

Hydrologic and biotic influences on nitrate removal in a subtropical spring-fed river

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Abstract

We use a long-term chemical and hydrologic record in combination with longitudinal sampling and high-frequency nitrate (NO_3^-) measurements from in situ sensors to describe temporal and spatial patterns of nitrogen (N) inputs and removal in the spring-fed Ichetucknee River (Columbia County, Florida) and to determine the hydrological, geomorphic, and biological factors that influence those dynamics. Over a 20-yr period of record, NO_3^- -N removal averaged 118 kg N d^{-1} ($0.77 \text{ g N m}^{-2} \text{ d}^{-1}$) over the upper 5 km of the Ichetucknee River. Three independent estimates of gross autotrophic N assimilation (from gross primary production, diel NO_3^- variation, and standing biomass) agreed closely but accounted for less than 20% of observed N removal. Longitudinal surveys indicate negligible or negative dissolved organic nitrogen and ammonium (NH_4^+) production, suggesting that denitrification is the predominant mechanism of N removal in this river. A positive relationship between discharge and the magnitude of NO_3^- -N removal shows that interactions with the surrounding floodplain exert considerable influence at high flows, and longitudinal NO_3^- patterns indicate that N removal may be influenced by channel morphology. These results suggest a greater role for dissimilatory processes and hydrologic connectivity with hyporheic and floodplain sediments than has been previously recognized in highly productive spring-fed rivers of north Florida. While hydrologic variation is the primary determinant of variation in NO_3^- removal within the Ichetucknee River, comparison across systems indicates that biotic characteristics can cause significant deviation from predictions based on purely physical models of relationships between river size and N removal.

Stream networks are an important vector for delivery of nutrients to downstream systems, but they are also sites of significant nutrient removal during transport from land to sea (Peterson et al. 2001; Seitzinger et al. 2006; Mulholland et al. 2008). The magnitude and efficiency of this removal are influenced by biotic and physical structures and processes that vary (and covary) at multiple spatial and temporal scales. The interplay of these features has been well-studied in small streams of mesic, temperate biomes, primarily via single or infrequent solute injections (Mulholland et al. 2008). Less is known about how spatial and temporal variation in flow, channel geometry, and biotic activity influence nutrient dynamics (Fisher et al. 2004; but see Roberts and Mulholland 2007), particularly in tropical and subtropical systems and in larger rivers. Anticipating the responses of river networks and watersheds to future changes in land use and climate will require a more general understanding of the circumstances under which physical or biotic factors are the primary determinants of nutrient removal across channel sizes and among river systems at a range of temporal scales.

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Because discharge generally increases more quickly than channel width as a function of catchment area (Church 1992), larger channels have higher discharge per unit stream width (specific discharge: $\text{m}^3 \text{ s}^{-1} \text{ m}^{-1}$ or $\text{m}^2 \text{ s}^{-1}$). As a result, models of nitrogen (N) removal in river networks indicate that large rivers remove less N per unit length than do small streams (Alexander et al. 2000; Seitzinger et al. 2002; but see Ensign and Doyle 2006) as a result of reduced interaction between the water column and biogeochemically active benthic sediments. However, at the scale of whole basins, the total magnitude of N removal in higher-order rivers can be considerable (Seitzinger et al. 2002; Wollheim et al. 2006). Importantly, these models generally assume a constant uptake rate across channel sizes (but see Wollheim et al. 2006). Although there is little evidence that uptake efficiency varies as a function of stream size, variation within channel orders is considerable, and existing studies are strongly biased toward small- to medium-sized channels (Ensign and Doyle 2006). These models also assume that efficiency declines rapidly with increasing discharge, an assumption that may not hold in larger systems, where high flows may promote interaction with reactive floodplain sediments (Pinay et al. 2000).

In addition to total N removal, the relative magnitude of assimilatory uptake and denitrification as mechanisms of N removal in streams and rivers has important consequences for the delivery of N to downstream ecosystems (Mulholland et al. 2008). Whereas most assimilated N is ultimately exported downstream in some form (Newbold et al. 1982;

Peterson et al. 2001), denitrification and other pathways of N_2 production result in permanent removal. In smaller streams, isotopic tracers allow relatively precise, methodologically consistent, and process-specific estimates of biotic and hydrologic fluxes (Peterson et al. 2001; Mulholland et al. 2008). In large rivers, where tracer studies are generally impractical, few studies address the absolute and relative importance of assimilatory and dissimilatory N removal. Most estimates of whole-reach denitrification in large rivers are based on either scaling up of chamber measurements or differences in N flux between upstream and downstream sampling stations (Seitzinger et al. 2002). Open channel measurements of denitrification (Laursen and Seitzinger 2002; Pribyl et al. 2005) can provide whole-system estimates of dissimilatory N removal; however, few studies of large rivers have assessed whole-system autotrophic N assimilation, which can be estimated based on metabolism measurements in conjunction with stoichiometric assumptions (Hall and Tank 2003) or via recently developed approaches based on diel nitrate (NO_3^-) variation (Johnson et al. 2006; J. B. Heffernan and M. J. Cohen unpubl.).

Spring-fed rivers of north Florida as model ecosystems

Northern Florida has the highest density of large springs in the world, including 33 first-magnitude (defined as nominal discharge greater than $2.8 \text{ m}^3 \text{ s}^{-1}$) and 191 second-magnitude ($0.28\text{--}2.8 \text{ m}^3 \text{ s}^{-1}$) springs (Scott et al. 2004). These springs are fed by the Floridan Aquifer, an eogenetic carbonate karst system, the porous matrix of which stores large volumes of water relative to other karst systems (Floreana and Vacher 2006). As a consequence of the significant storage and active conduit-matrix exchange (Martin and Dean 2001), these large springs exhibit relatively low variability of discharge, temperature, and chemical composition over periods of weeks to months and years (Odum 1957b). Over longer time scales, spring chemistry (e.g., NO_3^- , dissolved oxygen [DO]) may vary in response to major climatic events and oscillations that alter the relative contribution of short- and long-residence-time groundwater flowpaths (Martin and Gordon 2000; Toth and Katz 2006; Heffernan et al. in press).

Because of their hydrologic, thermal, and chemical stability, plus their abundant light and high water clarity, many Florida spring systems support dense, productive macrophyte communities (Odum 1957a; Duarte and Canfield 1990). These communities provided a model system for important early studies of ecosystem energetics and trophic structure (Odum 1957b), and recent studies have continued to emphasize reciprocal interactions between water column chemistry and the metabolism and structure of autotrophic communities (Canfield and Hoyer 1988; De Brabandere et al. 2007). Despite the widespread recognition of the importance of hyporheic, riparian, and floodplain processes in streams and rivers generally (Ward and Stanford 1995; Boulton et al. 1998), the magnitude and biogeochemical influence of these flowpaths in Florida's spring runs and rivers has received little attention.

Since the mid-20th century, NO_3^- concentrations in Florida springs have risen more than an order of magnitude from historic concentrations of approximately 0.05 mg N L^{-1} to greater than 1 mg N L^{-1} or higher in many springs (Scott et al. 2004; Upchurch et al. 2007). Isotopic signatures and mass balance models indicate that agricultural sources (principally fertilizer, but also animal waste) are primarily responsible for increased N loads (Katz et al. 2005). Elevated NO_3^- concentrations have been linked to the proliferation of algal blooms in springs (Stevenson et al. 2007, but see Heffernan et al. [in press] for a critical review of evidence supporting that link), and spring discharge accounts for a significant proportion of the N load that reaches coastal oceans via the Suwannee and St. Johns Rivers (Pittman et al. 1997; Cohen 2008; Magley and Joyner 2008). Downstream declines in NO_3^- are observed in many spring runs, although in some cases total nitrogen (TN) remains relatively constant (De Brabandere et al. 2007; WSI 2008). Whether such patterns represent production of dissolved organic nitrogen (DON) by autotrophs or input of DON from external sources remains unclear.

Florida spring-fed rivers possess several characteristics that allow construction of well-constrained element budgets over a wide range of temporal scales. Because N inputs are dominated by NO_3^- ($> 75\%$ of total N inputs for N-enriched springs; Cohen 2008), measurements of NO_3^- concentration capture most ecological N metabolism. In addition, stable discharge, low stream gradients, and dense aquatic vegetation minimize episodic export of mineral and organic sediments and associated nutrients, which dominate material budgets in many fluvial systems (reviewed by Webster and Meyer [1997]). Finally, in the specific case of the Ichetucknee River, close balance between discharge from source springs and downstream river discharge reduces the uncertainty associated with estimates of groundwater fluxes and chemistry.

The goal of this study is to quantify spatial and temporal patterns of N fluxes in the Ichetucknee River, a large, benthically productive, spring-fed river. We use a long-term hydrologic and chemical record, in conjunction with high-resolution measurements from in situ NO_3^- sensors, to describe variation in N flux at timescales ranging from hours to decades and to evaluate the hydrologic, geomorphic, and biotic factors that influence N removal. To determine the mechanisms of N removal, we compare total N removal to estimates of heterotrophic and autotrophic assimilation, the latter of which is determined via multiple methods. We use longitudinal patterns of NO_3^- concentration to identify spatial patterns in N removal. These observations provide a basis to evaluate current conceptual models of spring ecosystems and the more general relationships among biotic and hydrologic variation and N removal in lotic ecosystems.

Methods

Study site—The Ichetucknee River, a tributary of the Santa Fe River and part of the Suwannee River drainage (Fig. 1A), drains a 770-km^2 catchment (or springshed)

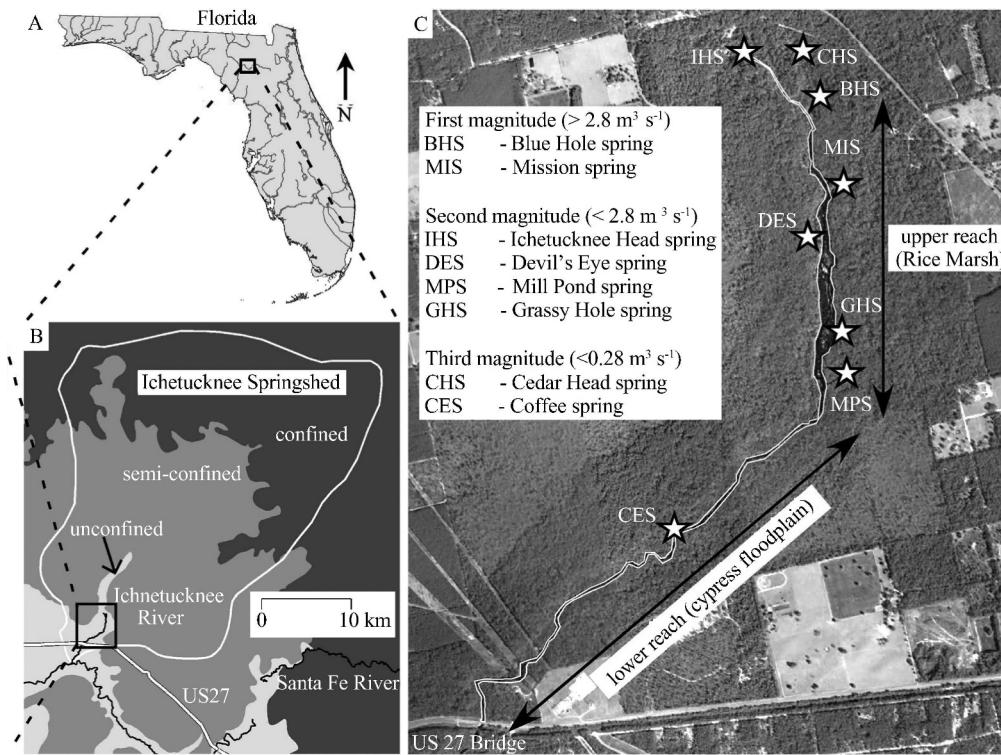


Fig. 1. Maps of the Ichetucknee River and springshed. (A) Location of the Ichetucknee River in north Florida. (B) The Ichetucknee springshed, which occupies most of Columbia County. Shaded regions indicate the distribution of Floridan Aquifer confinement by the Hawthorne Group. (C) The upper 5 km of the Ichetucknee River, contained within Ichetucknee Springs State Park, including locations of major springs, geomorphically distinct Rice Marsh and Cypress Floodplain reaches, and the downstream sampling location just above the US 27 bridge.

encompassing most of Columbia County, Florida (Champion and Upchurch 2003). Upland land use is dominated by row-crop agriculture and managed forests but includes the municipality of Lake City (population 10,000). The river is fed by six major, named springs (and multiple smaller springs) that emerge in the southernmost portion of the catchment (Fig. 1B), where the karstic Floridan Aquifer is unconfined as a result of the erosion of the clay-rich Hawthorn Group (Scott 1988). Runoff that is generated in the confined region reaches the Ichetucknee River via surface drainage during unusually large events (e.g., 2004 hurricanes), but during most storms overland and ephemeral-channel flow in the confined region is captured by numerous sinkholes that connect directly to subsurface conduits. Tracer studies indicate that flow between sinkholes and springs can be rapid ($< 10\text{--}120 \text{ d}$; KES 1997; Butt and Murphy 2003), but water discharging from springs is generally 10–40 yr old (Katz et al. 2005).

The morphology of the Ichetucknee River varies considerably along its 8-km length (Fig. 1C). Within 1000 m of its origin at the Ichetucknee Headspring (median discharge = $1.3 \text{ m}^3 \text{ s}^{-1}$), the river is joined by the combined flow of Blue Hole ($3.6 \text{ m}^3 \text{ s}^{-1}$) and Cedar Head Springs ($0.3 \text{ m}^3 \text{ s}^{-1}$), followed by the Mission Springs complex ($2.6 \text{ m}^3 \text{ s}^{-1}$) and Devil's Eye spring ($1.4 \text{ m}^3 \text{ s}^{-1}$). The upper reach between Ichetucknee Headspring and Mission Springs is $\sim 15 \text{ m}$ wide, relatively shallow (0.5–1 m), and slow-moving (0.15 m s^{-1}). Downstream of Mission Springs, in a reach

referred to as the “rice marsh,” the river widens to between 50 and 100 m for a distance of ca. 1200 m. The rice marsh has a central advective channel that is 20–25 m wide and 1–2 m deep; the remainder of the channel cross section is 20–50 cm deep. Grassy Hole ($0.2 \text{ m}^3 \text{ s}^{-1}$) and Mill Pond Springs ($0.8 \text{ m}^3 \text{ s}^{-1}$) enter the river at the downstream end of the rice marsh, below which the channel narrows to approximately 30 m and deepens to 2–3 m, while flow velocities increase to $\sim 0.25 \text{ m s}^{-1}$. This constrained section has steep channel margins and is surrounded by a floodplain (75–150 m wide) dominated by bald cypress (*Taxodium distichum*). Under median flow conditions ($\sim 9\text{--}12 \text{ m}^3 \text{ s}^{-1}$), the floodplain surface sits 10–30 cm above the river level. Coffee Spring ($0.1 \text{ m}^3 \text{ s}^{-1}$) enters the river approximately halfway between the rice marsh and the downstream boundary of Ichetucknee State Park (U.S. Highway 27; US 27). Beyond the park boundary, the Ichetucknee flows ca. 3 km to its confluence with the Santa Fe River. Because of the river's low gradient ($< 2 \text{ m km}^{-1}$), flooding in the Santa Fe and Suwannee Rivers can raise water levels in the lower Ichetucknee by several meters; these events reduce flow velocity in the Ichetucknee and inundate the floodplain.

Vegetation varies with geomorphic features as well as human activity in the Ichetucknee River. Spring vent pools and advective portions of the main channel (including the rice marsh) support abundant native submerged macrophytes, especially strapleaf sagittaria (*Sagittaria kurziana*)

and tapegrass (or eelgrass; *Vallisneria americana*), that typify Florida springs (Odum 1957a,b). Shallow lateral portions of the rice marsh also support emergent vegetation, notably wild rice (*Zizania aquatica*). Emergent and floating species (*Cicuta maculata*, non-native *Pistia stratiotes*) occur along channel margins and are subject to occasional manual removal as part of park management. Algal abundance is variable, but epiphytic and benthic mats are most often observed in spring pools and run immediately downstream of Blue Hole, Devil's Eye, Mission, and Mill Pond Springs (Frydenbourg 2006). Historically, recreational use led to significant losses of vegetation, particularly in the shallow upper reach (DuToit 1979). Subsequent restrictions have allowed recovery of macrophyte density (Kurz et al. 2004).

Hydrologic and chemical measurements—Daily discharge of the six largest springs and of the Ichetucknee River at US 27 has been measured continuously by the U.S. Geological Survey (USGS) since February 2002. Field measurements of discharge date back to 1976 for the Ichetucknee River at US 27, but field measurements of springs discharge are rare prior to 2002. Summed hydrologic inputs from the two ungauged third-magnitude springs (Coffee and Grassy Hole Springs) are less than 1% of discharge from gauged springs; other smaller, unnamed springs and boils are evident along the river. Over the period of record, discharge of the Ichetucknee River at US 27 has averaged ~11% less than the combined flow of the gauged springs (Fig. 2A), indicating that diffuse groundwater inputs (including inputs from smaller springs) are minimal and that the Ichetucknee River is typically a losing river. Comparison of discharge at the US 27 bridge and an additional gauge midway through the lower floodplain reach indicates that most water loss occurs below the rice marsh (V. de Montety unpubl.). The algorithm for discharge at the US 27 gauge includes a correction for water levels in the Santa Fe River to account for periods when stage increases but discharge slows or ceases as a result of backwater effects.

Nutrient chemistry has been measured in the Ichetucknee River at US 27 intermittently since 1968, with approximately monthly sampling since 1989. Concentrations of dissolved N species (NO_3^- , NH_4^+ , and total Kjeldahl N [TKN]) from the six gauged spring vents have been measured approximately quarterly since 2002 and on a monthly basis during 2 year-long periods (October 1991–September 1992, October 2001–September 2002), with occasional additional samples collected since 1998. These data were obtained from national water quality databases (U.S. Environmental Protection Agency [EPA] STORET: <http://www.epa.gov/storet/>; USGS National Water Information System: <http://waterdata.usgs.gov/nwis>). To supplement these records, we sampled spring vent water on 10 dates between June 2007 and August 2008 and river water during two longitudinal surveys of N chemistry (14 September 2007 and 27 March 2008), during which we sampled at approximately 500-m intervals (nine stations). Water samples from synoptic spring sampling and longitudinal surveys were collected in acid-washed polyethylene bottles

and stored on ice until return to the laboratory. Samples were filtered, subsampled, and frozen until analysis for NO_3^- (EPA Method 353.2), NH_4^+ (EPA Method 350.1), and TKN (EPA Method 351.2). We supplemented the aforementioned records with data from state agency reports, which included an additional longitudinal survey conducted on 28 April 2004 (Kurz et al. 2004; Frydenbourg 2006).

To evaluate finer-scale temporal dynamics of NO_3^- and DO in the Ichetucknee River, we deployed in situ sensor arrays in the Ichetucknee River at US 27 for four periods of varying duration during the spring (14 April–07 May and 14–18 May) and fall (01–04 October, 19 November–01 December) of 2008. Details concerning instrumentation and temporal patterns are described by J. B. Heffernan and M. J. Cohen (unpubl.). Here we report the hourly mean concentrations of each solute during the spring and fall deployments.

Nitrogen budgets and longitudinal patterns—The majority of observations of both NH_4^+ (76%) and TKN (52%) from the long-term record at the US 27 station were below stated method detection limits (which ranged from 0.004 to 0.05 mg $\text{NH}_4\text{-N L}^{-1}$ and 0.05 to 0.2 mg TKN L^{-1} , respectively, over the duration of the long-term record). Low total organic carbon (TOC) concentrations and the absence of a correlation between TKN and TOC ($r < 0.01$) further indicates that observed variation in TKN concentration largely reflects analytical measurement noise near detection limits. The role of particulate organic C and N export is unknown, but observations from similar spring systems indicate that river reaches generally act as particle sinks rather than sources (WSI 2008). In constructing N mass balance from the long-term record, we therefore use changes in NO_3^- concentration as our primary metric of changes in total N concentration, and we rely on synoptic and longitudinal surveys, for which detection limits were lower, to assess DON dynamics in the Ichetucknee River.

Low variance and strong temporal auto- and cross-correlations of discharge and NO_3^- concentration within and among the Ichetucknee River and its feeder springs allowed us to calculate N mass balance when observations were incomplete or asynchronous. Springs were nearly always sampled within 1 d of one another, but on five occasions we pooled data from a 14-d window, a period over which NO_3^- concentration in spring vents is extremely stable (T. J. Rayfield unpubl.). Asynchrony of spring and river samples was more common. Our record included 35 dates on which spring and river samples were collected within 1 d, 20 within 7 d, and 16 within 14 d. Autocorrelation of monthly river NO_3^- concentration was high ($r = 0.84$), and extended sensor deployments indicated little day-to-day change over monthly intervals (J. B. Heffernan and M. J. Cohen unpubl.). When two measurements bracketed observations from springs within a 2-week window, we interpolated linearly (16 of 36 observations with > 1-d window).

Covariation among springs also allowed two approaches for estimating N inputs for dates when all springs were not sampled. We used regression analysis to quantify bivariate

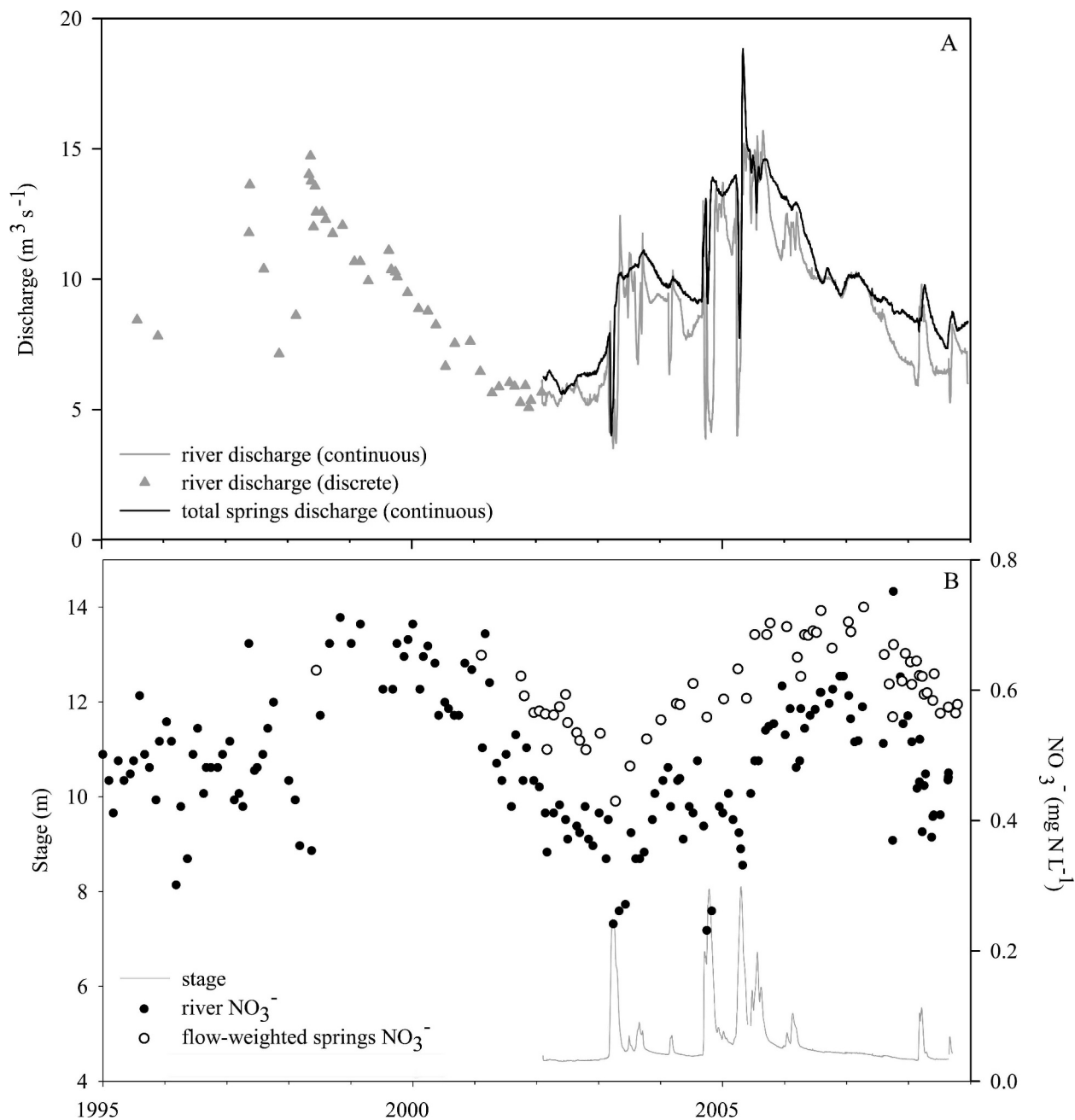


Fig. 2. Temporal dynamics of (A) discharge and (B) river stage and NO_3^- concentration in the Ichetucknee River and contributing springs between 1995 and 2009. (A) Comparison of spring inflow and river discharge indicates that the Ichetucknee River loses an average of 11% of its discharge prior to the US 27 bridge. Comparison of discharge at other gauges indicates that most water loss occurs in the lower cypress floodplain reach. Sharp declines in discharge are caused by reduced hydraulic gradient during floods in the Santa Fe River. Note that discharge varies only ca. fourfold measure over the entire 15-yr period of record. (B) NO_3^- concentrations in the Ichetucknee River (at the US 27 bridge) track flow-weighted springs input concentrations but are consistently lower and exhibit marked declines coinciding with backflooding by the Santa Fe and Suwannee Rivers that produce steep increases in river stage. NO_3^- concentrations in the river are maximally correlated with river discharge at a lag of 6–18 months.

relationships of NO_3^- concentration among all springs based on all samples from the long-term record collected within a 7-d window of one another (Fig. 3). In cases where measurements of one or two springs were missing, we used the relationships between the missing spring and its strongest correlate to estimate NO_3^- concentration. The two springs most frequently omitted were Cedar Head

Spring, whose contribution to total NO_3^- inputs is small, and Devil's Eye Spring, whose NO_3^- concentration was closely correlated with that of Mission Springs ($r = 0.88$). When three or fewer springs were measured, we used NO_3^- concentrations of individual springs (Blue Hole, Mission, or Mill Pond Springs) to estimate the flow-weighted NO_3^- concentration of total spring inflow; NO_3^- concentrations

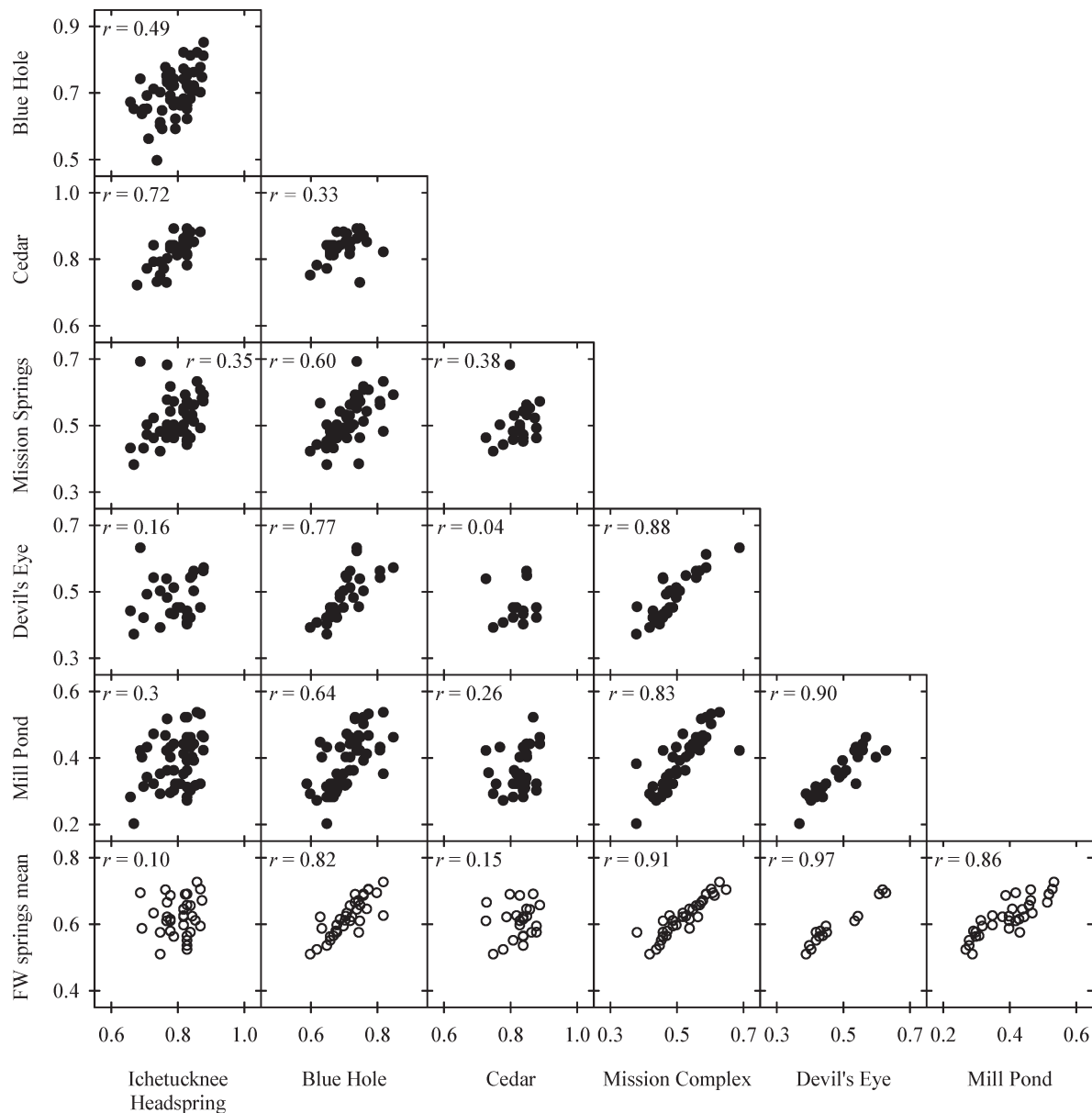


Fig. 3. Correlations of NO_3^- concentration (mg N L^{-1}) among springs and between individual springs and the flow-weighted mean concentration of all springs (lower row, open symbols). These correlations were used to estimate NO_3^- inputs on dates when all springs were not sampled.

in each of those three springs were closely correlated ($r > 0.8$) with the flow-weighted mean NO_3^- concentration of all springs (Fig. 3).

We calculated total mass of N removal as the difference between NO_3^- fluxes from springs and in the Ichetucknee River at US 27 for a total of 60 dates. We categorized N removal calculations as complete (for calculations based on NO_3^- concentration from four or more springs and the river collected within a 7-d period, $n = 33$) or estimated (when either three or fewer springs were sampled and/or measurements were within a 8–14-d window, $n = 27$). We determined areal rates of NO_3^- removal using channel dimensions measured by Kurz et al. (2004), which yielded a streambed area of 0.154 km^2 upstream of the US 27 bridge. During periods of high stage, this is undoubtedly an

underestimate, since wetted area will include an unknown proportion of the lower floodplain (3 km [length] \times 100 m [width] = 0.3 km^2). Physical and chemical characteristics of the Ichetucknee River and its feeder springs on the subset of dates for which we calculated mass balance were similar to those observed over the entirety of our long-term record and were also similar between dates of complete and estimated mass balance (data not shown).

Because the hydrologic input of combined springs discharge is generally greater than the downstream discharge of the Ichetucknee River, direct comparison of NO_3^- inputs and NO_3^- outputs will tend to overestimate NO_3^- removal, because this comparison implicitly assumes that all NO_3^- is removed from water that reenters the groundwater system. Alternatively, if we calculate NO_3^-

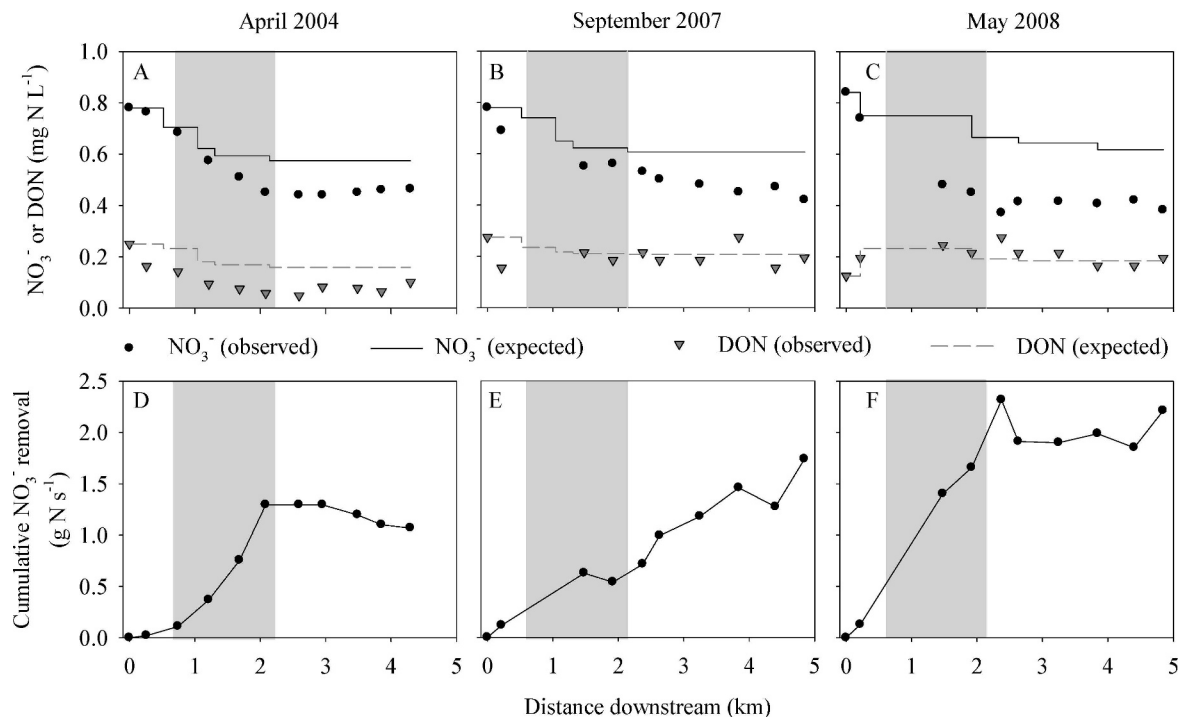


Fig. 4. (A–C) Longitudinal patterns of NO_3^- and DON concentration and (D–F) cumulative NO_3^- removal in the Ichetucknee River on three dates. (A–C) Lines indicate concentrations expected based on mixing of spring inflows. Symbols indicate observed concentrations. (D–F) Symbols indicate the difference between expected NO_3^- fluxes based on spring inflows and observed fluxes based on NO_3^- concentration and discharge of upstream springs. Gray areas in all panels indicate the longitudinal extent of the Rice Marsh reach. April 2004 survey proceeded in an upstream direction over the course of an entire day. September 2007 and March 2008 surveys were conducted over the course of 2 h in a downstream direction beginning at 14:00 h and 08:00 h, respectively. Discharge on all three dates was between 9 and $10 \text{ m}^3 \text{ s}^{-1}$.

inputs as the product of river discharge and flow-weighted spring NO_3^- concentration, then the difference between this estimate of inputs and measured export implicitly assumes that no NO_3^- is removed from water that reenters the groundwater system and will therefore tend to underestimate NO_3^- removal. A third, intermediate alternative is to estimate NO_3^- export as the product of spring discharge and river NO_3^- concentration. This approach implicitly assumes that the same proportion of NO_3^- is removed from water that reenters the groundwater system as is removed from river discharge. We calculated NO_3^- removal in the Ichetucknee River using all three approaches, but we used the third, intermediate estimates in all statistical analyses.

To evaluate the spatial distribution of N removal, we determined expected longitudinal patterns of NO_3^- and DON concentrations based on spring inflows over the length of the Ichetucknee River for comparison to our longitudinal surveys. We calculated the flow-weighted average concentration (of NO_3^- or DON) of all springs upstream of each sampling point and determined cumulative N removal as the difference between expected and observed NO_3^- fluxes. Since discharge at each sampling point is unknown, these longitudinal null models assume, as do our whole-river mass balance estimates, that N processing in ‘missing’ water occurs at the same rates as in observed water. Longitudinal surveys differed in the direction (upstream vs. downstream) and duration of sampling, leading to variability in the time of day of collection from

each location. Error in longitudinal patterns due to diel variability is not known but would be expected to increase with distance downstream.

Estimates of N assimilation—We used three independent approaches to estimate autotrophic N assimilation. The first of these, which also was the basis for our estimates of heterotrophic N assimilation, is based on application of stoichiometric ratios and growth efficiencies to measurements of gross primary production (GPP) and ecosystem respiration (ER) from 2008 sensor deployments (as described by Hall and Tank [2003]). We used the same approach to estimate autotrophic assimilation using data from Duarte and Canfield (1990), who reported GPP but not ER from two smaller reaches of the Ichetucknee in June 1987. A second, independent estimate of autotrophic N assimilation was calculated for spring and fall from mean diel NO_3^- variation during the 2008 sensor deployments. Similar to methods for calculating gross primary productivity, this approach uses the difference between the varying diel curve and a baseline interpolated between diel maxima to estimate autotrophic assimilation (J. B. Heffernan and M. J. Cohen unpubl).

Standing crops of vegetation biomass, in combination with tissue N content and biomass turnover, provided the basis for our third set of N assimilation estimates. We obtained estimates of standing aboveground biomass in the Ichetucknee River from published literature and reports

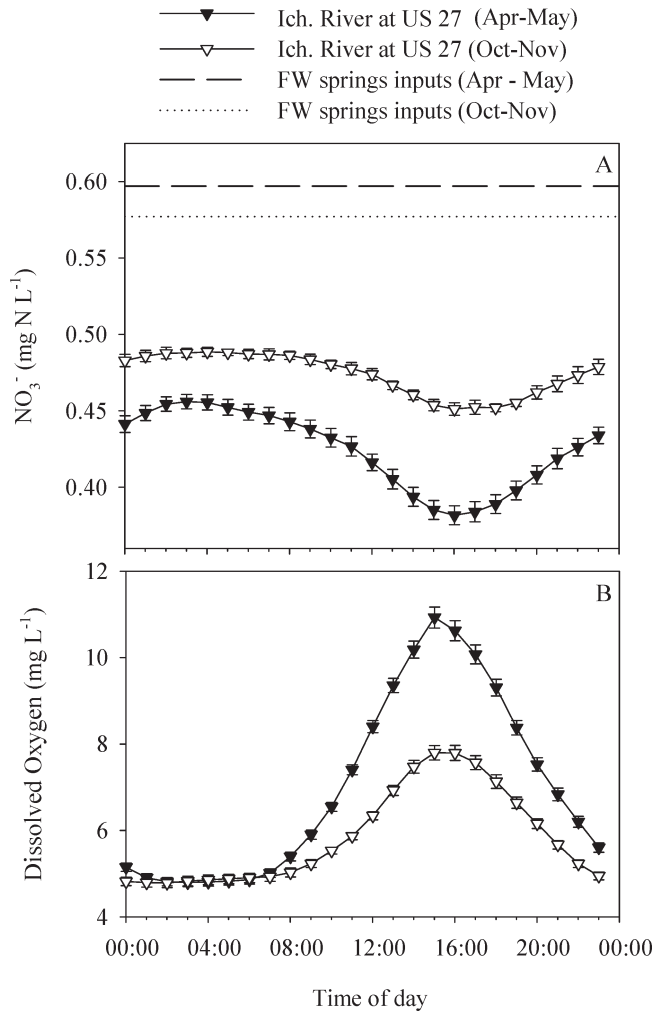


Fig. 5. Mean hourly concentrations of (A) NO_3^- and (B) DO in the Ichetucknee River during sensor deployments in April–May 2008 (closed symbols) and October–November 2008 (open symbols). Each symbol indicates the mean at hourly time steps over 25 (April–May) and 16 d (October–November), respectively. During both seasons, nighttime NO_3^- maxima were considerably lower than the flow-weighted mean of NO_3^- inputs from contributing springs ($0.597 \text{ mg N L}^{-1}$ on 24 April and $0.577 \text{ mg N L}^{-1}$ on 21 October). Error bars are $\pm 2 \text{ SE}$.

(Canfield and Hoyer 1988; Duarte and Canfield 1990; Kurz et al. 2004). These studies did not differentiate between macroalgal and vascular macrophyte biomass. We calculated biomass N standing crops assuming a dry mass N content of 2.3% by mass (Odum 1957b; Canfield and Hoyer 1988), and we converted standing crops to uptake based on autotrophic biomass turnover, which ranges from 5 to 8 yr^{-1} (Odum 1957b; Canfield and Hoyer 1988; Hauxwell et al. 2007).

Statistical analyses—We evaluated long-term changes in NO_3^- concentration by comparing the early (1965–1977) and recent (1989–2008) records using a Student's *t*-test. We calculated cross-correlation as a function of lag between discharge and NO_3^- concentration in the Ichetucknee River based on monthly averages (usually single monthly obser-

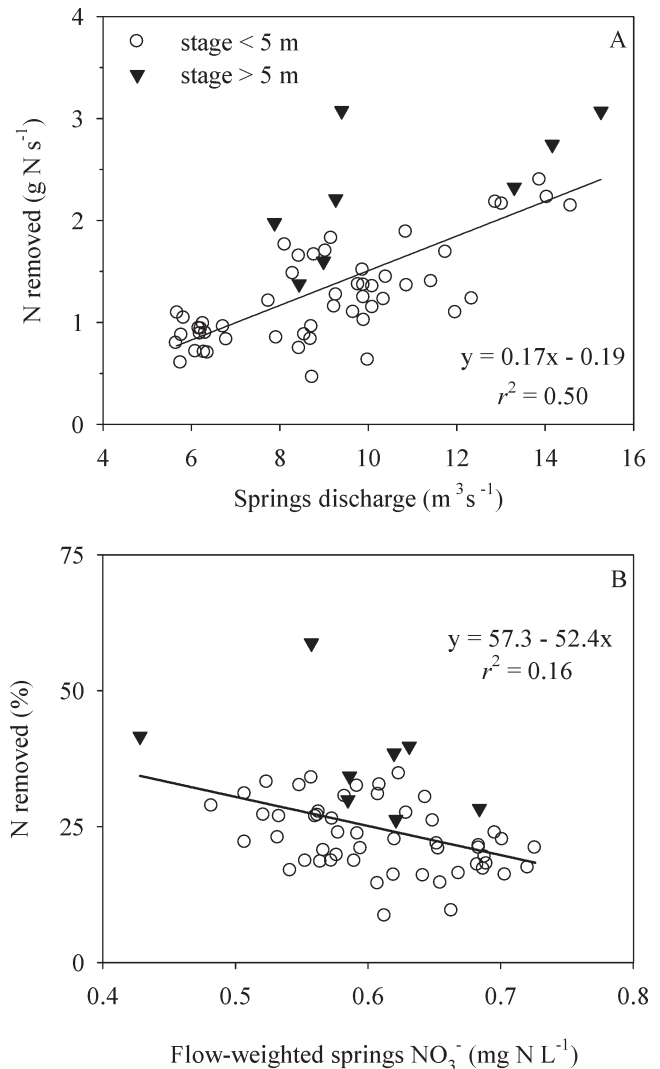


Fig. 6. Relationship between (A) discharge and mass of N removed and (B) flow-weighted springs NO_3^- concentration and N removal as a percent of inputs in the Ichetucknee River. Open symbols represent dates on which river stage is less than 5 m. Closed symbols indicate dates on which stage exceeded 5 m, generally because of flooding in the downstream Santa Fe River. Solid lines indicate best-fit univariate least-squares regression on all data.

vations for NO_3^- and for discharge prior to 2002) from 1998 to 2008, the period over which both discharge and NO_3^- measurements were available at monthly intervals or more frequently. The available density of spring vent NO_3^- measurements over periods greater than a single year was insufficient for a parallel analysis at each vent.

We used multiple regression analysis to evaluate the strength of spring discharge, spring-river discharge imbalance, river stage, time of day, DO, pH, temperature (with time of day effects removed), and flow-weighted spring NO_3^- concentration as predictors of river NO_3^- removal (as calculated when we assumed that river discharge was equal to that of springs). In this analysis we included a dummy variable that identified mass balance estimates as measure-

Table 1. Results of multiple regression analysis for prediction of nitrogen (N) removal (by mass and as a percent of inputs) in the Ichetucknee River. Models were selected based on adjusted R^2 from all subsets of the identical lists of predictors. Variables excluded from both models were pH, river DO at time of measurement, difference between spring inflow and river discharge, and NO_3^- flux from springs. The temperature variable was transformed prior to analysis to remove correlation with time of day and thus primarily describes seasonal variation in insolation and ambient temperature.*

| Effect | SS | df | MSE | F | p | β |
|---|--------|----|--------|------|--------|---------|
| N removal (g N s^{-1})—whole-model adjusted $R^2 = 0.76$ | | | | | | |
| Intercept | 4.023 | 1 | 4.023 | 46.2 | <0.001 | |
| Time of day | 0.606 | 1 | 0.606 | 7.0 | 0.01 | 0.036 |
| Temperature | 1.203 | 1 | 1.203 | 13.8 | <0.001 | 0.148 |
| River stage | 4.705 | 1 | 4.705 | 54.0 | <0.001 | 0.470 |
| Springs discharge | 6.355 | 1 | 6.355 | 72.9 | <0.001 | 0.139 |
| Error | 4.793 | 55 | 0.087 | | | |
| N removal (%)—whole-model adjusted $R^2 = 0.65$ | | | | | | |
| FW springs NO_3^- | 0.0651 | 1 | 0.0651 | 26.1 | <0.001 | -0.524 |
| Time of day | 0.0196 | 1 | 0.0196 | 7.9 | 0.007 | 0.005 |
| Temperature | 0.0442 | 1 | 0.0442 | 17.8 | <0.001 | 0.027 |
| River stage | 0.1627 | 1 | 0.1627 | 65.4 | <0.001 | 0.087 |
| Error | 0.1369 | 55 | 0.0025 | | | |

* SS, sum of squares; MSE, mean square error; FW Springs NO_3^- , flow-weighted springs NO_3^- concentration.

ments or estimates based on coincidence and completeness of concentration data. Models were chosen from all possible subsets of predictor variables based on adjusted R^2 . For comparison of total N removal with estimates of autotrophic and heterotrophic assimilation, we grouped our long-term mass balance estimates into spring–summer (March–September) and fall–winter (October–February) periods. All statistical analyses were carried out using Statistica version 8.0 (StatSoft).

Results

Concentrations of NO_3^- in the Ichetucknee River increased between 1966 and 1977, ranging from 0.02 to 0.4 mg N L^{-1} (data not shown). Concentrations during the later period of record, beginning in 1989, were greater than those observed during the earlier period (t -test: $\text{df} = 242$, $t = -9.9$, $p < 0.0001$), ranging from 0.23 to 0.9 mg N L^{-1} , but they did not exhibit any monotonic trend in time. However, NO_3^- concentration was temporally correlated with river discharge. Cross-correlation peaked ($r = 0.6$) at lags between 6 and 18 months, but instantaneous correlation was relatively weak ($r = 0.3$). Sharp declines in NO_3^- concentration coincided with spikes in river stage associated with flooding in the Santa Fe River (Fig. 2B), but these backflooding events did not produce any changes in DOC or specific conductivity (data not shown).

All three longitudinal surveys indicated rapid declines in NO_3^- concentration with distance downstream in the Ichetucknee River (Fig. 4A–C). Approximately 50% of these declines could be attributed to mixing of lower- NO_3^- inflow from downstream spring vents (Mission, Devil's Eye, and Mill Pond Springs) with higher- NO_3^- water from upstream vents (Ichetucknee Headspring, Cedar, and Blue Hole). However, observed concentrations were consistently lower than those predicted by spring inflow mixing alone.

In April 2004 and March 2008, divergence of observed NO_3^- concentration from the mixing-only model primarily occurred within the rice marsh (Fig. 4D,F). In September 2007, when sampling occurred in the downstream direction during late afternoon, observed and expected NO_3^- fluxes diverged continuously with distance downstream (Fig. 4E). In September 2007 and March 2008, DON concentration exhibited no longitudinal pattern and was not significantly different from spring input concentrations (Fig. 4B,C). In April 2004, DON concentration declined in the upper portion of the river and was lower than spring inputs at all downstream locations (Fig. 4A). Concentrations of NH_4^+ were below detection limits (0.02 $\text{mg NH}_4\text{-N L}^{-1}$) in all samples from longitudinal surveys.

At finer temporal scales, mean diel variation in NO_3^- concentration exhibited strong concordance with mean variation in dissolved oxygen (DO) (Fig. 5). During spring 2008 deployments, the amplitude of diel variation in NO_3^- concentration averaged 0.075 mg N L^{-1} , while diel DO amplitude averaged 6.1 mg L^{-1} . Diel amplitudes of NO_3^- and DO were smaller during the fall deployments, averaging 0.041 mg N L^{-1} and 3.0 $\text{mg O}_2 \text{L}^{-1}$, respectively. At all times, NO_3^- concentrations in the Ichetucknee River were considerably lower than the flow-weighted springs concentration during both spring–summer (0.597 $\text{mg NO}_3\text{-N L}^{-1}$) and fall–winter (0.575 $\text{mg NO}_3\text{-N L}^{-1}$) periods of 2008.

Fluxes of NO_3^- at the Ichetucknee River outflow were lower than spring vent inputs on all 60 dates for which we calculated NO_3^- mass balance, regardless of assumptions of hydrologic balance or imbalance. Differences between spring influx of NO_3^- (5.69 g N s^{-1}) and river outflow (4.31 g N s^{-1}) averaged 1.38 g N s^{-1} (119 kg N d^{-1}) or 0.77 $\text{g N m}^{-2} \text{d}^{-1}$ (paired t -test: $\text{df} = 60$, $t = 17.6$, $p < 0.0001$) and ranged from 0.46 to 3.08 g N s^{-1} , representing an average decrease in NO_3^- load of 24.7% (range: 8.6–58.7%). Assumptions of 0% or 100% removal of NO_3^-

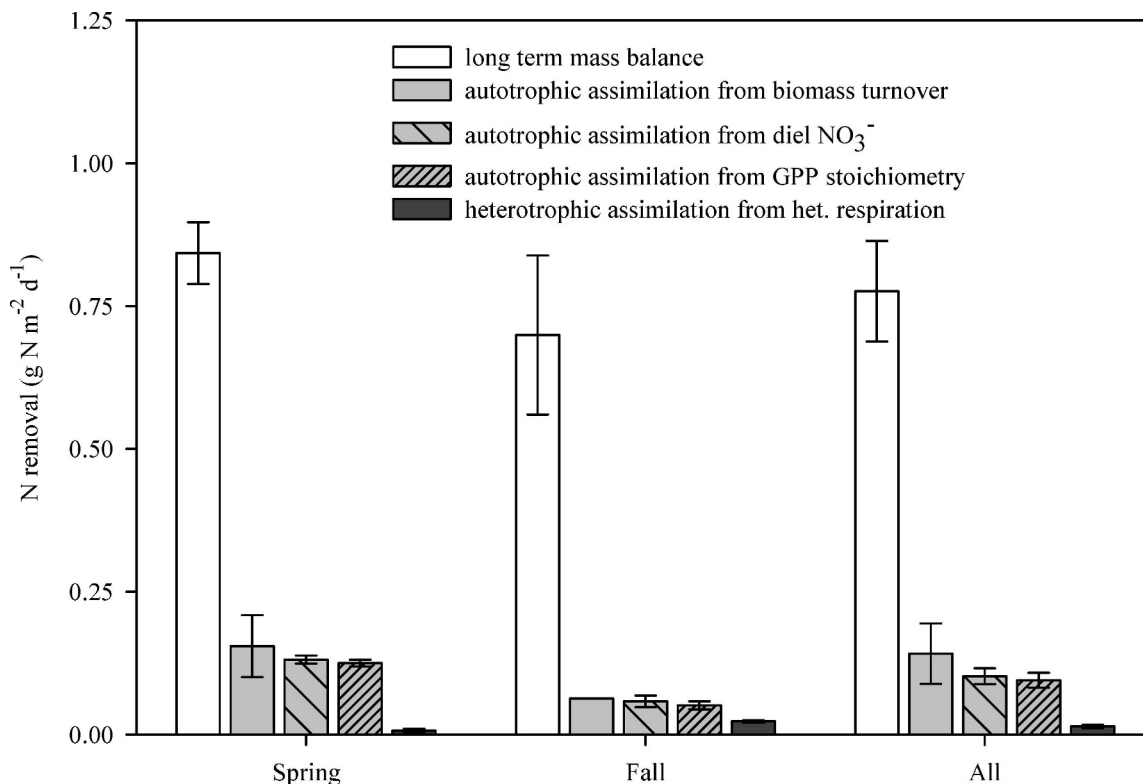


Fig. 7. Seasonal averages of total NO_3^- removal and gross assimilatory N uptake by autotrophs and heterotrophs in the Ichetucknee River. Mean NO_3^- removal estimates are based on mass balance calculations from the long-term record. Mean estimates of autotrophic uptake are derived from biomass, turnover, and plant N content; from daily measurements of diel NO_3^- variation in spring and fall of 2008; and from GPP stoichiometry. Mean estimates of heterotrophic uptake are derived from GPP and ER measurements. Error bars are ± 2 SE.

from the roughly 10% of river water that reenters the groundwater system yielded mean NO_3^- removal estimates that were 10% lower and 28% higher, respectively. Removal of N did not differ between calculations made using measured and estimated spring-vent concentrations, regardless of whether N removal was measured on a mass ($p = 0.74$) or percent ($p = 0.93$) basis.

The mass of N removed within the Ichetucknee River exhibited strong positive relationships with both river discharge and stage (Fig. 6A). Positive relationships with seasonal temperature and time of day accounted for additional, smaller amounts of variance in NO_3^- removal (Table 1). N mass removal was not affected by the difference between spring and river discharge or by the flow-weighted springs NO_3^- concentration. Mean (± 2 standard errors [SE]) areal N removal was slightly, but significantly, higher during the spring and summer (0.846 ± 0.088 g N m⁻² d⁻¹) than during the fall (0.699 ± 0.139 g N m⁻² d⁻¹). Nitrate removal efficiency (%) was not influenced by discharge but was positively correlated with river stage, seasonal temperature, and time of day and negatively correlated with flow-weighted springs NO_3^- concentration (Fig. 6B; Table 1).

Estimates of gross autotrophic assimilatory demand varied seasonally but were remarkably consistent across methods (Fig. 7). Estimates of N assimilation from GPP were 0.125 ± 0.006 and 0.051 ± 0.007 g N m⁻² d⁻¹ from spring–summer and fall–winter of 2008, respectively,

while N assimilation estimated from diel NO_3^- variation averaged 0.131 ± 0.007 and 0.058 ± 0.009 g N m⁻² d⁻¹ during the same periods. Earlier measures of GPP (June 1987) yielded an N assimilation estimate of 0.154 g N m⁻² d⁻¹. Based on biomass turnover, we estimated mean gross N assimilation of 0.089 ± 0.033 (range: 0.039–0.170) and 0.141 ± 0.053 g N m⁻² d⁻¹ (range: 0.063–0.272), assuming turnover rates of 5 and 8 yr⁻¹, respectively. Estimated heterotrophic assimilation was 0.007 ± 0.001 and 0.023 ± 0.001 g N m⁻² d⁻¹ during the spring–summer and fall–winter of 2008, respectively.

Discussion

H. T. Odum's seminal studies (Odum 1957*a,b*) of spring ecosystems emphasized interactions between the metabolism of aquatic vegetation and water column chemistry in a 'chemostat-like' environment. Our analysis of long-term chemical and hydrologic variation in the Ichetucknee River supports the view of Florida springs as notably stable relative to other river systems; nonetheless, we also observed significant relationships between hydrologic variation and nutrient delivery and removal. Even in such relatively homogeneous and temporally stable systems, interactions between hydrologic variability and geomorphic structure appear to alter connectivity among the advective channel and backwater, hyporheic, and floodplain flowpaths.

Despite the high productivity of these spring-fed rivers, our observations indicate that dissimilatory processes account for the predominance of NO_3^- removal.

Temporal variation in NO_3^- inputs and removal—The dynamics of NO_3^- inputs and removal in the Ichetucknee River reflect a suite of both biotic and hydrologic processes occurring at distinct temporal scales. Differences in NO_3^- concentration between early (1966–1977) and recent (1989–2008) time periods are consistent both with estimates of background NO_3^- concentration of between 0.02 and 0.1 mg N L⁻¹ in Florida springs (Maddox et al. 1992) and with the timing and magnitude of increased NO_3^- in other Florida springs in response to changes in land use (Upchurch et al. 2007). However, we also observed strong lagged correlations between discharge and NO_3^- that to our knowledge have not previously been reported for other Florida springs. The lag in this relationship is consistent with other discharge–chemistry relationships that reflect differential mixing of water sources during event hydrographs (Evans and Davies 1998); however, the timescale of the lag in this relationship (6–18 months) is far longer than that observed in other systems. Discharge-driven variation in spring chemistry may reflect differential contributions of recent and older groundwater, as suggested by the widespread occurrence of low DO under historic low flows in Florida springs between 2002 and 2003 (Heffernan et al. in press). Regardless of the hydrogeologic basis for observed lags, one important consequence is that the instantaneous relationship between discharge and NO_3^- is weak, allowing us to make inferences about their independent effects on N removal.

The hydrologic dynamics of the Ichetucknee River are sensitive to both upstream and downstream processes, which influence NO_3^- removal in distinct ways. The positive relationship we observed between discharge and NO_3^- removal likely reflects increased interactions with the adjacent floodplain. The variation in discharge that occurs over the period of record in the Ichetucknee River corresponds to a variation in river stage of about 0.7 m, sufficient to inundate the floodplain, even in the absence of backflooding caused by downstream rivers. The negative relationship between input NO_3^- concentration and NO_3^- removal efficiency indicates that the processes responsible for N removal are not limited by N delivery per se but rather by reactive channel area (as influenced by discharge) and residence time, which probably increases considerably during periods of elevated stage in the Santa Fe and Suwannee Rivers as a result of increased water volume in the Ichetucknee and reductions in discharge (Fig. 2). Such episodic backflooding events are a common occurrence in these low-gradient systems and represent an interesting example of upstream influence in river networks (Pringle 1997).

Seasonal and diel variation in NO_3^- removal reflect the dynamics of biotic processes at these timescales, but these variables were less important than hydrologic conditions in predicting NO_3^- removal. The highly regular, inverse diel covariation of DO and NO_3^- observed via in situ sensors (Fig. 5) indicates that diel variation is largely due to

autotrophic assimilation (J. B. Heffernan and M. J. Cohen unpubl.). This diel variation is of sufficient magnitude that time of day was a significant, albeit minor, predictor of NO_3^- export in the long-term record. However, time of day of sample collection was evenly distributed between 08:00 h and 18:00 h, a time span that accounted for nearly the entire range of diel NO_3^- variation during both spring–summer and fall–winter sensor deployments. As a result, this variation is unlikely to have biased the long-term mean of our mass balance estimates of N removal in the Ichetucknee River.

Seasonal variation in NO_3^- removal, indicated by the effect of time-of-day–corrected temperature (Table 1), may reflect temperature dependence of ecosystem metabolism and/or denitrification, but it also may be due to the phenology of spring autotrophs. Hauxwell et al. (2007) observed minimal seasonal variation in *Vallisneria* productivity in the coastal, spring-fed Crystal River, and Odum (1957b) found little evidence of seasonal variation in productivity in Silver Springs. However, Quinlan et al. (2008) observed distinct seasonal patterns in algal biomass and productivity in Silver Springs, and J. B. Heffernan and M. J. Cohen (unpubl.) found seasonal differences in productivity and N removal in the Ichetucknee River. Seasonal variation in productivity in the Ichetucknee River may influence NO_3^- removal directly via autotrophic assimilation or indirectly via the effects of production on organic matter supply to denitrifying bacteria (J. B. Heffernan and M. J. Cohen unpubl.).

Mechanisms of N removal—Although autotrophic and heterotrophic assimilation account for most N removal in many streams and rivers (Arango et al. 2008), our data indicate that assimilation accounts for a small proportion of observed N removal in the Ichetucknee River (Fig. 7). Our several independent estimates of gross autotrophic assimilation during the spring fell within a narrow range (0.12–0.14 g N m⁻² d⁻¹) that was considerably lower than observed N removal (0.77 g N m⁻² d⁻¹). Heterotrophic assimilation was similarly unable to account for the magnitude of NO_3^- removal observed in the long-term record. Because all of these estimates represent gross autotrophic or heterotrophic assimilation, comparison between these values and observed NO_3^- removal effectively assumes that internal recycling is zero. As such, the sum of autotrophic and heterotrophic assimilation (which may be as high as ~ 0.2 g N m⁻² d⁻¹) represents a liberal upper bound of assimilation as a mechanism of net NO_3^- removal. In light of the hypothesized relationship between increases in NO_3^- concentration and algal abundance (Stevenson et al. 2007; but see Heffernan et al. in press), it is noteworthy that even in the absence of any remineralization, background NO_3^- of 0.05 mg $\text{NO}_3\text{-N}$ L⁻¹ (generating fluxes of 0.4 g N s⁻¹ at 8 m³ s⁻¹ discharge) would be sufficient to support current gross assimilatory demand over the length of the Ichetucknee River.

The disparity between gross assimilation and observed NO_3^- removal indicates that denitrification is the predominant mechanism of N removal in the Ichetucknee River. We can determine lower and upper bounds on the

magnitude of denitrification assuming that net assimilation is equal to gross assimilation—or equal to zero—respectively. Whether denitrification is closer to the upper or lower bound depends on the magnitude of net assimilation. Under assumptions of steady-state N storage in biota and sediments, the sum of particulate and dissolved organic N losses is equal to the magnitude of net N assimilation (Fisher et al. 1982; Newbold et al. 1982; Brookshire et al. 2009). In the Ichetucknee River and in spring-fed rivers generally, minimal disturbance indicates that the steady-state assumption is reasonable. From the productivity: respiration ratio (1.28) determined by J. B. Heffernan and M. J. Cohen (unpubl.), which is slightly higher than that observed from other studies of metabolism in spring-fed Florida rivers (Odum 1957a,b; WSI 2008), we can estimate net assimilation as $0.05 \text{ g N m}^{-2} \text{ d}^{-1}$ ($\sim 25\%$ of maximum assimilatory demand). This estimate, which is consistent with the limited data on dissolved and particulate organic matter production in spring-fed rivers, would account for less than 7% of observed N removal. In Silver Springs, by comparison, particulate losses of organic matter were approximately 12% of GPP (Odum 1957b). In addition, based on precise measurements of downstream DOC accrual in seven spring-fed rivers, C. M. Duarte (unpubl.) estimated that DOC production from submerged vegetation was between 0.04 and $0.30 \text{ mol C m}^{-2} \text{ d}^{-1}$ (14% of GPP on average), including an estimate of $0.07 \text{ mol C m}^{-2} \text{ d}^{-1}$ in the Ichetucknee. If that DOM had a molar C:N ratio of 25:1, DON production would be approximately $0.04 \text{ g N m}^{-2} \text{ d}^{-1}$, or 5% of observed removal. While our longitudinal surveys did not indicate any DON production in the Ichetucknee River, downstream persistence of high TN concentrations has been observed in other spring-fed rivers, despite concurrent declines in NO_3^- (De Brabandere et al. 2007; WSI 2008). If dissolved and particulate losses for the Ichetucknee are at all comparable to those in other systems, then inputs from other sources likely account for much of the apparent DON production in those systems. While further attention toward organic N dynamics is needed in spring-fed rivers, denitrification likely accounts for more than 90% of NO_3^- removal in the Ichetucknee River.

The magnitude of NO_3^- removal in the Ichetucknee is comparable to open-channel measurements of denitrification in N-enriched rivers of similar size. McCutchan and Lewis (2008) estimated that denitrification in the South Platte River (Colorado) ranged from 0.3 to $3.4 \text{ g N m}^{-2} \text{ d}^{-1}$ during summer months when temperatures were similar (20 – 25°C) to those that occur throughout the year in the Ichetucknee River. Laursen and Seitzinger (2002) reported denitrification rates from 0.1 to $5.3 \text{ g N m}^{-2} \text{ d}^{-1}$ in Millstone River, New Jersey, and the Iroquois River and Sugar Creek (Illinois). Rates of N removal in the Ichetucknee River are similar to mass balance estimates of N removal in the spring-fed Chassahowitzka River but nearly an order of magnitude higher than the NO_3^- removal rate estimated from benthic flux chambers (Saunders 2007).

Spatial and temporal dynamics of NO_3^- removal in the Ichetucknee River provide additional support for denitrification as the primary mechanism of N removal. Increased

N removal at elevated stage is consistent with studies from other large rivers, in which floodplains are important sites of N removal via denitrification (Pinay et al. 2000). Although inference from our longitudinal surveys is somewhat confounded by differences in the direction and timing of their collection, two of those surveys indicated that N loss occurred almost entirely within the wide uppermost section of the river (Fig. 4). Indeed, even if all N removal in the Ichetucknee River occurs in the rice marsh, which accounts for ca. 50–60% of the total channel area, observed N removal would translate to an areal rate of about $1.5 \text{ g N m}^{-2} \text{ d}^{-1}$ in that reach. This amount would still fall within the ranges of denitrification reported by Laursen and Seitzinger (2002) and McCutchan and Lewis (2008).

Longitudinal patterns also present a potential explanation for the positive relationship between discharge and NO_3^- removal that occurs even at low stage: specifically, that small changes in discharge disproportionately influence flow through the shallow, lateral flowpaths of the rice marsh, where increased benthic contact or hyporheic exchange permits greater N removal. Spring river sediments are rich in organic matter (Saunders 2007; WSI 2008), but the magnitude and distribution of surface–hyporheic exchange in spring rivers is almost entirely unknown. As a result, the relative importance of benthic and subsurface denitrification within the rice marsh and throughout the Ichetucknee River remains unclear.

Improving our understanding of the mechanisms underlying N dynamics in larger streams and rivers will require the use of multiple approaches and continued development of new approaches and tools. The integration of long-term observations and mechanistic studies based on high-resolution data from in situ sensors can be a fruitful component of those efforts. In our study of the Ichetucknee River, inference concerning the magnitude of assimilatory demand was strengthened considerably by the simultaneous use of multiple independent approaches. Future studies of N dynamics and removal pathways in large rivers should incorporate these approaches with direct measurement of denitrification and whole-system mass balance (Pribyl et al. 2005). Such a combination of approaches may eventually produce process-specific understanding of N dynamics comparable to that developed for small streams. Spring-fed systems such as the Ichetucknee River are a particularly valuable venue for development of these approaches because inputs are temporally stable and spatially discrete.

Large-river N dynamics—Scales of biotic and hydrologic control—Stream ecologists have long recognized that nutrient dynamics in lotic systems reflect the interaction of biotic processing and hydrologic transport. This insight is the foundation of the nutrient spiraling concept (Webster and Patten 1979; Newbold et al. 1982) and has been extended conceptually and empirically to lakes, riparian zones, and terrestrial systems (Essington and Carpenter 2000; Fisher et al. 2004; Ocampo et al. 2006). Recent efforts to synthesize and scale up nutrient removal processes to river networks have emphasized the importance of rela-

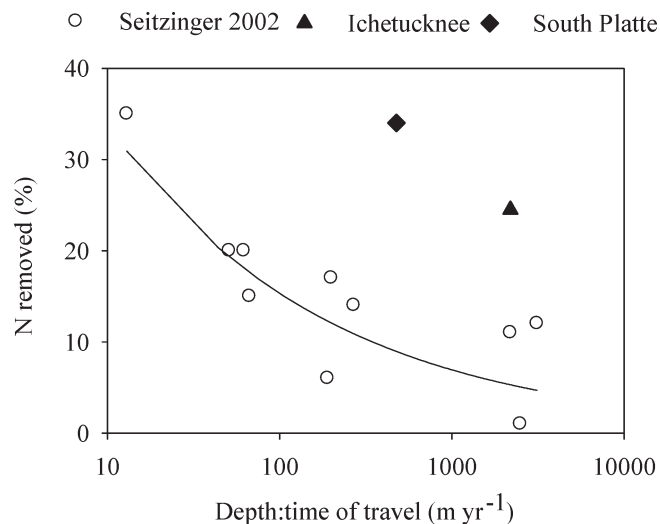


Fig. 8. Relationship between benthic contact (as described by depth:time of travel [TOT]) and N removal in aquatic ecosystems. Modified from Seitzinger et al. (2002) to include N removal in the Ichetucknee River (based on mean of mass balance estimates from this study) and South Platte River (based on open-channel measurements of denitrification and channel dimensions from Pribyl et al. [2005]). TOT for the Ichetucknee was calculated based on channel length, width, and depth, as reported by Kurz et al. (2004), and mean discharge on all dates of mass balance calculations. Data pooled by Seitzinger et al. (2002) are drawn from mesic temperate systems in North America, Europe, and New Zealand. Higher productivity and resulting labile carbon availability in the Ichetucknee and South Platte may explain their high N removal relative to depth : TOT.

tionships among channel geometry, discharge, and N removal in time and across channel sizes (Alexander et al. 2000; Seitzinger et al. 2002). Our observations in the Ichetucknee River are consistent with this emphasis on hydrologic variation as the primary determinant of N removal within systems. However, the importance of hydrology as a predictor of NO_3^- removal may reflect the relative invariance of biotic processes in this thermally and chemically stable river and may not hold in systems in which biotic processes are more variable (Roberts and Mulholland 2007). Furthermore, the lack of a relationship between N removal efficiency and discharge in the Ichetucknee River indicates that the effects of hydrologic variation may differ between small headwater streams and larger, low-gradient systems with intact floodplains.

Comparison of nutrient dynamics in streams of different biomes has demonstrated considerable differences in denitrification and autotrophic and heterotrophic assimilation among systems (Peterson et al. 2001; Mulholland et al. 2008). The results of this study indicate that large rivers may exhibit similar magnitudes of variation in N removal processes and that this variation may be linked to ecosystem metabolism, as is true for denitrification in small streams (Mulholland et al. 2009). Compared to the mesic, temperate streams and rivers used by Seitzinger et al. (2002) to develop river-network-scale models of N removal, both the Ichetucknee River (this study) and the South Platte River (Pribyl et al. 2005) have high rates of N retention

relative to expectations based on channel depth and residence time (Fig. 8). One potential explanation for this observation is that both systems have high benthic productivity, which may increase the availability of labile organic matter and alleviate C limitation of microbes responsible for denitrification. Regardless of the mechanism, hydrologic characteristics alone appear to be insufficient as predictors of N removal in river channels, particularly when comparing across biomes. More studies that address the temporal dynamics of N removal are needed, particularly in warm biomes and larger systems, to understand how interactions of hydrologic and biotic processes influence riverine N dynamics across spatial and temporal scales.

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