range expected for denitrification (Kendall et al., 2007). This enrichment factor also overlaps with the range for microbial uptake, which can occur autotrophically in the water column or heterotrophically throughout the stream ecosystem. Thus, the line may represent either biological process.

Substrate nitrate concentrations (C) were calculated by assigning values to $\frac{C}{C_0}$ ranging from 0.01 to 1 and multiplying by the initial substrate nitrate concentration (C_0). Again, nitrate to chloride ratios were used for concentration; in this case, chloride was merely a constant correction factor based on the initial sample downstream of the WTP. This hypothetical microbial enrichment provides the trend line for biological processing. The sample from the hyporheic zone, where denitrification is more likely, provides an additional biological end member. However, mixing

analysis was not conducted since flow paths in and out of the hyporheic zone were not identified. The end members form distinct trend lines, and samples falling along the hyperbolic curve for either groundwater or the tributary provide evidence of mixing as a controlling factor, while those plotting along the exponential curve suggest biological processing.

RESULTS AND DISCUSSION
High Flow Longitudinal Trends

The WTP discharge caused an increase in nitrate from 1.2 mg-l^{-1} as N to 6.6 mgl^{-1} (Table 1) during the high flow sampling period (June). The δ^{15}N-NO$_3$ of the WTP discharge was 10.0‰, within the range expected for wastewater and sewage (Kendall et al., 2007). Mixing of upstream Pennypack Creek water (δ^{15}N-NO$_3$ = 8.8‰) with WTP water yielded δ^{15}N-NO$_3$ of 9.7‰ downstream of the WTP (Table 1). Nitrate concentrations decreased to 4.5 mg-l^{-1}over the next 8 km, with 0.5 mg/L of decline occurring in the first study reach and 1 mg/L in the second, downstream study reach (Figure 2). The δ^{15}N-NO$_3$ of samples along the first study reach (between 4.5 and 5.5 km) varied by only 0.2‰, which is small relative to analytical error (0.10‰) and any possible error associated with sample filtration and storage. However, in the second study reach (between 7 and 8 km) the δ^{15}N-NO$_3$ increased from 9.7‰ to 10.5‰ and remained greater than 10‰ throughout this reach (Figure 2). Furthermore, nitrate concentrations decreased along this reach, possibly indicating biological processing.

The largest decrease in nitrate concentration during high flow, from 5.5 mg-l^{-1} to 4.5 mg-l^{-1}, occurred between 7.0 and 7.5 km downstream of the WTP, the reach along which the longitudinal bar with potential hyporheic flow was located. However, δ^{15}N-NO$_3$ decreased slightly from 10.4‰ to 10.3‰, which is within analytical error and thus not consistent with biological processing (Figure 2). These results prompted more focused sampling around the longitudinal bar during low flow.

Across the reach with known groundwater discharge between 7.6 and 8 km, nitrate remained nearly constant despite low nitrate concentrations in the spring sample (1.3 mg-l^{-1}), suggesting little dilution from groundwater at this site (Table 1, Figure 2). Groundwater δ^{15}N-NO$_3$ was 9.4‰, slightly lower than nearby stream δ^{15}N-NO$_3$ values; thus, dilution from this source should have resulted in a decrease in stream δ^{15}N-NO$_3$. Although δ^{15}N-NO$_3$ decreased slightly from 10.3‰ to 10.2‰, this change was within analytical error. Thus, neither the nitrogen isotope signature nor the nitrate concentrations along this reach showed evidence of groundwater dilution.

Despite low nitrate and chloride in Terwood Run, neither nitrate nor chloride concentrations downstream of this tributary differed measurably from upstream concentrations (Table 1). Terwood Run δ^{15}N-NO$_3$ was 7.5‰, so any dilution of nitrate from this tributary should have resulted in a decrease in δ^{15}N-NO$_3$. This signature should provide a clear contrast with changes in δ^{15}N-NO$_3$ resulting from biological processing. However, there was no observable decrease in nitrate isotopes downstream of the tributary; instead, an increase was recorded (Table 1). Furthermore, there were no decreases downstream of tributaries in the upstream study reach (Figure 2 and described further in Klein, 2015).

Low Flow Longitudinal Trends

During the low flow sampling in September, nitrate concentrations rose sharply from 1.8 mg-l^{-1} to 14.7 mg-l^{-1} downstream of the WTP, more than double the high flow concentration at this location (Table 1). Similarly, the δ^{15}N-NO$_3$ of Pennypack Creek increased from 9.5‰ upstream of the WTP to 10.3‰ at the site directly downstream, showing input from the

isotopically heavier WTP discharge. The nitrate concentration was nearly double the concentration at high flow in the WTP discharge, and the δ^{15}N-NO$_3$ at low flow was greater than high flow by 0.3‰.

Along the first study reach (5.5 to 6.5 km), nitrate concentrations were 11 to 12 mg-l^{-1} (Figure 3). The δ^{15}N-NO$_3$ increased to about 10.8‰ and did not change appreciably throughout this reach. In the second reach, the nitrate concentrations were 11.9 mg-l^{-1} at 7.0 km and then declined, varying between 10.1 and 10.9 mg-l^{-1}. This decline in nitrate was not accompanied by an increase in δ^{15}N-NO$_3$, which varied between 10.2 and 10.7‰, suggesting input from isotopically lighter water. The largest decline in nitrate (1.3 mg-l^{-1}) was downstream of Terwood Run and was accompanied by a slight change in δ^{15}N-NO$_3$ from 10.4 to 10.3‰ (Figure 3, Table 1). While the NO$_3$ concentration suggests that there could be tributary dilution at low flow, the change in isotopic signature is within analytical error.

Nitrate and chloride concentrations in the sample collected from the well installed in the hyporheic zone of the longitudinal bar were 2.8 mg-l^{-1} and 180.2 mg-l^{-1}, respectively (Table 1). The chloride concentration in this sample was higher than either upstream or downstream samples from the stream, possibly indicating concentration of the hyporheic zone water due to evapotranspiration. The δ^{15}N-NO$_3$ of this sample was 12.6‰, the highest value measured among all samples collected for the study. The concomitant occurrence of low nitrate concentration and high δ^{15}N-NO$_3$ in this bar strongly suggests biological processing in the hyporheic zone (Table 1). Despite these chemically and isotopically distinct signatures of hyporheic zone water, however, both chloride and nitrate concentrations remained nearly constant from above to below the longitudinal bar, and the decrease in stream δ^{15}N-NO$_3$ across this reach from 10.6‰ to 10.5‰ was within analytical error (Table 1, Figure 3) and does not have a tributary signature.

The δ^{15}N-NO$_3$ at the spring in the groundwater discharge zone between 7.6 and 8 km remained the same in low flow as in high flow, and the δ^{15}N-NO$_3$ at the nearby streambed well confirmed this isotope signature. The spring had low concentrations of both nitrate (1.3 mgl^{-1} as N) and chloride (19.2 mg-l^{-1}); however, nitrate concentration in Pennypack Creek remained 10.9 mg-l^{-1} throughout the groundwater discharge zone, and δ^{15}N-NO$_3$ was only slightly lower than upstream. Thus, no clear signal for groundwater discharge was identified (Figure 3).

Mixing line analysis

The study reaches presented two distinct data clusters. During high flow, δ^{15}N-NO$_3$ increased between the upstream (mean = 9.7‰) and downstream (mean = 10.3‰) reach with a decrease in nitrate concentration. In contrast, at low flow a decrease in δ^{15}N-NO$_3$ between upstream (mean = 10.8‰) and downstream (mean = 10.4‰) reaches occurred with a decrease in nitrate concentration. These reaches were plotted as distinct groups in the mixing line analysis.

At high flow, the end members were distinct in their δ^{15}N-NO$_3$ and molar Cl/NO$_3$ signatures (Figure 4, Table 1). Terwood Run had a high Cl/NO$_3$ because it was impacted by road salt but not the WTP nitrate. The δ^{15}N-NO$_3$ was about 2‰ lower than the WTP as well. The groundwater spring in the Ledger formation had a lower Cl/NO$_3$ ratio, as it was not affected by road salt, and the δ^{15}N-NO$_3$ was about 0.5‰ lower than the WTP. Biological processing was expected to significantly increase the δ^{15}N-NO$_3$ even with the modest fractionation factor selected. The line for biological processing is shown departing from the sample downstream of the WTP, but at any point along the mixing lines, biological processing would increase δ^{15}N-NO$_3$ and Cl/NO$_3$ since NO$_3$ decreases during uptake.

The samples from the first study reach (5.4 to 6.5 km) followed the groundwater mixing line. Even though they were not in the zone of observed groundwater discharge, it is possible

that groundwater discharge with an isotopically similar signature to the measured spring occurred throughout this reach. In the second study reach (7 to 8 km), the samples had an isotopically heavier signature suggestive of biological processing. However, the Cl/NO_3 was more typical of groundwater discharge. Given that biological processing and groundwater discharge have opposite effects on the Cl/NO_3, these samples may have undergone a combination of processes leading to attenuation along this reach. None of the samples showed a tributary mixing signal.

At low flow, the end member Cl/NO_3 ratios were not as distinct because the NO_3 was higher in the WTP-affected waters (Figure 5, Table 1). However, the isotope signature of the groundwater end member was distinctly lighter than the WTP water, and the tributary water still had light $\delta^{15}N$-NO_3 and high Cl/NO_3. The samples from the first study reach (5.4 to 6.5 km) followed the biological processing line assuming the modest fractionation factor. It is possible that a larger biological fractionation occurred but a combination of groundwater discharge and biological processing occurred along this reach which muted the isotope signal. In the second study reach (7 to 8 km), the samples had slightly lower $\delta^{15}N$-NO_3, pointing to enhanced groundwater discharge at low flow along this reach. However, biological processing could also be occurring, which would mute the groundwater discharge signal in the reverse direction of the previous reach. Given the larger fractionation seen in the hyporheic zone well, along with a high Cl/NO_3 ratio, it seems unlikely that hyporheic zone processing contributes significantly to nitrate attenuation along either of these reaches. Instead, biological processing with a lower fractionation factor seems to occur in the water column.

Contrasts between high flow and low flow

Changes in the contribution of groundwater discharge to attenuation between high and low flow were indicated by the difference in isotope signatures of the two study reaches. At high flow, the $\delta^{15}N$-NO_3 signatures shifted to heavier values in the second reach. The Cl/NO_3 ratio suggested groundwater discharge occurred along both reaches, but biological processing was greater in the second reach based on the isotope signature. At low flow, the pattern was reversed; the first reach had heavier $\delta^{15}N$-NO_3 signatures that shifted to lighter values in the second reach. The initially heavier isotope signature suggests enhanced biological processing at low flow, but in the second reach, groundwater discharge increased relative to total stream discharge, decreasing the isotope signature. Barnes and Raymond (2010) found $\delta^{15}N$-NO_3 values more closely resembled groundwater nitrate nitrogen isotope signatures during periods of low discharge in urban watersheds throughout Connecticut when there is an increase in the relative contribution of groundwater discharge. Because the second study reach included carbonate bedrock with observable springs, groundwater discharge may have been greater there than upstream.

Biological processing was enhanced at low flow compared to high flow based on the increased number of samples with enriched $\delta^{15}N$-NO_3. The influence of biological processing may be even higher than apparent based on the nitrogen isotope signatures because any groundwater discharge occurring along these reaches shifts the isotope signature in the opposite direction. Several factors may enhance biological processing at low flow, including high nitrate concentrations (Baker and Vervier, 2004), higher residence times (Royer et al., 2004), and greater light availability and temperature (Beaulieu et al., 2013). These factors are not all applicable to Pennypack Creek, however. For example, increased residence time in the hyporheic zone would not be a factor because the primary biological processing appears to occur in the water column based on the observed modest fractionation. Hyporheic zone denitrification

would likely cause a larger shift to heavier δ^{15}N-NO$_3$ signatures based on the large fractionation observed in the sample from the hyporheic zone well. On the other hand, lower flows can enhance biological uptake through water column interaction with microfilms on the streambed (Claessens et al., 2010). Another factor, nitrogen concentration, is unlikely to be rate limiting in the WTP-affected water. Temperature was slightly lower in September during low flow (Klein, 2015); however, increased light in shallower water relative to high flow may have enhanced biological processing.

Contrasts between processes

Spatially discrete zones or hot spots were not identified for tributary input, groundwater discharge, or biological processing despite fine scale sampling. Tributary input, as well as supplemental nitrate sources, may create increases or decreases in nitrate concentration that do not persist downstream after mixing occurs (Table 1, Figure 3). Similarly, hyporheic zone water with a high δ^{15}N-NO$_3$ signature does not result in reach-scale shifts in isotope signature. The amount of water processed through the hyporheic zone may not be a large portion of the main stem (Wondzell, 2011; Mulholland et al., 2008). Instead, as mentioned above, the biological processing observed likely occurs primarily in the water column. The second reach showed decreasing δ^{15}N-NO$_3$, providing evidence of groundwater discharge. However, the two samples bracketing the groundwater discharge zone in the Ledger Formation between 7.6 and 8 km did not show measurable changes in nitrate concentration or δ^{15}N-NO$_3$ despite substantial evidence of concentrated groundwater from springs and the streambed. The combination of groundwater discharge and biological processing may have worked to moderate any changes in δ^{15}N-NO$_3$ that would have otherwise been observed in the isotope signature; furthermore, nutrient spiraling (Ensign and Doyle, 2006) could moderate nitrate decreases.

CONCLUSIONS

This study revealed that collocated physical and biological processes can mute each other's signals. The mixing lines provided evidence that a combination of groundwater discharge and biological processing occurred along the study reaches. Rather than data falling along one mixing line, the samples started along a mixing line and then shifted direction (Figures 4 and 5). Furthermore, the samples clustered close to the source end member (DS WTP) despite evidence of both groundwater discharge and biological processing. This clustering likely occurred because the mixing lines for these two processes have opposite trends, thus muting isotope signatures of the individual processes. Nitrate attenuation downstream of theft in Pennypack Creek occurred through both groundwater discharge and biological processing, and these interactions varied at low and high streamflow.

This collocation of processes prohibited the isolation and quantification of any single process using longitudinal sampling alone. Ironically, fine-scale spatial sampling was not effective for identifying hot spots of nitrate attenuation. Unfortunately, the collocation of processes adds uncertainty to quantifying the importance of biological processing. Consequently, it is difficult to predict how urbanization as well as stream restoration influence nitrate attenuation. Reach comparisons and up-scaling are common techniques, but care should be taken not to over-interpret small scale reach behavior without understanding all the processes influencing attenuation.

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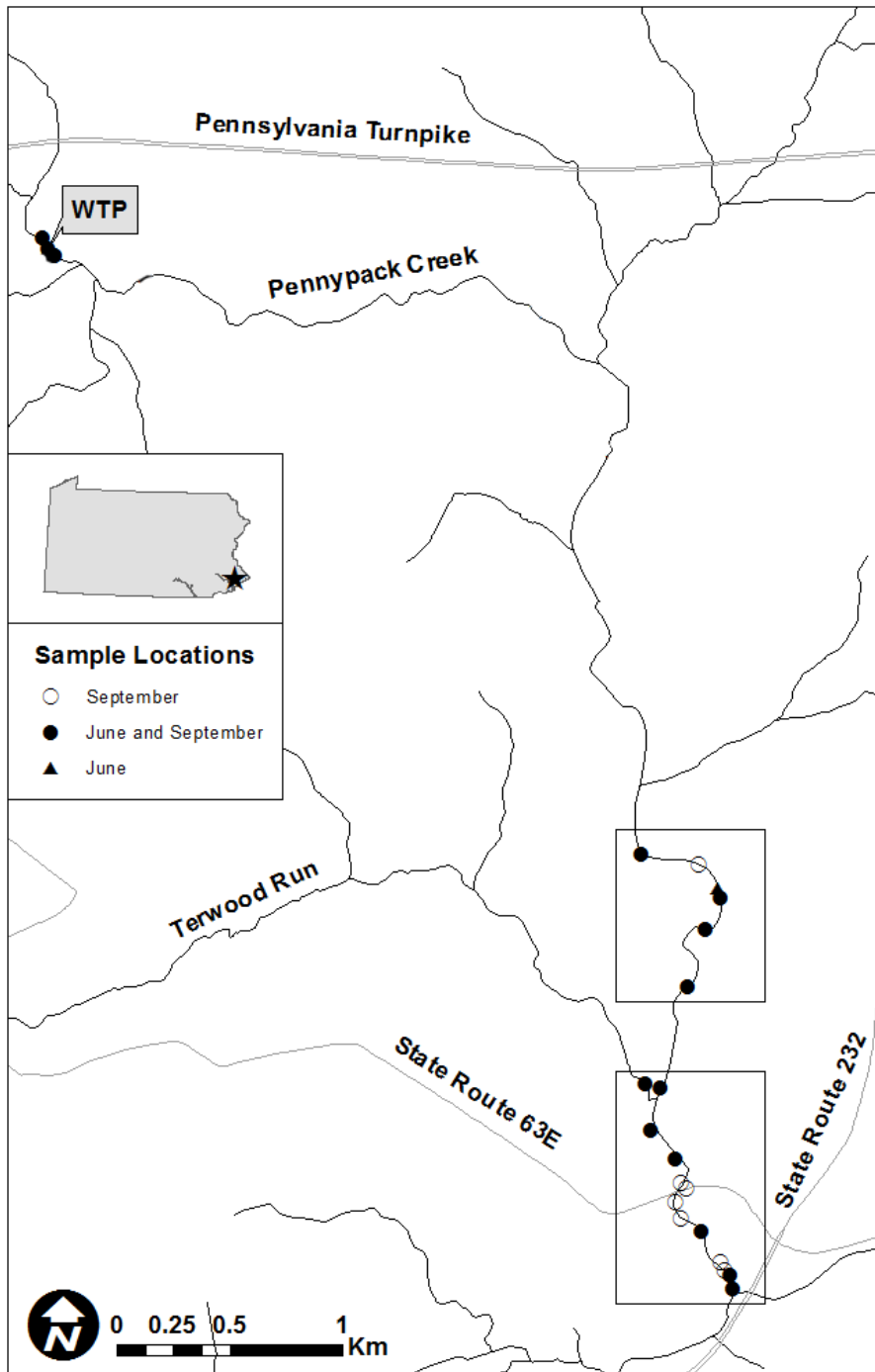
FIGURES

Figure 1: Site map showing approximately 8 km of Pennypack Creek downstream of the wastewater treatment plant (WTP). Boxes indicate two study reaches. June samples collected at high flow, September samples at low flow. The additional samples in Sept south of State Route 63E were along longitudinal bar.
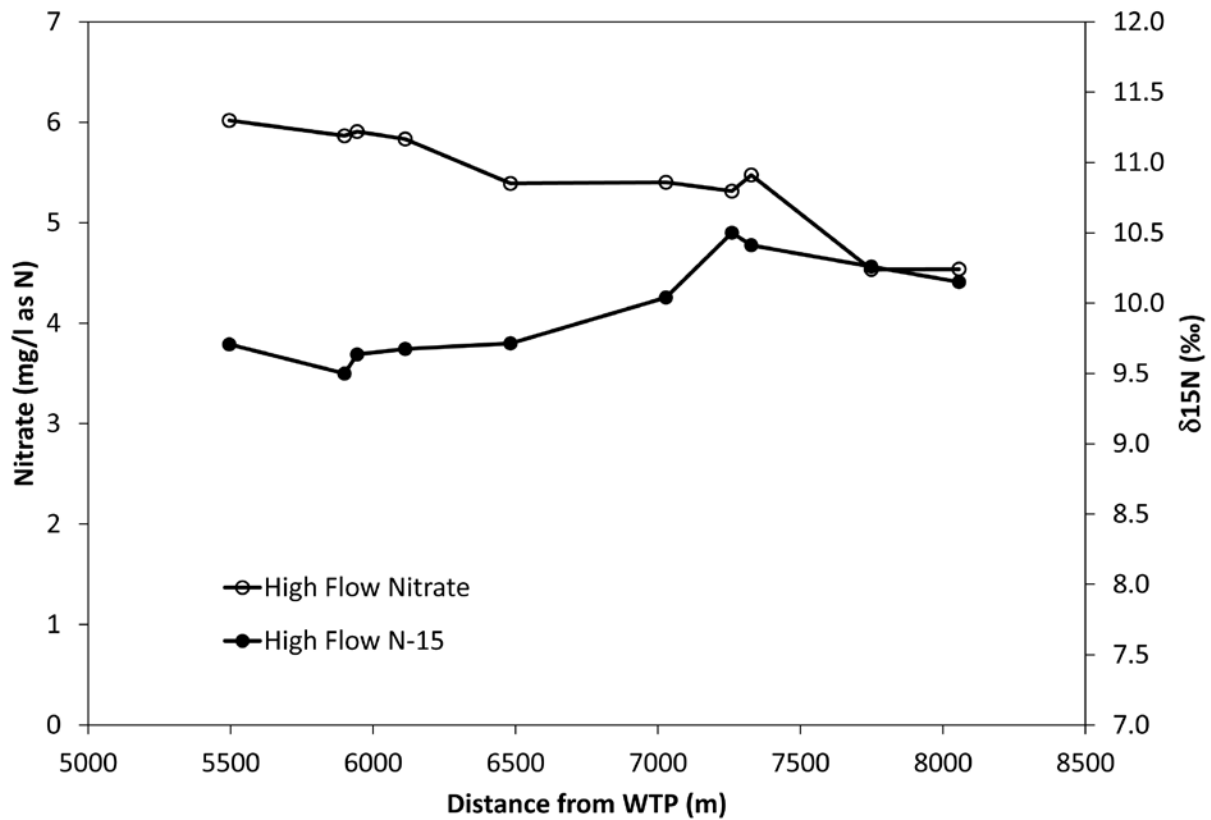
Figure 2: Longitudinal trends in nitrate and δ^{15}N-NO$_3$ at high flow, showing the trends in the study reaches. The nitrate decreased along the study reaches while the δ^{15}N-NO$_3$ increased.
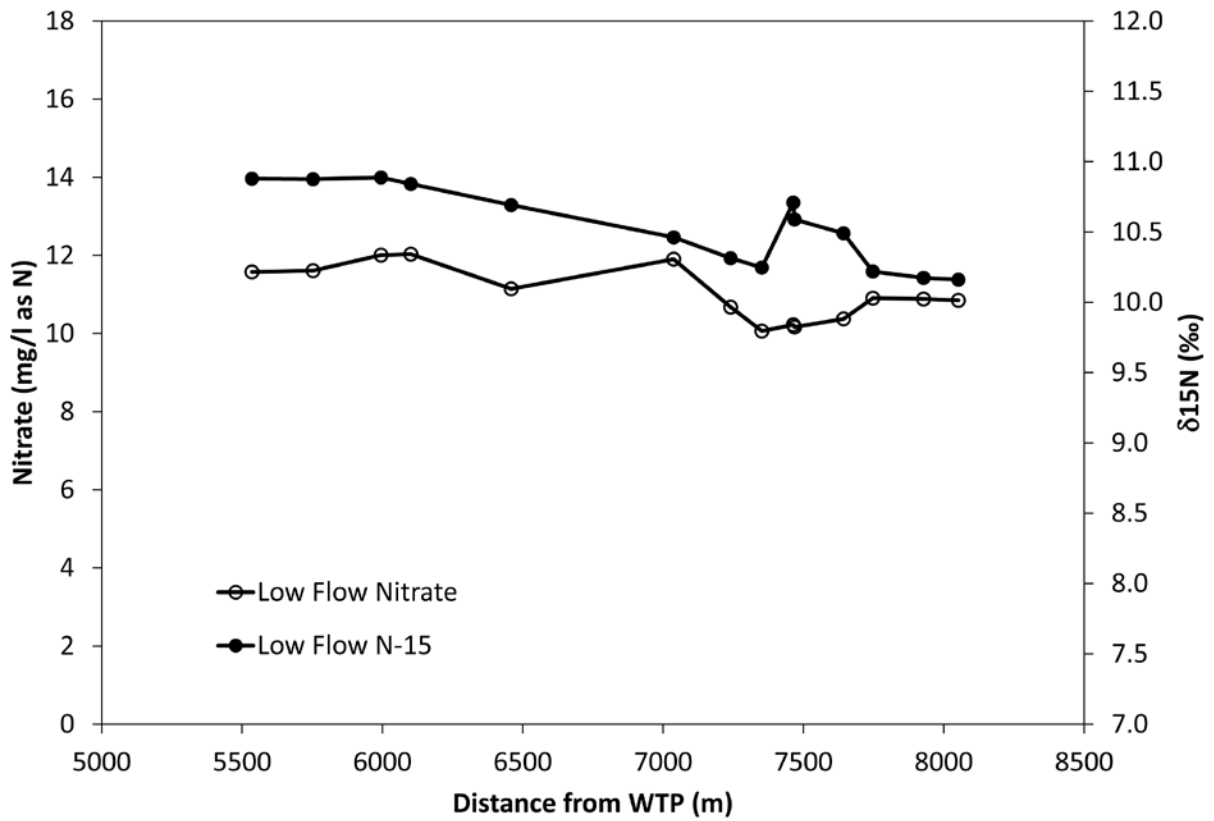
Figure 3: Longitudinal trends in nitrate and δ^{15}N-NO$_3$ at low flow, showing the trends in the study reaches. The nitrate concentration at the WTP is nearly double the high flow concentration. There were both increases and decreases in δ^{15}N-NO$_3$ along the study reaches, indicating collocated processes.
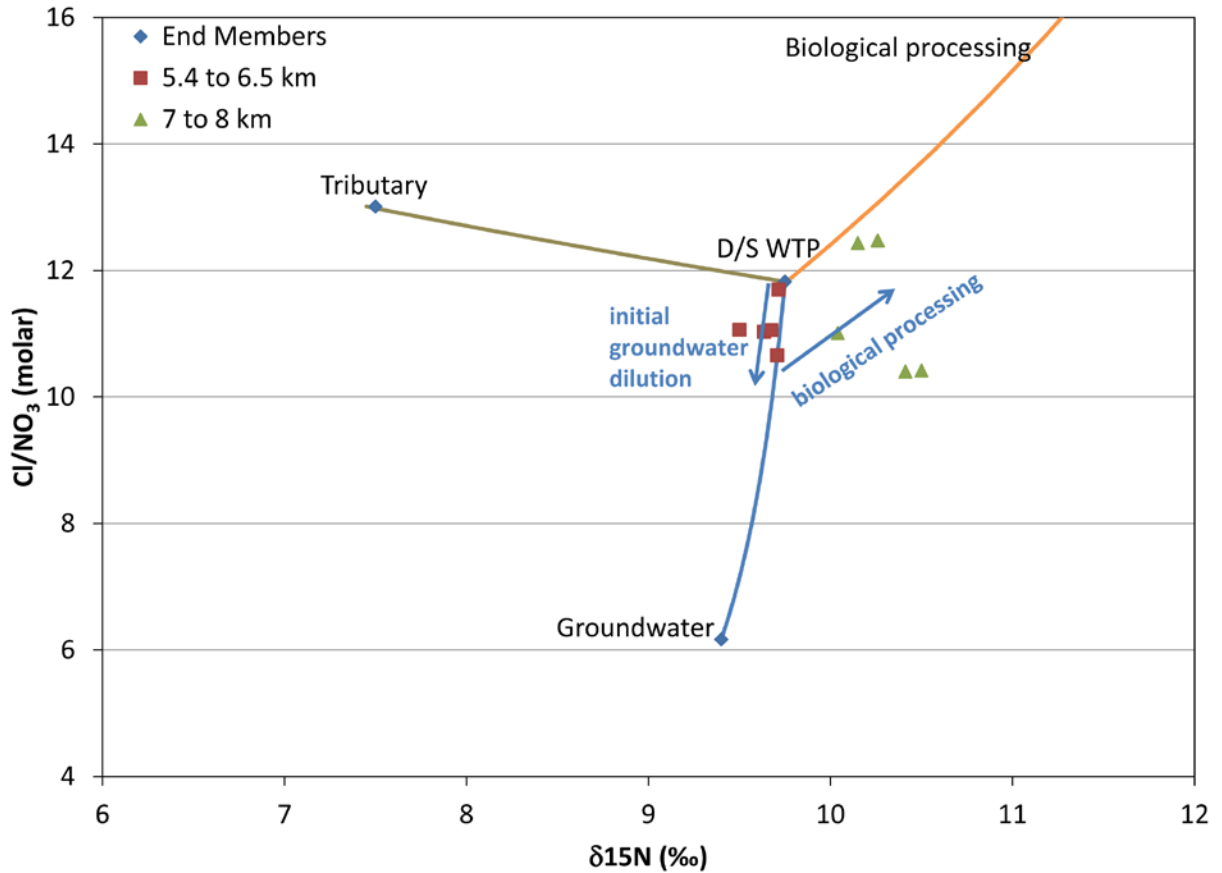
Figure 4: Mixing analysis for Cl/NO₃ ratio and δ^{15}N-NO₃ for high flow samples grouped by the two study reaches. Using the ion ratios helps distinguish tributary, groundwater and biological processing end members and accounts for dilution effects. The upstream reach showed groundwater dilution based on lower Cl/NO₃ ratio (downward arrow). The downstream reach had heavier δ^{15}N-NO₃ indicating more biological processing in this reach (arrow shifting to the right).
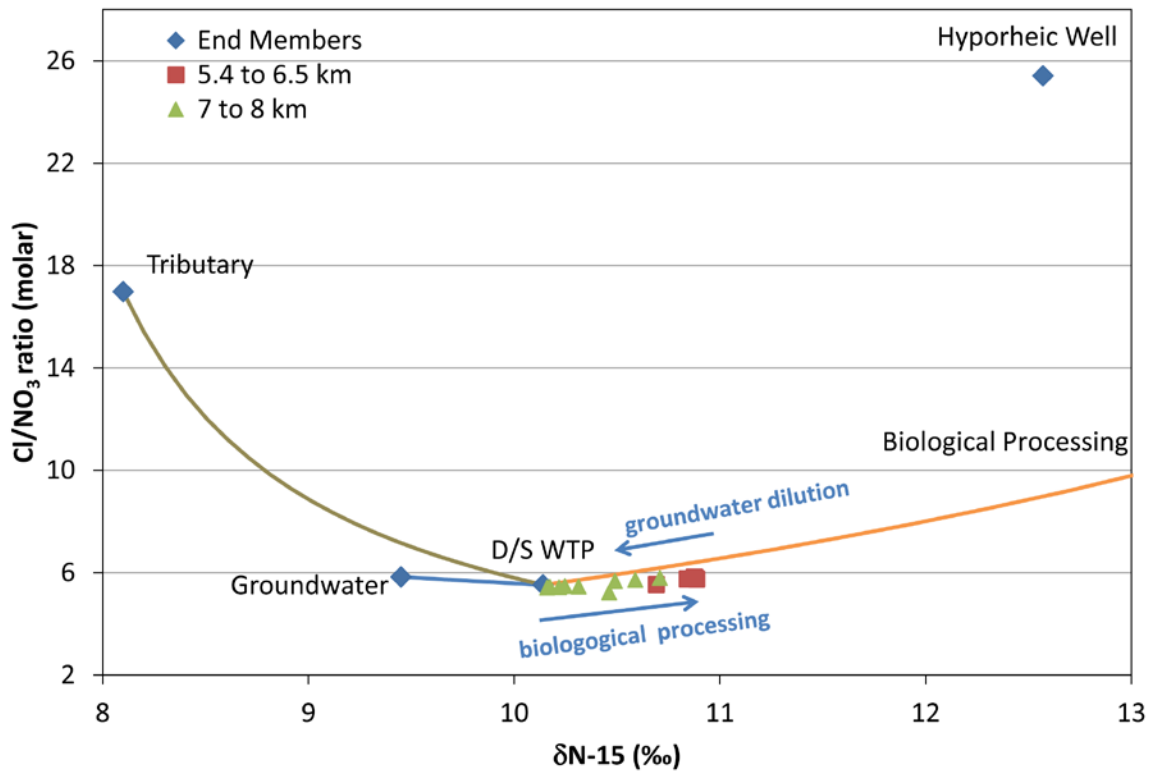
Figure 5: Mixing analysis for Cl/NO₃ ratio and δ^{15}N-NO₃ for low flow samples grouped by the two study reaches. The well sample from the hyporheic zone has high δ^{15}N-NO₃ and low NO₃, suggesting denitrification. None of the stream samples have a comparable signature. At low flow the upstream reach showed biological uptake in the water column based on a smaller shift to heavier δ^{15}N-NO₃ (right arrow). The downstream reach showed more groundwater dilution at low flow based on lighter δ^{15}N-NO₃ (arrow shifting to the left). However, collocation of processes mutes the isotope signal.

Table 1: End members and paired samples for low and high flow. US is upstream. DS is downstream.

	NO_3 (mg-l^{-1})	Cl (mg-l^{-1})	$\delta^{15}N$ (‰)	Cl/NO_3, (molar)
HIGH FLOW END MEMBERS (June)				
US of WTP	1.2	211.5	8.8	68.7
WTP	10.9	174.7	10.0	6.3
DS of WTP*	6.6	195.9	9.8	11.7
Groundwater (Ledger Spring)	1.3	20.3	9.4	6.1
Tributary (Terwood Run)	1.9	62.6	7.5	13.1
HIGH FLOW PAIRED SAMPLES (June)				
US of Terwood Run	5.4	150.6	10.0	11.0
DS of Terwood Run	5.3	140.2	10.5	10.4
US of Ledger Spring	4.5	143.3	10.3	12.4
DS of Ledger Spring	4.5	142.9	10.2	12.4
LOW FLOW END MEMBERS (Sept)				
US of WTP	1.8	239.5	9.5	52.2
WTP	17.0	183.8	10.3	4.3
DS of WTP	14.1	197.5	10.3	5.5
Groundwater (Ledger Spring)	1.3	19.2	9.5	5.8
Tributary (Terwood Run)	1.4	60.2	8.1	17.0
Hyporheic Zone Well in Longitudinal Bar	2.8	180.2	12.6	25.4
LOW FLOW PAIRED SAMPLES (Sept)				
US of Hyporheic Zone Well in Longitudinal Bar	10.2	147.2	10.6	5.7
DS of Hyporheic Zone Well in Longitudinal Bar	10.4	148.9	10.5	5.7
US of Terwood Run	11.9	157.6	10.5	5.2
DS of Terwood Run	10.7	147.5	10.3	5.5
US of Ledger Spring	10.9	150.0	10.2	5.4
DS of Ledger Spring	10.8	148.8	10.2	5.4

*Ion data from the second downstream sample were combined with isotope data from the first downstream sample because the first sample showed incomplete mixing. These samples were the same at low flow.