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Identification of nitrogen sources and transformations within karst springs using isotope tracers of nitrogen

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Abstract Isotope analyses of nitrate and algae were used to gain better understanding of sources of nitrate to Florida's karst springs and processes affecting nitrate in the Floridan aquifer at multiple scales. In wet years, δ^{15}N and δ^{18}O of nitrate ranged from +3 to +9‰ in headwater springs in north Florida, indicating nitrification of soil ammonium as the dominant source. With below normal rainfall, the δ^{15}N and δ^{18}O of nitrate were higher in almost all springs (reaching +20.2 and +15.3‰, respectively) and were negatively correlated with dissolved oxygen. In springs with values of δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ greater than +10‰, nitrate concentrations declined 40–50% in dry years and variations in the δ^{15}N and δ^{18}O of nitrate were consistent with the effects of denitrification. Modeling of the aquifer as a closed system yielded in situ fractionation caused by denitrification of 9 and 18‰ for Δ^{18}O and Δ^{15}N, respectively. We observed no strong evidence for local sources of nitrate along spring runs; concentrations declined downstream (0.42–3.3 μmol-NO$_3$ L^{-1} per km) and the isotopic dynamics of algae and nitrate indicated a closed system. Correlation between the δ^{15}N composition of nitrate and algae was observed at regional and spring-run scales, but the relationship was complicated by varying isotopic fractionation factors associated with nitrate uptake (Δ ranged from 2 to 13‰). Our study demonstrates that nitrate inputs to Florida's springs are derived predominantly from non-point sources and that denitrification is detectable in aquifer waters with relatively long residence time (i.e., matrix flow).

Keywords Florida · Karst springs · Isotope · Nitrate · Algae · Denitrification

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Introduction

Growing human population and land-use change have increased nitrate in the Floridan aquifer (De Brabandere et al. 2007; Munch et al. 2006; Katz et al. 2001), a groundwater system which extends through the United States southeast. Nitrate in many Florida karst springs has increased over the last 50 years, from less than 0.1 mg N-NO_3 L^{-1} to as high as 5 mg L^{-1}, but phosphorus levels have remained stable (Hornsby and Ceryak 1999; Scott et al. 2004; Strong 2004). While phosphate sorbs onto metal oxides and is precipitated with carbonate minerals in Florida's calcitic soils and in the limestone matrix of the aquifer (Rhue et al. 2006), nitrate is transported in the deep aquifer system (Katz 2004). Thus, Florida's spring ecosystems are susceptible to land applications of nitrogen (Bacchus and Barile 2005; Katz et al. 2008), but the partitioning of nitrogen sources is difficult owing to the complexity of land use patterns (Vasques et al. 2010) and hydrologic flowpaths within the aquifer (Martin and Dean 2001). Increases in filamentous algae have been observed in many springs, particularly the cyanobacteria *Lyngbya wollei* (Cyanophyceae) and the yellow-green alga, *Vaucheria* sp. (Xanthophyceae) (Stevenson et al. 2004; Pinowska et al. 2007) and may be the consequence of increased nitrate loading.

Anthropogenic sources of nitrate to springs include inorganic fertilizers, confined animal feeding operations (CAFOs), septic effluent, and atmospheric deposition of NO_3^- (Bacchus and Barile 2005; Katz et al. 2008). Natural sources include the products of microbial mineralization of organic matter and subsequent nitrification and biological dinitrogen fixation, which can contribute 28% of the total N input to pine savannas (Van Breemen et al. 2002; Hiers et al. 2003; Cohen 2008). In budgets calculated for springsheds in Florida, inorganic fertilizers (containing ammonium) are the largest anthropogenic source of nitrogen, however, the amount of nitrogen that actually reaches the Floridan aquifer from non-point sources is difficult to determine. Urban development along spring runs, is another potential source of nitrogen, but has received relatively little attention.

Isotope tracers provide a tool to elucidate sources of nitrate in aquatic ecosystems, but their use is complicated by mixing of multiple N sources with overlapping isotopic composition as well as biogeochemical processes that obscure source signatures (Kendall 1998; De Brabandere et al. 2007; Einsiedl and Mayer 2006; Derse et al. 2007). The δ^{15}N of groundwater nitrate can be attributed to mixtures of the following sources: (1) inorganic fertilizer (−7 to +7‰), (2) soil nitrate (−3 to +14‰), (3) animal and septic waste (+2 to +25‰) and (4) atmospheric deposition (NH_4^+ and NO_3^- in rain, −7 to +8‰) (Einsiedl and Mayer 2006; Kendall 1998; Aravena and Robertson 1998; Kreitler 1979; Kendall et al. 2007).

Dual isotope analysis of nitrate (δ^{15}N-NO_3 and δ^{18}O-NO_3) provides additional information to differentiate watershed sources of nitrate and helps to overcome the problems of overlapping source signatures and fractionation processes (Fukada et al. 2004; Dahnke et al. 2008; Pellerin et al. 2009; Wankel et al. 2009). The δ^{18}O values of nitrate are typically within the following ranges (1) inorganic fertilizer (−15 to 25‰), (2) cultivated and natural soils (−15 to 15‰), (3) atmospheric deposition (NH_4 and NO_3 in rain, 20 to 95‰) and (4) animal and septic waste (−15 to +15‰) (Kendall et al. 2007; Einsiedl and Mayer 2006; Kendall 1998; Durka et al. 1994). In aquifers, the denitrification process produces isotopically lighter N_2 and N_2O gases, leaving behind ^{15}N-enriched residual nitrate that can be isotopically similar to that of animal and septic waste (Panno et al. 2001). Additionally, because the δ^{18}O and δ^{15}N of the residual nitrate increase systematically as a result of denitrification (the expected isotopic enrichment of δ^{18}O relative to δ^{15}N is 1:2), dual-isotope analysis potentially allows for the detection of denitrification in aquifers (Böttcher et al. 1990; Kendall 1998; Chen and MacQuarrie 2005).

Katz (2004) used several lines of evidence to conclude that denitrification in the Floridan aquifer is negligible: (1) spring waters are aerobic and contain low concentrations of dissolved organic carbon (DOC) (i.e., redox levels are above those required for denitrification and there is little substrate to support heterotrophic respiration) and (2) the ratio of N_2:Ar gases dissolved in spring waters was consistent with atmospheric equilibration during groundwater recharge (excess N_2 would indicate a non-atmospheric source of N_2 gas in the aquifer, e.g., denitrification). In a later study conducted in the Wakulla Springs region, Katz et al. (2008) found evidence that denitrification processes had occurred in some wells that had enriched δ^{15}N-NO_3 and δ^{18}O-NO_3, low NO_3, and slightly excess N_2:Ar ratios. More research is needed to identify sources of nitrate to Florida springs and to understand the occurrence and magnitude of denitrification in the Floridan Aquifer.

The goal of our study was to elucidate sources of nitrate to springs across north Florida and to assess whether denitrification is altering the isotopic signature of nitrate in the Floridan Aquifer. We hypothesize that fertilizers are the main source of excess nitrate in the aquifer and that denitrification can be detected using isotopic analysis of nitrate. We used dual isotope analysis of nitrate in spring water and δ^{15}N of algal tissues to examine nitrate sources at two spatial scales: (1) regional surveys of nitrate and algal tissue δ^{15}N conducted during 3 years (2005, 2006, and 2008), and (2) longitudinal sampling of nitrate and algal tissue δ^{15}N along river runs of four springs on a single date (2006). The sampling for the synoptic study was conducted during years with both above and below normal rainfall and we were able to observe the effect of varying hydrology on nitrate concentrations and stable isotopes of nitrate. The longitudinal study was designed to detect point sources of nitrate along spring runs (e.g., septic systems) and test the hypothesis that biological uptake of N is relevant along spring river runs.

Materials and methods

Synoptic and longitudinal surveys of springs

Synoptic surveys of first magnitude springs (discharge $> 2.8\ \mathrm{m}^3\ \mathrm{s}^{-1}$) in north Florida were conducted during the summer of 2005 (13 springs), July 2006 (10 springs) and summer of 2008 (15 springs) (Table 1 and see Electronic Supplementary Material). Spring names are listed as found in Scott et al. (2004). Seven springs were sampled in all 3 years: Fanning, Lafayette Blue, Madison Blue, Rainbow No. 1, Troy and Wakulla and Silver (Main) Spring (hereafter called Silver Spring). At all springs during all years, water samples were collected for nitrate concentration analysis and dual isotopic analysis of nitrate (δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$). Dissolved oxygen (DO) was measured at each site using a YSI 556 Multi-probe System (YSI Incorporated, Yellow Springs, OH, USA). Water samples and water quality measurements were collected at the boil (the region of the spring where groundwater first reaches the surface). During 2006, samples of macroalgae were collected from mats near the boil for identification, stable isotope analysis (δ^{15}N) and determination of elemental composition (%N and %P).

Longitudinal surveys were conducted in four spring river runs during January 2006 to determine whether local point-source pollution could be detected: Silver River (7.7 km transect), Rainbow River (7.2 km transect), Wakulla River (3.2 km transect) and the Weeki Wachee River (8.1 km transect). The sites sampled along each river run and their geographic locations and site codes are presented in the Supplementary Materials. The sites chosen for sampling overlapped with stations in Stevenson et al. (2004). At each site, water samples were collected for nitrate analysis (concentration and isotopes) and samples of macroalgae were obtained for identification, isotope analysis and elemental composition (%N and %P).

Collection of algae and water

Individual samples of each of the most common algal species were collected (by snorkeling or from a kayak), shaken in the water to remove any loosely attached debris and placed into 1 gallon Ziploc bags filled with site water. A duplicate algal sample (ca. 0.5–1 g fresh mass) was also collected and placed in a scintillation vial to confirm the accuracy of field identification. Samples were transported to the laboratory on ice.

Water samples were collected at the same time at a depth of 0.5 m from the surface. Samples were filtered in the field through 0.45-μm cartridge filters (Millipore model number—GWSC04550, Millipore, Billerica, MA, USA) using a peristaltic pump or through 0.45-μm polycarbonate membranes (Whatmann Inc., Florham Park, NJ, USA) using a 47 mm filter holder and syringe. Filtered aliquots were collected for δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ determination and NO$_3$ + NO$_2$ analysis. Samples for NO$_3$ + NO$_2$ analysis were acidified to pH 2 with concentrated H$_2$SO$_4$ and stored at 4°C for a maximum of 28 days until analyzed. All samples for δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ determination were stored frozen at −20°C and analyzed in 2008.

Processing and analysis of algae and water

Algae samples were identified, and picked clean of invertebrates and debris within 24 h of field

Table 1 Stable isotopes of nitrate (δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$) and nitrate concentrations from individual Florida head springs sampled during synoptic surveys in 2005, 2006 and 2008

Spring	Year sampled	NO$_3$ µmol L^{-1}	δ^{15}N-NO$_3$‰	δ^{18}O-NO$_3$‰
Fanning	2005	321	8.0	5.5
	2006	350	7.9	5.5
	2008	220	7.7	5.7
Guaranto	2006	70.0	5.1	8.0
	2008	81.4	5.4	7.2
Ichetucknee Blue Hole	2005	50.7	4.4	7.4
	2008	48.6	4.2	7.2
Ichetucknee Head	2005	45.0	3.5	3.0
	2008	73.6	3.9	6.3
Jackson Blue	2005	231	2.9	4.9
Lafayette Blue	2005	202	8.0	8.8
	2006	157	9.3	9.3
	2008	105	13.3	11.4
Little Fanning	2006	349	7.9	5.4
Little River	2006	85.7	5.7	7.8
	2008	80.0	11.0	11.1
Madison Blue	2005	158	3.6	6.7
	2006	107	4.2	6.4
	2008	278	4.9	6.4
Manatee	2005	115	5.7	7.7
	2008	145	6.6	7.0
Rainbow no. 1	2005	122	3.9	7.2
	2006	107	4.0	6.0
	2008	146	4.2	5.8
Silver (Main Spring)	2005	45.0	6.8	7.7
	2006	92.9	7.4	7.4
	2008	109	7.6	7.4
Troy	2005	219	7.1	10.2
	2006	157	7.2	10.1
	2008	111	20.2	15.3
Volusia Blue	2008	31.4	14.5	10.8
Wakulla	2005	42.9	8.8	3.8
	2006	5.36	9.2	5.5
	2008	101	9.7	5.1
Weeki Wachee	2005	57.9	6.2	4.5
	2008	56.4	6.2	4.6
Wekiwa	2005	87.9	10.3	10.2
	2008	50.0	15.9	13.9

collection and stored frozen. Prior to isotopic analysis and elemental analysis, algal samples were lyophilized and picked clean again of any debris initially missed, and ground and homogenized in a ball mill. Nitrogen isotopic composition of algae was measured on a Thermo Delta-Plus XP, isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at the University of Florida using an elemental analyzer inlet system (Costech) and continuous flow of helium. The International Atomic Energy Association reference material, N1, was used for isotopic standardization in each run. Nitrogen isotope values are reported in δ notation relative to atmospheric N_2. Percent nitrogen of the dried algal tissue was measured by high temperature combustion using a Flash EA 1112 Nitrogen/Carbon Analyzer with MAS 200 R Autosampler (Thermo Fisher Scientific Inc., Waltham, MA, USA). Phosphorus content of dried algal tissues and sediment was measured on combusted (550°C) and acid digested (6 N HCl) samples as SRP (Anderson 1976) on a Technicon Autoanalyzer (Technicon Instruments Corporation Wilmington, MA, USA).

Nitrate plus nitrite were measured on a Bran + Luebbe Auto Analyzer 3 (Bran + Luebbe, Norderstedt, Germany) using EPA Method 353.2. In all samples, nitrate represented greater than 98% of the oxidized inorganic nitrogen, hence we present only the nitrate concentrations in the manuscript. All samples for the dual-isotope analysis of nitrate (δ^{15}N-NO_3 and δ^{18}O-NO_3) were analyzed at the University of Florida using the bacterial denitrifier method (Sigman et al. 2001; Casciotti et al. 2002) in which nitrate is converted to N_2O by the bacteria *Pseudomonas aureofasciens*. The δ^{15}N and δ^{18}O of the N_2O produced was measured on a Thermo Delta-Plus XP isotope ratio mass spectrometer at the University of Florida using the GasBench interface and a continuous flow of helium. Based on Sigman et al. (2001) and Casciotti et al. (2002), the International Atomic Energy Association standard, N3, was included in each analytical run and used for isotopic standardization. Percent incorporation of oxygen from H_2O into N_2O during denitrification by *P. aureofaciens* was not measured for each culture batch, therefore 5% incorporation was assumed, resulting in ±0.6‰ uncertainty in δ^{18}O-NO_3 values (Casciotti et al. 2002). Uncertainty of δ^{15}N-NO_3 values was ±0.3‰. Nitrogen isotope values are reported in δ notation relative to atmospheric air; oxygen isotope values are reported in δ notation relative to the standard Vienna Standard Mean Ocean Water (VSMOW).

Results

Regional patterns of spring water nitrate

For the 17 individual springs sampled during the synoptic surveys, δ^{15}N of spring water nitrate ranged from +2.9 to +20.2‰, δ^{18}O ranged from +3.0 to +15.3‰ and nitrate concentrations varied from 5.4 to 350 μmol L^{-1} (Table 1). Relatively high δ^{15}N and δ^{18}O values (δ^{15}N and δ^{18}O ≥ 10‰) were observed during 2008 at Troy, Wekiwa, Volusia Blue, Lafayette Blue, and Little River Spring. Relatively low values (δ^{15}N and δ^{18}O ≤ 5‰) were observed during 2005 at Ichetucknee Head Spring, Jackson Blue, Ichetucknee Blue Hole, Madison Blue and Rainbow No. 1. At the seven springs sampled in all 3 years, all except Fanning exhibited increasing δ^{15}N and δ^{18}O values with time, while nitrate concentrations increased at four springs (Madison Blue, Rainbow No. 1, Silver and Wakulla), and decreased at three springs (Fanning, Lafayette Blue and Troy) (Table 1).

The relationship between δ^{15}N and δ^{18}O of nitrate varied over the 3 years (Fig. 1). During 2005 and 2006, regressions describing the linear relationship were non-significant with slopes not significantly different from zero (Fig. 1a, b). During 2008, the linear regression was significant ($p < 0.001$) and the slope of the line was 0.59 (the 95% confidence interval for the slope was 0.41–0.77) (Fig. 1c). Springs that were sampled in all 3 years exhibited the same trends in the relationship between δ^{15}N-NO_3 and δ^{18}O-NO_3 as the whole sample population (solid circles in Fig. 1). Additionally, in 2008 significant logarithmic relationships were observed between dissolved oxygen concentrations in spring water and both δ^{15}N-NO_3 and δ^{18}O-NO_3 ($p < 0.001$) (Fig. 2), while in other years there was no correlation between isotopes of nitrate and dissolved oxygen of spring water ($p > 0.05$). During 2008, the δ^{15}N and δ^{18}O of nitrate was highest in springs with the lowest DO concentrations.

Precipitation in north Florida varied from 2005 to 2008 and sampling in 2005 occurred during and following a period with greater incidence of tropical storms and hurricanes while in 2008, sampling occurred when the aquifer was in a condition of lower flow owing to below normal precipitation (Fig. 3). Therefore we classified 2005/2006 as wet years while 2008 was considered a dry year. Based on differences in precipitation and the observed differences in nitrate concentrations and isotope values over time at several spring sites (Table 1), we split our sample population into two sets: (1) springs where nitrate declined over time (i.e., nitrate concentrations were higher in 2005 and 2006 relative to 2008) (Fig. 4a, c) and (2) springs where nitrate

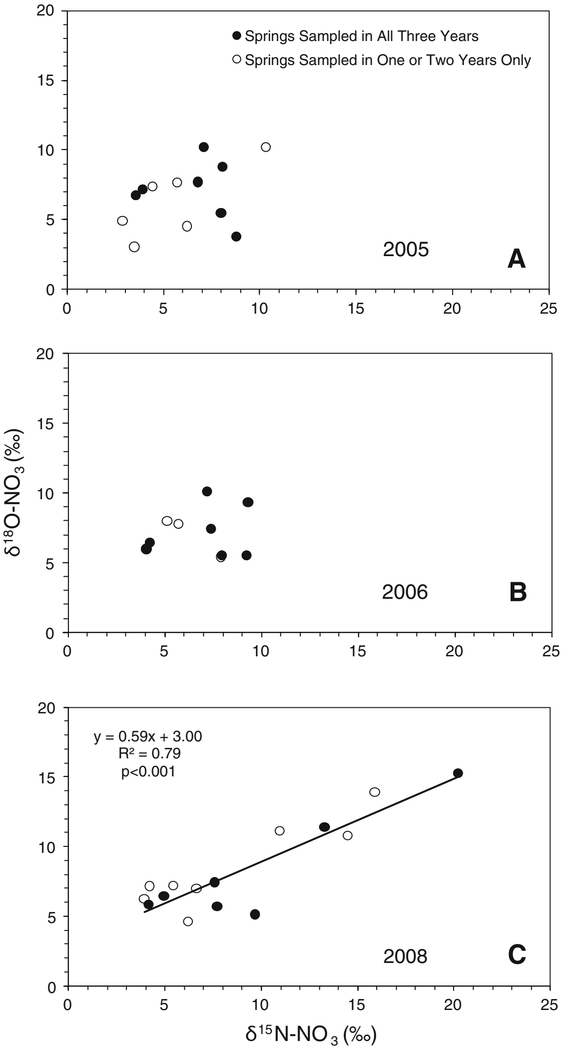


Fig. 1 Stable isotopes of nitrate (δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$) in Florida springs sampled during synoptic surveys in 2005 (**a**), 2006 (**b**) and 2008 (**c**). Springs sampled in all 3 years are denoted with *solid circles*, while springs that were sampled in only one or two years are denoted with *open circles*. The relationship between the δ^{15}N and δ^{18}O of nitrate was not significant ($p > 0.05$) in 2005 and 2006

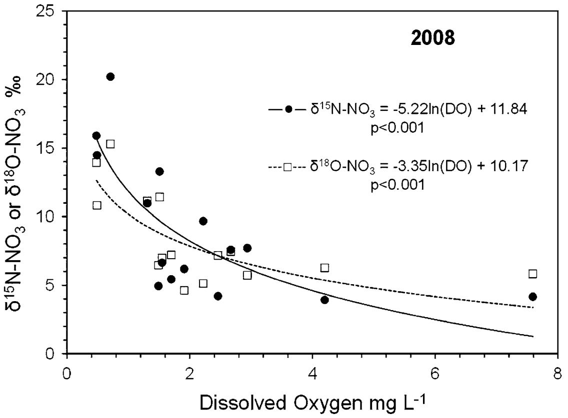


Fig. 2 Relationship between dissolved oxygen in water and δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ in Florida springs sampled during the 2008 regional survey. During other years, no significant correlation between isotopes of nitrate and dissolved oxygen was observed ($p > 0.05$)

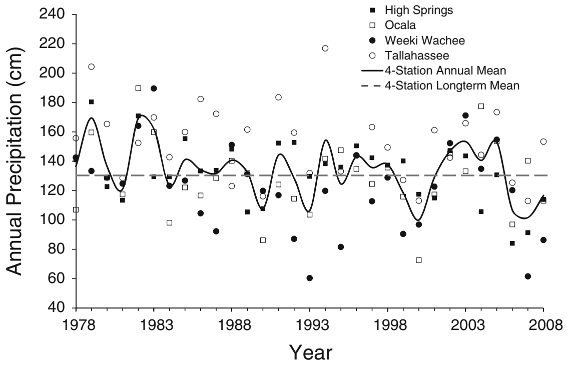
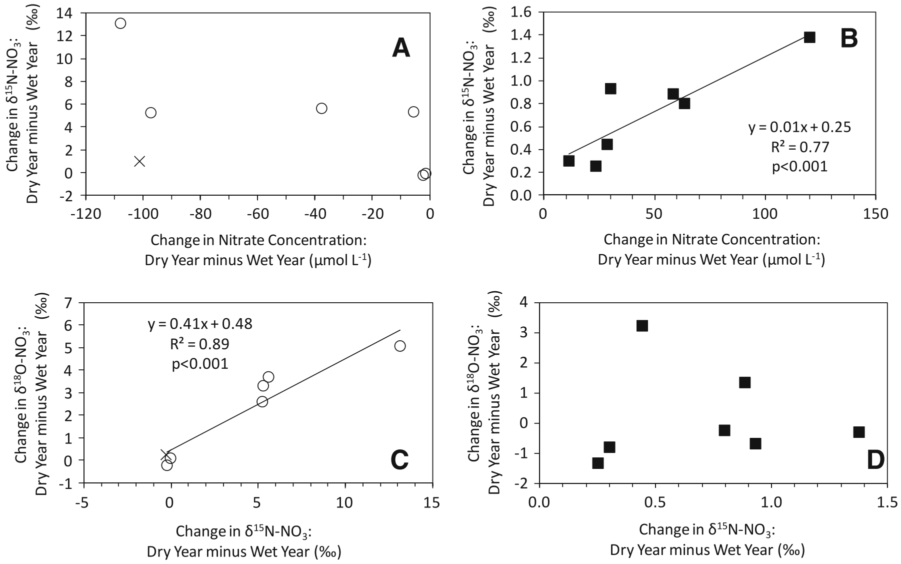


Fig. 3 Annual rainfall for meteorological stations located nearby springs sampled during synoptic surveys. Data are from the Southeast Regional Climate Center (2010, http://www.sercc.com/). Interannual patterns of precipitation were primarily driven by variations in the frequency of tropical storms

increased over time (Fig. 4b, d). The springs in which nitrate declined were Fanning, Ichetucknee Blue Hole, Lafayette Blue, Little River, Troy, Weeki Wachee and Wekiwa while the springs in which nitrate increased were Guaranto, Ichetucknee Head Spring, Madison Blue, Manatee, Rainbow, Silver and Wakulla. In springs where nitrate declined, we observed relatively large enrichments in δ^{15}N (up to 13‰) and these enrichments were somewhat proportional to the amount of nitrate decline; the main exception was Fanning Spring (denoted with an X) where nitrate declined by 100 μmol L^{-1} with only a 0.3‰ change in δ^{15}N (Fig. 4a). In the springs with decreasing nitrate, the increase in δ^{15}N of nitrate was accompanied by an increase in δ^{18}O and produced a slope of 0.41 (Fig. 4c). The springs where nitrate increased from 2005/2006 to 2008 showed much less change in δ^{15}N-NO$_3$ (Fig. 4b) and no significant relationship between δ^{15}N and δ^{18}O (Fig. 4d).

Fig. 4 Relationships between the δ^{15}N values of nitrate and nitrate concentration in years with above normal (“Wet”) and below normal (“Dry”) precipitation. We considered 2005/2006 to be wet years and 2008 was considered a dry year. In these analyses we separated springs in which nitrate decreased from 2005/2006 to 2008 (**a** and **c**) from springs in which nitrate increased (**b** and **d**)



Potential fractionation factors for denitrification

As a thought experiment to estimate the potential isotopic fractionation factor for denitrification in the Floridan aquifer, we modeled the isotopic behavior of nitrate in the water feeding springs such as Troy, Lafayette and Wekiwa springs (which showed nitrate concentration declines of 49, 48 and 43%, respectively, and increases in δ^{15}N and δ^{18}O from 2005 to 2008) using closed-system Rayleigh equations (Fry 2006) (Fig. 5):

$$\delta_{\mathrm{RS}} = \delta_{\mathrm{INPUT}} - \Delta \times \mathrm{Ln}(1 - f) \tag{1}$$

where δ_{RS} is the δ of the residual substrate, δ_{INPUT} is the initial δ value of the substrate, Δ is the fractionation factor, and f is the fraction of the substrate that is reacted. We started with an initial nitrate source with δ^{15}N and δ^{18}O of 7.1 and 10.2‰, respectively, based on the isotopic values observed in Troy Spring in 2005 (Table 1). Next we varied the fractionation factors (Δ) for denitrification, keeping the ratio of Δ^{18}O:Δ^{15}N at 0.5, so that at a fraction reacted of 0.5 (i.e., when 50% of nitrate substrate is lost to denitrification) we observed residual nitrate with δ^{15}N of ca. +20‰ and δ^{18}O of ca. +15‰, which coincide with the values observed in 2008 at Troy Springs. Setting Δ^{18}O:Δ^{15}N at 9:18 produced the best agreement with the isotopically enriched nitrate (Fig. 5).

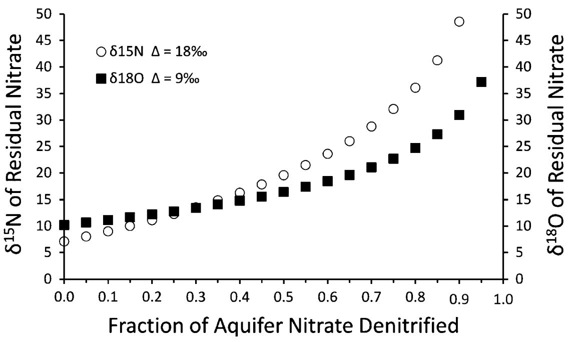


Fig. 5 Results from modeling the isotopic dynamics of nitrate in the Floridan aquifer as a closed system with single source with δ^{15}N of 7.1‰ and δ^{18}O of 10.2‰ which is affected by denitrification. *Open circles* denote δ^{15}N and *filled squares* denote δ^{18}O behavior. Closed system Rayleigh equations were used to model the isotopic dynamics (Fry 2006). Setting the fractionations factors for denitrification to 18‰ for ^{15}N and 9‰ for ^{18}O yielded residual nitrate with δ^{15}N-NO$_3$ of approximately +20‰ and δ^{18}O-NO$_3$ of +15‰, when 50% of the nitrate was lost to denitrification

Relationship between the δ^{15}N of algae and δ^{15}N of spring water nitrate

The range of algal δ^{15}N values measured in the ten head springs sampled during 2006 was −8 to +6‰, while the range in δ^{15}N of spring water nitrate was narrower, from +4 to +10‰. The lowest δ^{15}N values of algal tissue were found for *Vaucheria* sp. at Little

River Springs (−7.3‰) and Troy Springs (−6.4‰), while the highest values were measured in *Spirogyra* sp. (Chlorophyceae), a green alga, along the Wakulla River run and at Lafayette Blue Springs (+5.9 and +6.4‰). There was a significant, positive correlation between the δ^{15}N of algae and the δ^{15}N-NO$_3$ in the ten springs sampled in 2006 ($R^2 = 0.39$, $p = 0.01$; Fig. 6a). While there are relatively few data, there does not appear to be any species-specific relationship between the nitrogen isotopic composition of algae and nitrate. The average offset between the δ^{15}N of nitrate and algae was 5.7‰ (range 2.0–13.6‰) with the algae being isotopically lighter in all cases.

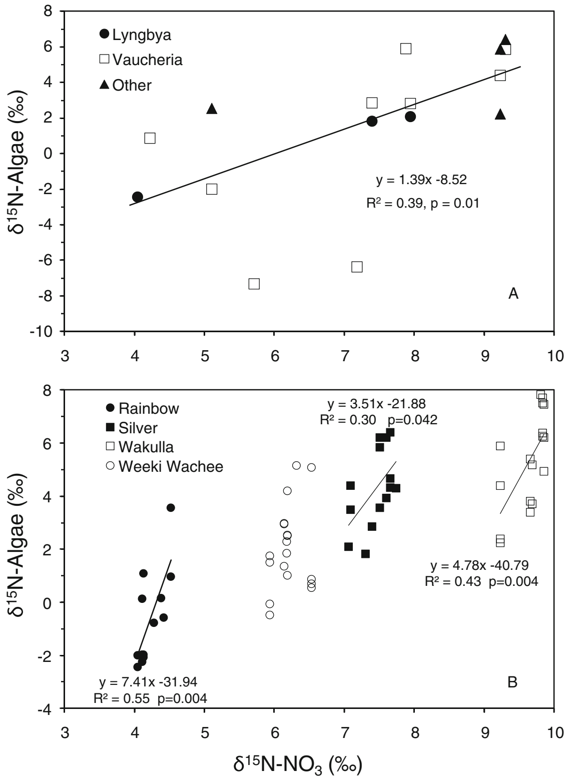


Fig. 6 Relationship between δ^{15}N-NO$_3$ and δ^{15}N of macro-algae in springs sampled in 2006 during regional surveys (**a**) and along four spring runs (**b**). There was a significant, positive correlation between the δ^{15}N of algae and the δ^{15}N-NO$_3$ in 2006 ($R = 0.58$, $p = 0.01$) (**a**). With the exception of Weeki Wachee Spring, there were statistically significant ($p < 0.05$), positive relationships between δ^{15}N-NO$_3$ and δ^{15}N of macro-algae in samples collected along spring runs (**b**)

Variation in stable isotopes and algal stoichiometry along longitudinal gradients

Similar to the regional surveys, we observed statistically significant relationships between the δ^{15}N of nitrate and algae along three of the four river runs; along the Weeki Wachee River there was no significant correlation (Figs. 6b, 7). In all rivers, the variations in algal δ^{15}N were on the order of 5–6‰ whereas δ^{15}N-NO$_3$ varied by less than 1‰. At Rainbow, Silver and Wakulla rivers, consistent patterns were observed in the behavior of nitrate concentration and the isotopes of nitrate moving from the boil towards downstream reaches. Nitrate declined by 2.5–3.3 μmol L^{-1} per km in the Rainbow and Silver rivers while slower losses were observed at Wakulla and Weeki Wachee Rivers (0.74 and 0.42 μmol L^{-1} per km) (Fig. 7). Coincident with nitrate decline, we observed increasing δ^{15}N of both algae and nitrate, with stronger isotopic enrichment seen in the algae (Fig. 7). For the Rainbow and Silver Rivers, *Lyngbya wollei* was found at each sampling location, whereas other species were not and are therefore not shown in Fig. 7a and c. At Wakulla Springs, *Compsopogon* sp. (Rhodophyceae), a red alga, and *Spirogyra* sp., a green alga, were most commonly observed and are included in Fig. 7b. Along the Weeki Wachee River, *Dichotomosiphon* sp. (Chlorophyceae,), a green alga, and *L. wollei* were the most widely distributed species and are shown in Fig. 7d. With the exception of nitrate concentration and δ^{15}N-NO$_3$ at Weeki Wachee, all longitudinal trends along the spring runs were significant at the 0.05 level and all slopes were significantly different from zero ($p < 0.05$).

Statistically significant Pearson product-moment correlation was observed between the N:P molar ratios of algal tissue and the fractionation factor, Δ (Fry 2006), for algal assimilation of nitrate along the Rainbow and Wakulla Rivers ($r = +0.73$, $p = 0.025$ and $r = +0.920$, $p < 0.001$, respectively, data not graphed). Values of Δ and N:P molar ratio decreased moving downstream from the boils which suggests that fractionation during nitrate assimilation lessened as algal growth became more limited by nitrogen availability. In the Rainbow River, N:P molar ratios of *L. wollei* ranged from 23 to 32, with corresponding fractionation ranging from 4 to 6‰. In the Wakulla River, N:P ratios of *Compsopogon* sp.

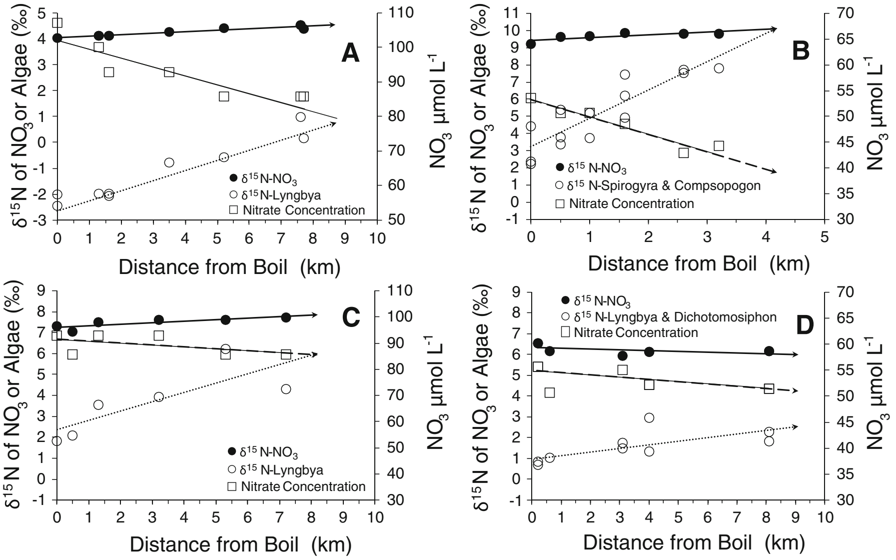


Fig. 7 Longitudinal trends in nitrate concentration, δ^{15}N-NO$_3$ and δ^{15}N of dominant macro-algae in Rainbow Springs (**a**), Wakulla Springs (**b**), Silver River (**c**), and Weeki Wachee Spring (**d**) during 2006. In general, nitrate concentrations declined along the spring runs while δ^{15}N-NO$_3$ and δ^{15}N of macroalgae increased. All longitudinal trends along the spring runs were significant at the 0.05 level and all slopes were significantly different from zero ($p < 0.05$), except for nitrate concentration and δ^{15}N-NO$_3$ at Weeki Wachee

ranged from 26 to 37, with corresponding fractionation from 4 to 7‰ and *Spirogyra* sp. N:P ratios ranged from 18 to 21, and fractionation from 2 to 7‰. The correlation between *Lyngbya wollei* N:P ratios and fractionation was not as strong in the Silver River ($r = 0.54$, $p = 0.27$). N:P ratios ranged from 26 to 38 and fractionation of N showed no correlation to distance from the boil. No relationships were found between N:P and algal fractionation along the Weeki Wachee River.

Discussion

Nitrate sources and processes affecting nitrate in the Floridan aquifer

We attempted to discern sources of nitrate to springs at the regional scale using synoptic surveys conducted over 3 years and at the local scale using longitudinal surveys conducted on a single date at four springs. Variations in isotopes of nitrate measured in Florida springs allow inferences to be made about the dominant sources of nitrate to the springs in our regional surveys. Five springs sampled in 2008, Troy, Wekiwa, Volusia Blue, Lafayette Blue and Little River had δ^{15}N and δ^{18}O between 10 and 20‰, which could indicate inputs from manure/septic waste or nitrate that has been affected by denitrification (Table 1, Fig. 1) (Kendall 1998; Panno et al. 2001). The remaining springs (and samples from Troy, Wekiwa, Volusia Blue, Lafayette Blue and Little River collected in 2005 and 2006) had δ^{15}N and δ^{18}O values between 3 and 9‰, indicating an inorganic N-source, such as oxidation of ammonium fertilizer and/or soil nitrogen (Kendall 1998),which supports our initial hypothesis. Katz and Griffin (2008) found that inorganic fertilizers were the major source of nitrogen at Ichetucknee Head Springs and Blue Hole, which is consistent with isotopic signatures observed in our study. Additionally, Katz (2004) obtained a δ^{15}N-NO$_3$ value for Wakulla Springs of 8‰ (similar to our study, 9‰ in 2005 and 2006), which he attributed to a mixture of inorganic fertilizers and septic/animal sources based on N mass balance calculations for the spring by Chelette et al. (2002).

Isotope values were higher for both δ^{15}N and δ^{18}O in samples collected in 2008 versus those collected in 2005 for several springs (Table 1) and we observed an inverse relationship between nitrate isotopic composition and DO. Troy, Lafayette, and Little River showed relatively large increases in isotopic values (changes of 3–13‰ in both δ^{15}N and δ^{18}O), coupled with decreasing nitrate concentration, which is suggestive of denitrification which supports our initial hypothesis. In 2008, sampling occurred when the aquifer was in a condition of lower flow owing to below normal precipitation while the 2005 sampling followed a period with greater incidence of tropical storms and hurricanes (Fig. 3). We speculate that these differences in precipitation altered the hydrology of the aquifer and changed the relative proportion of discharge deriving from conduit flow and matrix flow (Martin and Dean 2001). It is likely that denitrification is occurring to a greater extent in matrix flows (e.g., within inter-granular pores within rocks) that have longer residence time relative to conduit flows, which facilitate the rapid movement of water (Martin and Dean 2001, Ritorto et al. 2009). Thus, the isotopic composition of nitrate reaching Troy, Lafayette, and Little River springs had likely been altered by denitrification in 2008. While DOC concentrations in most springs were typically less than 1–2 mg L^{-1} and the waters were oxygenated (Albertin 2009), denitrification can occur with ferrous iron and pyrite (H_2S) as electron donors (Pauwels et al. 2000; Einsiedl and Mayer 2006; Einsiedl et al. 2005) at anoxic microsites within the aquifer.

We hypothesize that spring discharge at some locations in 2008 had greater contribution from regions of the Floridan aquifer with longer residence time, and therefore reflected more clearly the process of denitrification, while water discharged in 2005 may have been “younger” and primarily reflected mixing of multiple nitrate sources across the springshed. Katz (2004) indicates that water contributions from local conduit-flow systems (e.g., sinkholes) increase during conditions of high recharge, like in 2005. Groundwater discharged from springs located in unconfined portions of the Floridan Aquifer has estimated residence times from 11 to 66 years (Happell et al. 2006) and 10–30 years (Katz et al. 2001). However, using dye tracer studies, Wilson and Skiles (1988) showed that water can move through conduit systems to springs in as little as days to weeks. In contrast, using radiocarbon dating of dissolved inorganic carbon, Plummer and Sprinkle (2001) found that the majority of the water in confined parts of the Floridan Aquifer was recharged in the past 15,000 to 30,000 years.

While higher δ^{15}N-NO_3 during 2008 could be interpreted as greater influence of septic/CAFOs, the fact that δ^{18}O-NO_3 also increased and the slope of the line in Fig. 1c closely approximated the expected slope from denitrification of groundwater nitrate of 0.5 leads us to conclude that denitrification is an important process in the aquifers feeding some springs. In more recent literature, questions have arisen about the expected 0.5 slope from denitrification (e.g., the expected isotopic enrichment of δ^{18}O relative to δ^{15}N is 1:2) (Kendall et al. 2007). Work by Sigman et al. (2005) and Granger et al. (2004) with laboratory cultures of denitrifying bacteria resulted in slopes near 1 and it has been hypothesized that older methods for determining δ^{15}N-NO_3 and δ^{18}O-$NO_,$ (sealed tube combustions or pyrolysis rather than denitrifying bacteria) may have produced biased δ^{18}O-NO_3 data (Kendall et al. 2007). However, in experiments using two strains of denitrifying bacteria, Knöller et al. (2011) produced a mean ratio of 0.5 for multiple laboratory experiments (with a range of 0.33–0.79) and Kendall et al. (2007) state that they have obtained slopes of <1 in groundwater where denitrification occurs.

Figure 4 provides additional support for denitrification being a significant nitrate sink in portions of the Florida aquifer during dry years. In the springs with decreasing nitrate, the increase in δ^{15}N of nitrate was accompanied by an increase in δ^{18}O and a plot of the changes in isotope composition of nitrate (Fig. 4b) yields a slope of 0.41 which is close to the expected slope resulting from denitrification (the 95% confidence interval for the regression slope was 0.26–0.56). In contrast, the springs where nitrate increased from 2005/2006 to 2008 showed much less change in δ^{15}N-NO_3 (Fig. 4b) and the small isotope changes observed were highly correlated to the change in concentration which is suggestive of a two-component mixing system (Fry 2006). When changes in δ^{15}N-NO_3 were plotted against changes in δ^{18}O-NO_3 for the springs in Fig. 4b, we observed no relationship (Fig. 4d). We hypothesize that isotope dynamics in the springs with increasing nitrate were controlled primarily by mixing of different sources and that the source contribution of water and nitrate changed only modestly going from a wet period to a relatively dry period. In comparison, we hypothesize that the isotope dynamics in the springs with decreasing nitrate were a function of mixing of different nitrate sources and denitrification.

We speculate that the springs in Fig. 4a receive proportionally more matrix flow during dry years than those in Fig. 4b, based on a study of temporal changes in spring chemistry by Martin and Gordon (2000). Furthermore, the strong logarithmic relationship between both δ^{15}N and δ^{18}O of nitrate and DO concentrations for springs sampled in 2008 (Fig. 2) suggests that isotopic enrichment of nitrate within the aquifer varies as a function of redox condition and that isotope dynamics approximate a closed system (Fry 2006). Katz et al. (1999) found that DO concentrations of springs in the Suwanee River Basin were negatively correlated with groundwater age, as did Dietrich and Herbert (1997) in a karst aquifer in Germany thus one could interpret the *x*-axis on Fig. 2 as groundwater age and conclude that older waters (i.e., lowest DO concentration) more fully express the isotopic effects of denitrification. Denitrification rates in aquifers are difficult to measure and vary widely (5–40‰, Kendall 1998), but it has been suggested that higher Δ values will occur in aquifers with longer residence time and slower rates of denitrification (Kendall 1998). The results of our model estimating the fractionation factors for denitrification (shown in Fig. 5) further suggest that denitrification should be considered when using isotopes of nitrate to distinguish sources to the Floridan aquifer and that denitrification can be an important sink for nitrate along flowpaths with longer residence time.

δ^{15}N and nitrate gradients in spring-fed river runs

Based on our longitudinal study, we did not observe local inputs of nitrate at three of the four springs; nitrate isotope behavior at Weeki Wachee spring may suggest some localized sources of nitrate, but was inconclusive. Instead nitrate concentrations declined due to algal uptake supporting our initial hypothesis. Coherence among nitrate concentrations and the δ^{15}N of algae and nitrate suggests that nitrate from spring boils is the major source of nitrogen to spring algae, supporting the findings of Sickman et al. (2009) who found that nutrient recycling within algal mats at Manatee and Ichetucknee Springs was a minor source of nitrogen for mat algae relative to the flux of nitrate from the boil.

The results from our longitudinal surveys are similar to those of De Brabandere et al. (2007) who found that the δ^{15}N of epiphytic periphyton, macrophytes and dissolved nitrate increased as nitrate concentrations decreased moving downstream in two spring-fed river systems. Decreasing nitrate concentrations (ca. 6–7 μmol L^{-1} per km) were attributed to biological uptake of nitrogen and De Brabandere et al. (2007) hypothesized that increases in the δ^{15}N of nitrate and aquatic plants moving downstream were a consequence of isotopic fractionation associated with preferential use of $^{14}NO_3^-$ by aquatic plants. Our findings support this hypothesis, however it is noteworthy that we observed a generally greater change in algal δ^{15}N with decreasing nitrate concentration compared to De Brabandere et al. (Fig. 7), which could result from differences in algal species and nutrient limitation status.

In both the Rainbow and Wakulla rivers, significant correlations were found between the N:P molar ratios of algae and fractionation factors, and a marginally significant correlation was found along the Silver River; as N:P ratios decreased (i.e., N content decreased relative to P), the algae became less selective for $^{14}NO_3^-$. These findings are supported by Albertin (2009), who observed that algal δ^{15}N was negatively correlated to water column total N and NO_2/NO_3-N concentrations and positively correlated to P availability indices, i.e., algal δ^{15}N was higher when N was in shorter supply, but P was available. Under conditions of lower N availability, more complete assimilation of water column nitrate is expected, resulting in less isotopic fractionation and creating algal tissues with δ^{15}N more similar to the source nitrate. In contrast, when N supplies are relatively greater than P demand, then algal cells can be more discriminating in the isotope form of their nitrate source (^{14}N vs. ^{15}N), thereby producing greater fractionation and comparatively “lighter” algal tissues.

Utility of δ^{15}N measurements of algae

At both the regional and local scale we observed a significant positive correlation between the δ^{15}N of algae and the δ^{15}N-NO_3 in spring water discharged from the boil (Fig. 6a, b). Algal δ^{15}N was always lower than δ^{15}N-NO_3 of the source water, due to fractionation during algal uptake of nitrate. In the regional study values for Δ ranged from 2 to 13‰. Interestingly, we observed Δ values of about 5–6 at the boils in the spring runs, but the trend lines for δ^{15}N of nitrate and algae tended to converge moving down river, indicating substantial reductions in the Δ factor with distance from the boil, which may be an indication of the nutrient status of algae, as discussed above. Overall, however, linear regressions between δ^{15}N of algae and the δ^{15}N-NO$_3$, while statistically significant (Fig. 6a, b), have limited application in regional monitoring programs given the wide range and unpredictability of fractionation factors for algal assimilation of nitrate. De Brabandere et al. (2007) found that fractionation in periphyton attached to macrophytes in the spring-fed Chassahowitzka and Homossassa rivers of Florida varied from 0.7 to 2.5‰ and state that fractionation between algae and nitrate reported in the literature ranges from 2.5 to 10‰ (multiple sources listed therein). Fogel and Cifuentes (1993) state that fractionation values of up to 27‰ have been recorded for algae growing in culture. We would also note that the variability in fractionation factors could be due in part to the large diversity of algal genera and families found in our study, which have physiological diversity that could affect isotope dynamics.

Conclusions

Variation in the δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ within our study sites across multiple years demonstrates the complexity of Florida's karst springs, systems which integrate surface derived inputs of water and nutrients across wide areas and over multiple time scales. In wet years when mixing likely dominated the isotopic dynamics of nitrate in the aquifer, δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ values ranged from +3 to +9‰ in spring boils, which could be the result of nitrification of ammonium in natural or agricultural soils. This finding is supported by mass balances studies that have found that inorganic fertilizers are the largest anthropogenic input of nitrogen in most springsheds. During years with below normal rainfall, δ^{15}N-NO$_3$ and δ^{18}O-NO$_3$ values increased while nitrate concentrations decreased in some springs providing evidence for denitrification in the aquifer. We hypothesize that during dry years, the average age of water discharged from some springs increased and waters with longer residence time (and lower DO) had greater potential for denitrification to reduce nitrate concentrations and alter the isotopic composition of nitrate. At a finer spatial scale we found little evidence for localized sources of nitrate pollution along four spring runs; instead nitrate concentrations and the isotopic composition of nitrate and algae varied in a systematic and linear fashion with distance from the boil. Variations between fractionation factors for algal uptake of nitrate and algal stoichiometry indicate that the degree of nitrogen limitation of algae may strongly affect the fractionation factor. Correlation between the δ^{15}N composition of nitrate and algae was observed at both the regional and spring-run scale, but the wide range of fractionation factors observed, meant that measurements of algal δ^{15}N cannot be used to accurately infer the δ^{15}N of nitrate. Thus, the problem of evaluating anthropogenic nitrate sources to Florida springs is better addressed at the regional scale with tools that can provide information on the relative inputs of non-point sources of nitrate in springsheds as well as biogeochemical processes affecting nitrate in the Floridan aquifer.

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References

Albertin A (2009) Nutrient dynamics in Florida Springs and relationships to algal blooms. Dissertation, University of Florida

Anderson JM (1976) An ignition method for the determination of total phosphorus in lake sediments. Water Res 10:329–331

Aravena R, Robertson WD (1998) Use of multiple isotope tracers to evaluate denitrification in ground water: Study of nitrate from a large-flux septic system plume. Ground Water 36:975–982

Bacchus ST, Barile PJ (2005) Discriminating sources and flowpaths of anthropogenic nitrogen discharges to Florida springs, streams and lakes. Env Eng Geosci 11:347–369

Böttcher J, Strebel O, Voerkelius S, Schmidt HL (1990) Using isotope fractionation of nitrate nitrogen and nitrate oxygen for evaluation of microbial denitrification in a sandy aquifer. J Hydrol 114:413–424

Casciotti KL, Sigman DM, Hastings MG, Böhlke JK, Hilkert A (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem 74:4905–4912

Chelette AR, Pratt TR, Katz BG (2002) Nitrate loading as an indicator of nonpoint source pollution in the lower St. Marks–Wakulla Rivers watershed. Northwest Florida Water Management District Water Resources Special Report 02-1, Havana

Chen DJZ, MacQuarrie KTB (2005) Correlation of δ^{15}N and δ^{18}O in NO_3^- during denitrification in groundwater. J Environ Eng Sci 4:221–226

Cohen MJ (2008) Springshed nutrient loading, transport and transformations. In: Brown MT (ed) Summary and synthesis of available literature on the effects of nutrients on spring organisms and systems. University of Florida Water Institute, Gainesville

Dahnke K, Bahlmann E, Emeis K (2008) A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe estuary. Limnol Oceanogr 53:1504–1511

De Brabandere L, Frazer TK, Montoya JP (2007) Stable nitrogen isotope ratios of macrophytes and associated periphyton along a nitrate gradient in two subtropical, spring-fed streams. Freshw Biol 52:1564–1575

Derse E, Knee KL, Wankel SD, Kendall C, Berg CJ, Paytan A (2007) Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae. Environ Sci Technol 41:5217–5223

Dietrich PG, Herbert D (1997) Regional discharge of a Triassic artesian karst aquifer: Mixing and age of spring waters in the Thuringian basin, Germany, estimated by isotope methods. In: Gunay G, Johnson AI (eds) Karst waters and environmental impacts. AA Balkema, Rotterdam

Durka W, Schulze ED, Gebauer G, Voerkelius S (1994) Effects of forest decline on uptake and leaching of deposited nitrate determined from N-15 and O-18 measurements. Nature 372:765–767

Einsiedl F, Mayer B (2006) Hydrodynamic and microbial processes controlling nitrate in a fissured-porous karst aquifer of the Franconian Alb, Southern Germany. Environ Sci Technol 40:6697–6702

Einsiedl F, Maloszewski P, Stichler W (2005) Estimation of denitrification potential in a karst aquifer using the N-15 and O-18 isotopes of NO_3^-. Biogeochemistry 72:67–86

Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel MH, Macko SA (eds) Organic geochemistry. Plenum Press, New York, pp 73–98

Fry B (2006) Stable isotope ecology. Springer, New York

Fukada T, Hiscock KM, Dennis PF (2004) A dual-isotope approach to the nitrogen hydrochemistry of an urban aquifer. Appl Geochem 19:709–719

Granger J, Sigman DM, Needoba JA, Harrison PJ (2004) Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol Oceanogr 49:1763–1773

Happell JD, Opsahl S, Zafer T, Chanton JP (2006) Apparent CFC and 3H/3He age differences in water from Floridan Aquifer springs. J Hydrol 319:410–426

Hiers JK, Mitchell RJ, Boring LR, Hendricks JJ, Wyatt R (2003) Legumes native to longleaf pine savannas exhibit capacity for high N_2-fixation rates and negligible impacts due to timing of fire. New Phytol 157:327–338

Hornsby HD, Ceryak R (1999) Springs of the Suwanee River Basin in Florida. Suwanee River Water Management, Live Oak

Katz BG (2004) Sources of nitrate contamination and age of water in large karstic springs of Florida. Environ Geol 46:689–706

Katz BG, Griffin DW (2008) Using chemical and microbiological indicators to track the impacts from the land application of treated municipal wastewater and other sources on groundwater quality in a karstic springs basin. Environ Geol 55:801–821

Katz BG, Hornsby HD, Böhlke JF, Mokray MF (1999) Sources and chronology of nitrate contamination in spring waters, Suwannee River Basin, Florida. US Geological Survey Report 99-4252, Tallahassee

Katz BG, Böhlke JK, Hornsby HD (2001) Timescales for nitrate contamination of spring waters, northern Florida, USA. Chem Geol 179:167–186

Katz BG, Griffin DW, Davis JH (2008) Groundwater quality impacts from the land application of treated municipal wastewater in a large karstic spring basin: chemical and microbiological indicators. Sci Total Environ 407:2872–2886

Kendall C (1998) Tracing nitrogen sources and cycling in catchments. In: Kendall C, McDonnell JJ (eds) Isotope tracers in catchment hydrology. Elsevier, Lausanne

Kendall C, Elliott EM, Wankel SD (2007) Tracing anthropogenic inputs of nitrogen to ecosystems. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental science, 2nd edn. Blackwell, Oxford

Knöller K, Vogt C, Haupt M, Feisthauer S, Richnow HH (2011) Experimental investigation of nitrogen and oxygen fractionation in nitrate and nitrite during denitrification. Biogeochemistry. doi:10.1007/s10533-010-9483-9

Kreitler CW (1979) Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. J Hydrol 42:147–170

Martin JB, Dean RW (2001) Exchange of water between conduits and matrix in the Floridan aquifer. Chem Geol 179:145–165

Martin JB, Gordon SL (2000) Surface and groundwater mixing, flowpaths, and temporal variations in chemical composition of karst springs. In: Sasowsky ID, Wickes CM (eds) Groundwater flow and contaminant transport in carbonate aquifers. AA Balkema, Rotterdam, pp 65–92

Munch DA, Toth DJ, Huang, C, Davis JB, Fortich CM, Osburn WL, Phlips EJ, Quinlan EL, Allen MS, Woods MJ, Cooney P, Knight RL, Clarke RA, Knight SL (2006) Fifty year retrospective study of the ecology of Silver Springs, Florida. Special Publication SJ2007-SPA. St. Johns River Water Management District, Palatka

Panno SV, Hackley KC, Hwang HH, Kelly WR (2001) Determination of the sources of nitrate contamination in karst springs using isotopic and chemical indicators. Chem Geol 179:113–128

Pauwels H, Foucher JC, Kloppmann W (2000) Denitrification and mixing in a schist aquifer: influence on water chemistry and isotopes. Chem Geol 168:307–324

Pellerin BA, Downing BD, Kendall C, Dahlgren RA, Kraus TEC, Saraceno J, Spencer RGM, Bergamaschi BA (2009) Assessing the sources and magnitude of diurnal nitrate variability in the San Joaquin River (California) with an in situ optical nitrate sensor and dual nitrate isotopes. Freshw Biol 54:376–387

Pinowska A, Stevenson RJ, Albertin A, Sickman JO, Anderson M (2007) Integrated interpretation of survey for determining nutrient thresholds for macroalgae in Florida springs: macroalgal relationships to water, sediment and macroalgae nutrients, diatom indicators and land use. Florida Department of Environmental Protection, Tallahassee

Plummer LN, Sprinkle CL (2001) Radiocarbon dating of dissolved inorganic carbon in groundwater from confined portions of the Upper Floridan aquifer, Florida, USA. Hydrogeol J 9:127–150

Rhue RD, Harris WG, Nair VD (2006) A retardation-based model for phosphorus transport in sandy soil. Soil Sci 171:293–304

Ritorto M, Screaton EJ, Martin JB, Moore PJ (2009) Relative importance and chemical effects of diffuse and focused recharge in an eogenetic karst aquifer: an example from the unconfined upper Floridan aquifer, USA. Hydrogeol J 17:1687–1698

Scott TM, Means GH, Meegan RP, Means RC, Upchurch SB, Copeland RE, Jones J, Roberts T, Willet A (2004) Springs of Florida. Bulletin No. 66, Florida Geological Survey, Tallahassee

Sickman JO, Albertin AR, Anderson MW, Pinowska A, Stevenson RJ (2009) A comparison of internal and external supply of nutrients to algal mats in two first magnitude springs in Florida. J Aquat Plant Manag 47:135–144

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem 73:4145–4153

Sigman DM, Granger J, DiFiore PJ, Lehmann MM, Ho R, Cane G, van Green A (2005) Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. Global Biogeochem Cycles 19:GB4022. doi:10.1029/2005GB002458

Southeast Regional Climate Center (2010) Historical Climate Summaries Database. http://www.sercc.com/climateinfo/historical/historical.html. Accessed 22 Dec 2010

Stevenson RJ, Pinowska A, Wang Y (2004) Ecological condition of algae and nutrients in Florida Springs. Florida Department of Environmental Protection, Tallahassee

Strong WA (2004) Temporal water chemistry trends within individual springs and within a population of Florida Springs. Thesis, University of Florida, Gainesville

Van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger S, Latjha LK, Mayer B, Van Dam D, Howarth RW, Nadelhoffer KJ, Billen G (2002) Where did all the N go? Fate of N inputs to large watershed in the northeastern USA. Biogeochemistry 57(58):267–293

Vasques GM, Grunwald S, Comerford NB, Sickman JO (2010) Regional modeling of soil carbon at multiple depths within a subtropical watershed. Geoderma 156:326–336

Wankel SD, Kendall C, Paytan A (2009) Using nitrate dual isotopic composition (delta N-15 and delta O-18) as a tool for exploring sources and cycling of nitrate in an estuarine system: Elkhorn Slough, California. J Geophy Res Biogeo 114:G01011

Wilson WL, Skiles WC (1988) Aquifer characterization by quantitative dye tracing at Ginnie Spring, Northern Florida. The Association of Groundwater Scientists and Engineers, Dublin