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Nitrate uptake across biomes and the influence of elemental stoichiometry: A new look at LINX II: Nitrate Uptake across Biomes

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RESEARCH ARTICLE

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Key Points:

- Across biomes NO_3^- uptake is best predicted by DOC: NO_3^- ratios and photosynthetically active radiation
- No universal driver of NO_3^- uptake was identified
- Controls on NO_3^- uptake vary by biome

Supporting Information:

- Supporting Information S1
- Table S1
- Figure S1

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Nitrate uptake across biomes and the influence of elemental stoichiometry: A new look at LINX II

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Abstract Considering recent increases in anthropogenic N loading, it is essential to identify the controls on N removal and retention in aquatic ecosystems because the fate of N has consequences for water quality in streams and downstream ecosystems. Biological uptake of nitrate (NO_3^-) is a major pathway by which N is removed from these ecosystems. Here we used data from the second Lotic Intersite Nitrogen eXperiment (LINX II) in a multivariate analysis to identify the primary drivers of variation in NO_3^- uptake velocity among biomes. Across 69 study watersheds in North America, dissolved organic carbon: NO_3^- ratios and photosynthetically active radiation were identified as the two most important predictor variables in explaining NO_3^- uptake velocity. However, within a specific biome the predictor variables of NO_3^- uptake velocity varied and included various physical, chemical, and biological attributes. Our analysis demonstrates the broad control of elemental stoichiometry on NO_3^- uptake velocity as well as the importance of biome-specific predictors. Understanding this spatial variation has important implications for biome-specific watershed management and the downstream export of NO_3^- , as well as for development of spatially explicit global models that describe N dynamics in streams and rivers.

1. Introduction

Over the past several decades both terrestrial and aquatic ecosystems have experienced excessive nitrogen (N) loading due to anthropogenic activities [Vitousek *et al.*, 1997]. Streams and rivers in particular, export much of this N often with deleterious ecosystem effects [Carpenter *et al.*, 1998]. In-stream biological processing of inorganic N can mediate N export to coastal waters by removing substantial fractions of inorganic N from the water column [Peterson *et al.*, 2001]. However, the capacity for streams to remove N can saturate, resulting in increased export of N inputs from watersheds [Peterson *et al.*, 2001; Hall *et al.*, 2009]. Understanding the controls on N removal and retention in aquatic ecosystems is essential for controlling water quality in streams and downstream ecosystems.

Biological (assimilatory) uptake and denitrification are two major pathways by which nitrate (NO_3^-) is removed from stream ecosystems [Mulholland *et al.*, 2008]. The controls on NO_3^- uptake are often examined using nutrient addition experiments [Stream Solute Workshop, 1990] following the framework of nutrient spiraling [Newbold *et al.*, 1981]. Various studies have examined the predictors of NO_3^- uptake, revealing the importance of multiple drivers including discharge and stream size [Lautz and Siegel, 2007; Hall *et al.*, 2009, 2013], NO_3^- concentration [Earl *et al.*, 2007; Mulholland *et al.*, 2008; Ribot *et al.*, 2013], autotrophic [Mulholland *et al.*, 2006; Hall *et al.*, 2009] and heterotrophic [Bernhardt and Likens, 2002; Bernhardt and McDowell, 2008] demand for NO_3^- , and land use [Hall *et al.*, 2009]. Despite numerous studies examining the controls on NO_3^- uptake, no single predictor has emerged as a universal control of NO_3^- uptake. A variety of factors are known to be important in determining rates of NO_3^- uptake, but due to inconsistencies across studies and sites, it is difficult to determine their relative importance. More likely, several variables, and their interactions, are key predictors of NO_3^- uptake, and thus, a multivariate approach is needed.

The Lotic Intersite Nitrogen eXperiment II (LINX II) data set [Mulholland *et al.*, 2015] provides the unique opportunity to examine numerous predictor variables in a multivariate context while simultaneously examining the drivers of NO_3^- uptake across biomes. With the objective of developing quantitative predictive models on the controls of NO_3^- uptake, LINX II measured the uptake kinetics of $^{15}\text{NO}_3^-$ in 72 streams across seven biomes in North America and Puerto Rico [e.g., Mulholland *et al.*, 2008; Hall *et al.*, 2009]. Land use (reference, urban, and agriculture) was included in the experimental design, which included three sites of each land use type nested within each of eight regions representing seven distinct biomes.

While the primary objective of the LINX II project was to examine how NO_3^- uptake changed across land use [Mulholland *et al.*, 2008], there has yet to be an extensive analysis of this data set across biomes. Interbiome variation can drive differences in rates of important ecosystem processes. For example, stream metabolism varies by over an order of magnitude across biomes and is correlated with ambient concentrations of soluble reactive phosphorous [Mulholland *et al.*, 2001]. NO_3^- uptake ranges over three orders of magnitude across global biomes [Mulholland *et al.*, 2008; Arce *et al.*, 2014]. The LINX II study included conifer, desert, northern deciduous, southern deciduous, steppe, tallgrass, and tropical forest biomes. These contrasting biomes provide a gradient of variables that may be important predictors of nutrient uptake, such as light availability ($0.03\text{--}70 \text{ mol quanta m}^{-2} 24 \text{ h}^{-1}$) and dissolved organic carbon (DOC) concentration ($0.26\text{--}25.6 \text{ mg C/L}$) [Mulholland *et al.*, 2015]. Thus, we hypothesize that the important predictors of NO_3^- uptake will vary across the seven biomes represented in this study.

Our overarching goal was to examine whether the predictors of NO_3^- uptake varied across the distinct biomes represented in the LINX II data set. To accomplish this objective, we incorporated the multivariate method partial least squares (PLS), as it performs well when there is a high degree of collinearity among predictor variables as is the case with the LINX II data set. We also added new predictor variables to the data set based on recent work that demonstrates the relationship between elemental stoichiometry and nutrient uptake in aquatic ecosystems [Taylor and Townsend, 2010; Diemer *et al.*, 2015; Rodríguez-Cardona *et al.*, 2016]. The addition of these ratios provides new ecologically relevant predictor variables with which to examine variability in rates of NO_3^- uptake in the LINX II data set. We ask two guiding questions: (1) what are the predictors of NO_3^- uptake efficiency, and how are they related? and (2) do the relative contributions of the predictor variables change among biomes? We emphasize that this work differs from a previous multivariate analysis of the LINX II data set [Hall *et al.*, 2009] in four important ways: (1) we explore the dataset from an interbiome perspective as opposed to land use; (2) we use elemental stoichiometry to develop new and ecologically relevant predictor variables; (3) we use a different statistical approach; and (4) we use a different metric of nutrient uptake better suited for interbiome analyses. By exploring the LINX II data set with this novel framework, we are able to identify those drivers of NO_3^- uptake with greater confidence and shed new light on the controls of the nitrogen cycle in freshwater ecosystems across Earth's diverse biomes.

2. Methods

2.1. The LINX II Data Set

To identify the predictor variables of NO_3^- uptake efficiency, we used the publicly available LINX II data set [Mulholland *et al.*, 2015]. For further details regarding the LINX II experiment see Mulholland *et al.* [2008] and Hall *et al.* [2009]. Here we use the nutrient spiraling metric of uptake velocity [V_f], which is defined as the vertical movement of a molecule from the water column into the benthos and interpreted as the demand for a nutrient relative to the available concentration [Stream Solute Workshop, 1990]. We use the terms uptake velocity, V_f , and uptake efficiency interchangeably to describe this metric. Uptake velocity is ideal for comparisons among sites because it normalizes for stream depth and discharge [Wollheim *et al.*, 2001] as well as differences in ambient concentration [Peterson *et al.*, 2001; Hall *et al.*, 2013]. NO_3^- uptake velocity ranged from 0.00004 to 0.03 mm min^{-1} with a mean value across the 69 experiments of $0.003 \text{ mm min}^{-1}$.

2.2. Statistical Analyses

To identify predictors of $\text{NO}_3^- V_f$, we used partial least squares (PLS) analysis. PLS is a multivariate technique and an extension of multiple regression that uses latent variables (i.e., factors) to establish relationships between predictor variables and response variables. PLS performs well (identifies relevant variables) when there is a high degree of collinearity among predictor variables [Carrascal *et al.*, 2009]. We used a variable influence of projection (VIP) threshold of 1.0 to determine if a predictor variable contributed significantly to the model, with higher VIP values indicating relatively greater influence on the model. It is recommended that a VIP score of 0.8 be used to identify significant variables [Carrascal *et al.*, 2009]; however, we chose to use a more stringent standard due to the number of predictor variables included in our analysis. We further categorized VIP scores as *less influential* (1–1.5), *moderately influential* (1.5–2.0), and *highly influential* (>2) on the model.

We selected predictor variables to be included in PLS models based on a priori hypotheses. Metrics of stream water chemistry were included as individual solutes: mean dissolved oxygen (DO: mg L^{-1}), NO_3^- ($\mu\text{g N L}^{-1}$),

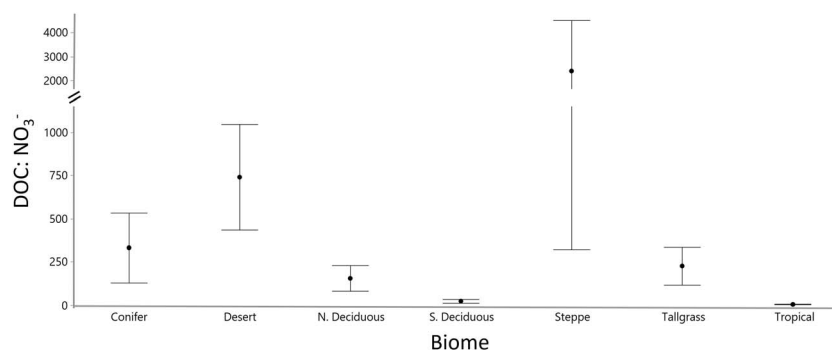


Figure 1. Stream water DOC:NO₃⁻ ratios across the seven biomes used in the second Lotic Intersite Nitrogen eXperiment (LINX II) study. Values reflect molar ratios and are means ±1 SE.

NH₄⁺ (μg NL⁻¹), dissolved organic nitrogen (DON) (μg NL⁻¹), soluble reactive phosphorus (SRP) (μg PL⁻¹), DOC (mg C L⁻¹); and as molar ratios: DOC:NO₃⁻, DOC:DON, DOC:SRP, NO₃⁻:SRP (see Table S1 in the supporting information for biome and stream-specific ratios). Stoichiometric ratios were not used in original analyses of the LINX II data set, and their use here thus represent a novel aspect of this study. Biological variables included in the model were gross primary productivity (GPP), respiration (*R*), total autotrophic standing stock (Tot Aut; g AFDM m⁻²), and total detritus (Tot Det; g ash free dry mass [AFDM] m⁻²). Physical characteristics included in the model were mean water temperature (°C), photosynthetically active radiation (PAR; mol quanta m⁻² 24 h⁻¹), stream gradient (Grad; m m⁻¹), specific discharge (*Q/w*; discharge/watershed area: m³ s⁻¹ m⁻¹), and storage zone cross-section area/main channel cross-sectional area (*A_s/A*). In the steppe model PAR data were missing from four of the nine sites. Channel shade (%) was included in the steppe model as a proxy for PAR measured via a densimeter. We excluded specific conductance and nitrification rate from analysis due to a high frequency of missing data.

To identify important predictors of NO₃⁻ *V_f* and how these predictors vary by biome, we used two approaches: an overall continental model (all biomes) that included all of the LINX II data and a series of separate models for each of the seven biomes. NO₃⁻ uptake was undetectable at three sites (two northern deciduous, one southern deciduous) resulting in 69 measurements of NO₃⁻ *V_f* available for modeling. We focus primarily on *V_f* (mm min⁻¹) in this study rather than areal uptake (*U*; mg m⁻² min⁻¹) because *V_f* normalizes for the effect of water column NO₃⁻ concentration on rates of areal uptake and more effectively represents the efficiency with which NO₃⁻ is removed. The number of latent variables included in the PLS models was selected to optimize cumulative variance captured for the *Y* block (*R*²*Y*) and *X* block (*R*²*X*) while minimizing *Q* residuals and maximizing Hotelling's *T*². Outliers were identified using the Hotelling's *T*² analysis if they exceeded the 95% confidence limit. In total, 11 outliers were removed from the overall model to improve model reliability with at least one, but not more than two, removed from any single biome-specific model. Across the biome-specific models we only removed one outlier, from the northern deciduous model. PLS analyses were performed using PLS Toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA) in MATLAB [MATLAB, 2014; version R2014b].

3. Results

3.1. Variation in Elemental Stoichiometry Across Biomes

Elemental molar stoichiometry varied considerably among the LINX II study streams. Values ranged as follows: DOC:NO₃⁻: 0.13–19,031 (Figure 1); DOC:SRP: 1.1–1361; DIN:SRP: 0.08–2889; TDN:SRP: 2.4–2332; DON:SRP: 1.1–1361, DOC:DON: 1.66–467. Median values were DOC:NO₃⁻: 19.2, DOC:SRP: 36.5, DIN:SRP: 35.3, TDN:SRP: 87.1, DON:SRP: 36.5, and DOC:DON: 17.8 (please see Table S1 for biome and stream-specific ratios).

3.2. Predictors of Nitrate Uptake Velocity

The stoichiometric ratio of DOC:NO₃⁻, PAR, and GPP loaded significantly into the continental model (Table 2), all correlating positively with NO₃⁻ *V_f* (Figure 2). DOC:NO₃⁻ ratios were approximately 2 times and 6 times more influential on the continental model than PAR and GPP, respectively (Table 2). Seven latent variables

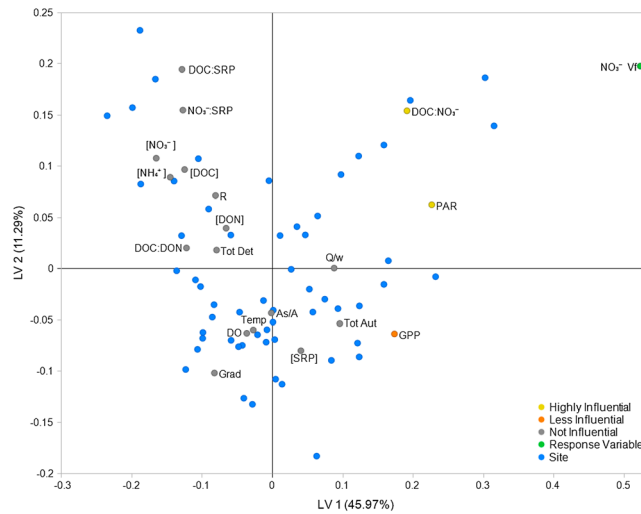


Figure 2. Partial least squares biplot predicting the variability of nitrate uptake velocity ($\text{NO}_3^- V_f$) across seven biomes. Highly influential variables have VIP scores >2 , moderately influential predictor variables have VIP scores between 2 and 1.5, and less influential predictor variables have VIP scores between 1.5 and 1.

(LV) were included in the continental model (Tables 1 and Table S2) and explained 62.0% of the variation in the predictor variables and 63.0% of the total variation in $\text{NO}_3^- V_f$ (Table 1). The first two latent variables accounted for the majority of the variation in $\text{NO}_3^- V_f$ explaining 57.2% of the variation (Figure 2).

Across the biome-specific PLS models, four latent variables explained 61.0–76.9% of the variation of the loaded predictor variables (R^2X ; Tables 2 and S3–S9). The amount of variation explained in rates of $\text{NO}_3^- V_f$ (R^2Y) ranged from 98.1 to 99.9% (Table 2). No universal predictor variable was identified across biomes and no biome had only one influential predictor variable. Three variables did not load significantly into any biome model including $[\text{NH}_4^+]$, total detritus, and DOC:SRP. Channel shade did not load significantly into the steppe model (see Figure S1–S4 for biome-specific PLS biplots).

Of the seven biome-specific models, DOC: NO_3^- ratio was highly influential in four models and PAR was highly influential in three models. The DOC: NO_3^- molar ratio was a highly influential predictor (Table 2) in desert, tallgrass, southern deciduous, and conifer biomes. In general, these streams had neutral to positive loadings along LV1 and moderately positive to negative loadings along LV2 in the continental model, indicative of lower DOC:SRP and NO_3^- :SRP ratios and higher light availability and DOC: NO_3^- . PAR was a highly influential predictor (Table 2) for the northern deciduous, tropical, and conifer models, and all of these biomes had among the lowest PAR and DOC: NO_3^- molar ratios (represented by strong positive loadings along LV1 in the continental model; Figure 2). The southern deciduous streams also had equally low light and DOC: NO_3^- molar ratios, but PAR was not an influential predictor in that biome. Light availability (as PAR or channel shade) was not an influential predictor of $\text{NO}_3^- V_f$ in tallgrass or steppe PLS models, both of which had high light availability and high DOC: NO_3^- ratios as represented by positive loadings along LV1

Table 1. Results of Partial Least Squares (PLS) Analyses Including the Overall Model and Seven Biome-Specific Models^a

Model	# of Latent Variables	R^2Y	R^2X	Q residuals	Hotellings T^2
Overall	7	63.0	62.0	38.2	62.0
Steppe	4	98.8	63.5	36.5	63.5
Desert	4	99.7	66.0	34.0	66.0
Tallgrass	4	98.4	72.5	27.5	72.5
Conifer	4	99.7	76.9	23.1	76.9
Northern Deciduous	4	98.1	60.9	39.1	60.9
Southern Deciduous	4	99.9	75.3	24.7	75.3
Tropical	4	99.7	71.3	28.7	71.3

^aResponse variable (R^2Y) is NO_3^- uptake velocity (V_f). Biomes are arranged from highest to lowest mean $\text{NO}_3^- V_f$.

Table 2. Variable Importance in Projection (VIP) Scores of Loaded Predictor Variables for the Overall Model and Seven Biome-Specific Models^a

Biome	Highly Influential		Moderately Influential		Less Influential	
Overall	DOC:NO ₃ ⁻	6.7			GPP	1.3
	PAR	2.9				
Steppe	NO ₃ ⁻ :SRP	2.7			A ₅ /A	1.5
	NO ₃ ⁻	2.7			Grad	1.3
	Temp	2.5			Tot Aut	1.1
	SRP	2.2				
Desert	DOC:NO ₃ ⁻	3.7	DOC:DON	1.6	DON	1.4
	SRP	2.1	Q/w	1.3	PAR	1.3
					GPP	1.3
					NO ₃ ⁻ :SRP	1.1
					NO ₃ ⁻	1.0
Tallgrass	DOC:NO ₃ ⁻	2.6	Grad	1.9	Temp	1.4
			A ₅ /A	1.9	Q/w	1.0
			DO	1.8		
			DOC	1.7		
Conifer	DOC:NO ₃ ⁻	3.1	DON	1.8	GPP	1.7
	PAR	3.0	NO ₃ ⁻	1.6		
			Temp	1.7		
Northern deciduous	PAR	4.7			NO ₃ ⁻	1.5
	Q/w	3.2				
	Grad	2.2				
Southern deciduous	DOC:DON	2.4	NO ₃ ⁻ :SRP	1.8	NO ₃ ⁻	1.4
	DOC:NO ₃ ⁻	2.4			Temp	1.3
	A ₅ /A	2.3			DO	1.3
Tropical	PAR	5.3			NO ₃ ⁻	1.5
	GPP	3.3			A ₅ /A	1.1
					DOC:DON	1.1

^aResponse variable is NO₃⁻ uptake velocity (V_f). Variables with VIP scores <1 were not considered influential and are not listed. See section 2 for definition of acronyms.

in the continental model (Figure 2). However, in the desert model, which also had high light availability and DOC:NO₃⁻ ratios, PAR modeled as less influential. SRP was a highly influential predictor in steppe and desert models. Streams from both of these biomes received the most PAR and had the greatest DOC:NO₃⁻ ratios.

The following variables were each highly influential in only one of the seven biome models: NO₃⁻:SRP (steppe; Figure 3a), specific discharge (northern deciduous; Figure 3b), GPP (tropical; Figure 3c), DOC:DON (southern deciduous), NO₃⁻ (steppe), A₅/A (southern deciduous), temperature (steppe), and stream gradient (northern deciduous).

4. Discussion

At the continental scale the results of our overall model reveal the importance of elemental stoichiometry and light availability in controlling rates of NO₃⁻ uptake velocity, parameters that capture both heterotrophic and autotrophic pathways of nutrient use efficiency. Specifically, the molar ratio of DOC:NO₃⁻ is the single best predictor of NO₃⁻ uptake velocity. This positive correlation reflects that under relatively high-energy availability (i.e., high DOC), inorganic nitrogen demand increases, driving higher rates of NO₃⁻ V_f . This is consistent with studies demonstrating elevated nutrient demand under high DOC conditions ranging from global to regional scales [Taylor and Townsend, 2010; Helton et al., 2015; Rodríguez-Cardona et al., 2016]. Our results also expand upon the original LINX II studies, which showed that NO₃⁻ concentration alone was a good predictor of V_f at the continental scale [Mulholland et al., 2008] but did not explicitly consider the importance of C:N stoichiometry. The ratio of DOC:NO₃⁻ is a better predictor of uptake velocity than the concentration of either solute alone [Rodríguez-Cardona et al., 2016] as it describes the balance between heterotrophic demand for energy versus the demand for nutrients. Strong effects of light have also been observed in multiple studies examining light availability and NO₃⁻ uptake [Hall and Tank, 2003; Fellows et al., 2006; Mulholland et al., 2006; Roberts and Mulholland, 2007]. A separate multivariate analysis examining

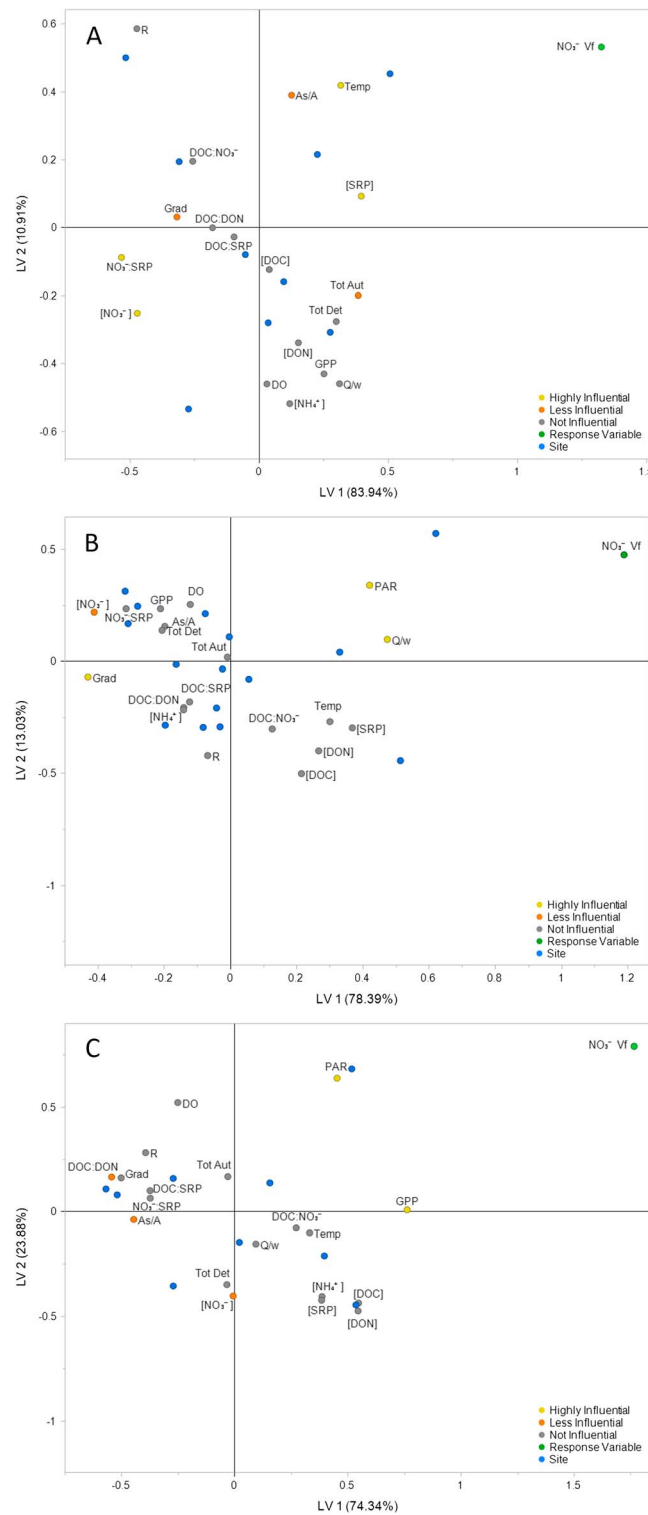


Figure 3. Partial least square biplots predicting the variability of nitrate uptake velocity ($\text{NO}_3^- \text{Vf}$) in streams from (a) steppe, (b) northern deciduous, and (c) tropical biomes. These biome-specific biplots are provided as examples where the influence of biological, physical, and chemical parameters were the strongest. See Figure 2 caption for VIP groupings.

the effect of land use on NO_3^- uptake length found a strong indirect effect of PAR through GPP [Hall et al., 2009], and our overall continental-scale results are consistent with this conclusion, as they demonstrate the relative strength of PAR and GPP across biomes. These patterns may also reflect an interaction between $\text{DOC}:\text{NO}_3^-$ and light where high PAR stimulates photoautotrophic activity, which would simultaneously increase both nutrient uptake and carbon fixation that can result in DOC production. The combined effect of this activity would raise $\text{DOC}:\text{NO}_3^-$ ratios, thereby leading to the ratio's predictive ability. However, our results do not allow us to tease apart these mechanisms or the role of denitrification in driving NO_3^- uptake. Regardless of the mechanistic drivers, the collective results from this study and others [e.g., Sobczak et al., 2003; Hall et al., 2009; Taylor and Townsend, 2010; Rodríguez-Cardona et al., 2016] reveal that at the global, continental, and regional scales, the ratio of $\text{DOC}:\text{NO}_3^-$ and the availability of light are the two strongest predictors of NO_3^- uptake in streams. Due to the integration of fully replicated land use types across biomes, our findings are likely applicable to a wide range of stream types.

Dissolved organic carbon to nitrate ratios and PAR are not universal predictors of NO_3^- use efficiency, however. The ratio of $\text{DOC}:\text{NO}_3^-$ was an influential predictor in only four of the seven biomes, and it is not clear why $\text{DOC}:\text{NO}_3^-$ was influential in some biomes but not others. Generally, biomes with highly influential $\text{DOC}:\text{NO}_3^-$ ratios had low specific discharge, high SRP, low NH_4^+ and DON, and high stream temperature, but $\text{DOC}:\text{NO}_3^-$ was not influential in all biomes with these characteristics. The specific combination of these factors may drive the influence of $\text{DOC}:\text{NO}_3^-$ ratios by providing sufficient quantities of other limiting nutrients (e.g., SRP), low concentrations of other sources of N, and high temperatures to support heterotrophic processes. These differences may be driven by biome-specific differences in DOC composition

and bioavailability, the dichotomous role of DON in streams [Wymore *et al.*, 2015], or differences in the composition of the algal and microbial communities [e.g., Martí *et al.*, 1997]. It is likely that the interaction of multiple biological, physical, and chemical characteristics determines when and where $\text{DOC}:\text{NO}_3^-$ ratios are highly predictive of NO_3^- uptake velocity.

Photosynthetically active radiation was only a highly influential predictor of NO_3^- uptake velocity in biomes with low mean PAR. However, PAR was not an influential predictor in all biomes with low light availability (e.g., southern deciduous). Our multivariate results indicate that $\text{NO}_3^- V_f$ in southern deciduous streams is tightly linked to stream chemistry and elemental ratios. It is important to emphasize that our analyses do not necessarily contradict other results that have found light and GPP to correlate with $\text{NO}_3^- V_f$ [e.g., Mulholland *et al.*, 2006]. Rather, our analyses indicate that when $\text{NO}_3^- V_f$ is analyzed in a multivariate context, certain variables emerge as influential while others do not. Thus, it is possible for one study to arrive at the conclusion that light is important to $\text{NO}_3^- V_f$ via a bivariate regression, while a multivariate study concludes that other variables are *relatively* more important.

In contrast to another multivariate analysis of the LINX II data [Hall *et al.*, 2009], our analysis, which used uptake velocity, as opposed to uptake length, did not identify discharge as a predictor of $\text{NO}_3^- V_f$ at the continental scale. Differences in our continental-scale model and Hall *et al.* [2009] are likely a result of using different response variables. V_f facilitates the direct comparison of nutrient use efficiency across sites which vary in discharge [Wollheim *et al.*, 2001] allowing us to more confidently demonstrate when and where discharge is a driving factor. As a result, the influence of discharge appears to be a relatively strong predictor of $\text{NO}_3^- V_f$ within certain biomes (e.g., northern deciduous) as well as a contributing factor in deserts and other global biomes (e.g., taiga [Diemer *et al.*, 2015]) [see Lautz and Siegel, 2007].

To provide a further comparison of our novel multivariate analysis to previous LINX II studies, we performed a posthoc PLS analysis using a different nutrient spiraling metric. Areal uptake (U) represents the mass of N removed per unit area ($\text{mg N m}^{-2} \text{min}^{-1}$). Our analysis revealed that the mass flux of NO_3^- is predicted by a slightly different set of variables at both the continental and biome scale than those identified in previous analyses of the LINX II data set (Table S10). In particular, $[\text{NO}_3^-]$ is the strongest predictor of U , confirming Hall *et al.* [2009]. Converting from U to V_f normalizes uptake for $[\text{NO}_3^-]$ in the water column, and thus, in our original analysis of V_f , $[\text{NO}_3^-]$ is no longer highly influential. However, when we expand the analysis to U , we still find $\text{DOC}:\text{NO}_3^-$ to be a highly influential predictor. This result demonstrates that the $\text{DOC}:\text{NO}_3^-$ ratio is not simply collinear with $\text{NO}_3^- V_f$ but has broad applicability as a predictor of the efficiency and overall rate of NO_3^- uptake in streams. With U as the response variable other stoichiometric ratios also emerge as influential predictor variables across biome-specific models, which highlight the need to consider elemental ratios in the development of robust nutrient uptake models. Understanding how drivers of nutrient uptake vary across space can be used to inform best management practices within specific biomes, thereby increasing both the resistance and resiliency of aquatic systems to excessive nutrient loading.

Although biotic processes are ultimately responsible for N uptake, physical and chemical variables indirectly control nutrient uptake by influencing biological parameters [Hall and Tank, 2003; Hall *et al.*, 2009]. We can characterize biomes by the types of physical and chemical variables that exert control on biotic uptake. For example, in northern deciduous streams only physical characteristics were highly influential predictors, while in desert and tallgrass streams only chemical characteristics were highly influential predictors of $\text{NO}_3^- V_f$. Streams in other biomes (e.g., steppe) had both physical and chemical characteristics as highly influential predictors. Our analyses provide little support for the direct control of biological predictors in the majority of biomes. Only in the tropical biome was metabolism a highly influential predictor of $\text{NO}_3^- V_f$; yet PAR, a physical characteristic, was still 50% more influential. In contrast, the role of biological predictor variables was considerably more apparent in predicting mass flux. For example, in both the steppe and tallgrass biomes, total autotrophic standing stock and total detritus contributed significantly to these models. And in the tropical biome, GPP and R were as influential as temperature, indicating a potential interaction between physical properties and biological processes to drive nutrient uptake. Such indirect pathways are consistent with the relationships observed via structural equation modeling where land use indirectly controlled NO_3^- uptake length by driving changes in GPP [Hall *et al.*, 2009]. Identifying those biomes where nutrient uptake is linked to physical parameters such as temperature or flow will be useful in predicting where the effects of climate change will most greatly impact nutrient cycling.

Biological processes and physical-chemical variables are inextricably linked within ecosystems. By using V_f in an interbiome approach, we were able to examine the controls on NO_3^- use efficiency across large environmental gradients. Recent large syntheses [Taylor and Townsend, 2010; Helton et al., 2015] have demonstrated a strong negative relationship between NO_3^- and DOC concentrations across terrestrial and aquatic ecosystems ranging from soils, groundwater, lakes, estuaries, and streams. Here we contribute to the broader understanding of DOC and NO_3^- interactions by presenting experimentally based results across a dynamic range of environmental gradients revealing how both the efficiency and areal rate of NO_3^- uptake can drive the overall negative relationship between DOC and NO_3^- . Consequently, we can use these results to hypothesize how the relative strength of the DOC- NO_3^- relationship changes across space and how environmental factors (e.g., light) contribute to the generation of high DOC and low NO_3^- concentrations in a wide range of environments. The spatial and biome-scale variation observed within the LINX II data set indicates that the underlying mechanism controlling the coupled biogeochemistry of DOC and NO_3^- is not universal as suggested by larger syntheses. In turn we can use results from this study to drive future mechanistic-based research and to point watershed management toward biome-specific practices that will have a greater chance of success. Mitigation of nutrient loading and export to downstream receiving water must be considered in not only a land use context but also a biome-specific context.

Acknowledgments

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