# Denitrification and inference of nitrogen sources in the karstic Floridan Aquifer

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## Abstract

Aquifer denitrification is among the most poorly constrained fluxes in global and regional nitrogen budgets. The few direct measurements of denitrification in groundwaters provide limited information about its spatial and temporal variability, particularly at the scale of whole aquifers. Uncertainty in estimates of denitrification may also lead to underestimates of its effect on isotopic signatures of inorganic N, and thereby confound the inference of N source from these data. In ~~this study~~, our objectives are to quantify the magnitude and variability of denitrification in the Upper Floridan Aquifer (UFA) and evaluate its effect on N isotopic signatures at the regional scale. Using dual noble gas tracers (Ne, Ar) to generate physical predictions of $N_2$ gas concentrations for 112 observations from 61 UFA springs, we show that excess (i.e. denitrification-derived) $N_2$ is highly variable in space and inversely correlated with dissolved oxygen ($O_2$). Negative relationship between $O_2$ and $\delta^{15}N_{NO3}$ across a larger dataset of 113 springs, well-constrained isotopic fractionation coefficients, and strong $^{15}N$:$^{18}O$ covariation further support inferences of denitrification in this uniquely organic-matter-poor system. Despite relatively low average rates, denitrification accounted for 32% of estimated aquifer N inputs across all sampled UFA springs. Back-calculations of source $\delta^{15}N_{NO3}$ based on denitrification

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progression suggest that isotopically-enriched nitrate ($NO_3^-$) in many springs of the UFA reflects groundwater denitrification rather than urban- or animal-derived inputs.

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## 1 Introduction

Anthropogenic increases in reactive nitrogen (N) availability have wide-ranging consequences including eutrophication of aquatic systems, acidification of soils and surface waters, loss of biodiversity, and facilitation of disease transmission (Vitousek, 1994; Galloway et al., 2003; Smith and Schindler, 2009). Denitrification, which reduces $NO_3^-$ to $N_2$ gas, mitigates this enrichment by returning N to long-residence-time atmospheric pools, and is an important component of the nitrogen cycle at local, regional, and global scales (David et al., 2006; Seitzinger et al., 2006; Townsend and Davidson, 2006; Schlesinger, 2009; Sigman et al., 2009). Although denitrification was once thought to occur only via the oxidation of simple organic compounds, more recent work has demonstrated that $NO_3^-$ reduction can involve multiple electron donors and end products (Burgin and Hamilton, 2007). Patchy and ephemeral distribution, diverse reaction modes, and challenges of direct measurement of $N_2$ all contribute to persistent high uncertainty in local, regional, and global estimates of denitrification (Davidson and Seitzinger, 2006; Groffman et al., 2009).

Aquifer denitrification is a potentially large component of regional and global nitrogen (N) budgets, with a recent global estimate of 44 Tg N $yr^{-1}$ (16% of land-based annual N inputs; (Seitzinger et al., 2006). However, existing estimates are based on simple models and are extremely poorly constrained (range of estimates from Seitzinger et al., [2006]: 0-138 Tg N $yr^{-1}$), in large part due to the limited number and spatio-temporal extent of available direct measurements of denitrification. Additional uncertainty arises because existing studies of groundwater N concentrations and denitrification are potentially biased by preferential study of aquifers with high N loading and high rates of denitrification (Green et al., 2008; Schlesinger, 2009). Moreover, measures of denitrification based on nitrate loss appear to provide much higher estimates than those based on direct measurement of $N_2$ gas accumulation (Green et al., 2008). Despite their limited numbers, directly-measured aquifer denitrification rates nonetheless span several orders of magnitude, and associated reductions in $NO_3^-$ range from negligible to weg?

complete across aquifer systems (Green et al., 2008). The extent to which these outcomes vary in space and time within individual aquifers is poorly understood.

Estimation of denitrification from $N_2$ gas concentrations in groundwaters requires determination of physical parameters (recharge temperature [$T_{rec}$] and excess air [$A_{ex}$]) that influence the quantity and composition of dissolved gases (Vogel et al., 1981; Wilson and McNeill, 1997; Cey et al., 2009). Recharge temperature, rather than ambient temperature at the time of sample collection, is what determines the solubility of atmospheric gases at the time when infiltrating groundwater loses contact with the atmosphere. Depending on the seasonality of temperature, precipitation, and infiltration, as well as other factors, recharge temperatures can diverge substantially from mean annual air temperatures (Hall et al., 2005; Castro et al., 2007). Dissolution of excess air occurs when bubbles of atmospheric air are entrained beneath the saturated zone; supersaturation of gases with respect to surface conditions is enabled by hydrostatic pressure at depth. Direct simultaneous estimation of these parameters requires two tracers (typically noble gases; Feast et al., 1998; Cey et al., 2009), but most measurements of $N_2$ are made only in conjunction with Ar (Kana et al., 1994). Studies that estimate denitrification via direct measurement of $N_2$ thus typically rely on assumed constant values of either recharge temperature or excess air entrainment to estimate biologically-derived $N_2$ (e.g., Green et al., 2008). Since recharge temperature and excess air entrainment can vary at broad scales due to variation in climate and geological structure, assessment of denitrification at regional or broader scales requires estimation of these physical parameters for each study site. + pyrite

Across diverse ecosystems, availability of organic matter is the primary driver of denitrification (Taylor and Townsend, 2010). Within ecosystems, spatial and temporal variability in the concentration of organic matter and nitrate and anoxic conditions produce heterogeneous mosaics of denitrifying activity (e.g., Harms and Grimm, 2008). In groundwater environments with strong directional flow, denitrification and other redox processes can follow distinctive spatial patterns reflecting the gradual downstream depletion of electron donors and acceptors (Chapelle et al., 1995; Hedin et al., 1998; Tarits et al., 2006). However, a growing body of research suggests that denitrification in most aquifers depends on matrix-derived, solid-phase electron donors (e.g., $Fe^{2+}$, $H_2S$) rather than surface-derived solutes (Green et al., 2008; Schwientek et al., 2008; Zhang et al., 2009; Torrento et al., 2010; Torrento et al., 2011). As a result, concentrations of dissolved organic matter and other electron donors may be a poor indicator of denitrification rates across aquifers, and spatial patterns within aquifers may reflect the distribution of these reactants within the aquifer matrix rather than substrate depletion along advective flowpaths.

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Efforts to understand and manage N enrichment of aquatic ecosystems have relied heavily on the distinctive isotopic signatures of potential sources (Kendall, 1998; Kendall et al., 2007), particularly the difference in $\delta^{15}N_{NO3}$ between inorganic fertilizers (typically 0-3‰) and organic N pools (i.e. animal and human waste; typically 9-12‰). More recently, methodological developments that permit determination of both N and O allow greater separation of sources with overlapping $^{15}N$ signatures (e.g., atmospheric deposition and inorganic fertilizer). Biogeochemical reactions such as denitrification and assimilation can alter these isotopic signatures during transport along soil-ocean flowpaths, potentially confounding N source inference (Kendall et al., 2007). Despite pleas for caution (Bedard-Haughn et al., 2003), observed $\delta^{15}N_{NO3}$ are commonly used to infer N sources and guide management and policy related to point and non-point inputs (Fogg et al., 1998;Survey, 2003; Harrington et al., 2010). While the potential effect of denitrification on isotope signatures is widely acknowledged, few studies to date have quantified its influence on source inference at the scale of a regional aquifer.

In addition to their utility in separating potentially counfounded N sources, dual isotopic tracers ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) of $NO_3^-$ can also be used to infer nitrogen transformations. Although insufficient to directly estimate rates of denitrification, coupled enrichment of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ are now widely used to infer the occurrence of fractionating processes (Burns et al., 2009). Among these are recent studies that suggest denitrification in the Upper Floridan Aquifer and other karst groundwater systems (Panno et al., 2001; Albertin et al., 2011). One potential value of dual isotopic measurements is the ability to partition removal into its component processes (e.g. assimilation, denitrification) based on the ratio of $^{15}N$:$^{18}O$ enrichment. Theoretical and laboratory studies have suggested that denitrification results in 2:1 fractionation of $^{15}N$:$^{18}O$ (Aravena and Robertson, 1998; Lehmann et al., 2003), but other studies have recently suggested a 1:1 ratio (Granger et al., 2008), in which case dual isotopes would be unable to distinguish between assimilation and denitrification.

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In ~~this~~ study, our objectives are (1) to quantify the magnitude and variability of denitrification at the regional scale in a karstic groundwater system (the Upper Floridan Aquifer

[UFA]), and (2) to assess the influence of denitrification on isotopic signatures of nitrate in the UFA and its influence on apparent N sources. To these ends, we measured dissolved gases and other biogeochemical characteristics of 35 Florida springs, conducted a 3 year study (quarterly-monthly samples) of 6 springs that feed the Ichetucknee River, and assembled published data describing dissolved gas concentration, nutrient chemistry, and/or nitrate isotope composition from over 100 additional springs. From a subset of 31 of these springs for which dual noble gas tracers (Ne and Ar) were available, we derived statistical predictors of excess air entrainment. These data enable an extensive and robust assessment of denitrification and its influence on nitrate isotopic composition at the scale of the entire UFA.

## 2 Methods

### 2.1 Study System

The karstic Upper Floridan Aquifer (UFA) supports the highest density of large natural artesian springs in the world (Fig. 1), and is a major regional and global economic resource (Notholt et al., 1989; Miller, 1990; Bonn and Bell, 2003; Bonn, 2004). Throughout parts of northern Florida, the UFA is confined by low-permeability, high-clay deposits that preclude infiltration except via sinkholes and fractures; these confining layers are largely absent in the central-western portion of the state (Scott et al., 2004). Springs are concentrated along drainage features, especially near boundaries of confining layers. Land use throughout the study region includes variable mixtures of row crop agriculture, urban and suburban development, and secondary forest (Katz et al., 2001).

Geochemistry of the UFA can be characterized as a mixture of two end members. Older water, characteristic of matrix porosity and deep flowpaths, is generally anoxic, low in $NO_3^-$, and enriched in minerals; younger water characteristic of conduits and more shallow~~er~~ flowpaths is generally oxic, enriched in $NO_3^-$, and sometimes subsaturated in mineral chemistry (Toth and Katz, 2006). vague Over event-driven and decadal timescales, the contribution of these water sources can vary considerably among springs as changes in flow drive exchange between primary and secondary porosity (i.e., the limestone matrix and karst conduits; Martin and Dean, 2001; Heffernan et al., 2010a,b). Except during runoff and backflow events that deliver organic-matter-rich waters to conduits (Gulley et al., 2011), dissolved organic carbon (DOC) levels in

UFA springs are among the lowest measured globally (Duarte et al., 2010). In conjunction with oxic conditions of many springs, low DOC concentrations undoubtedly contribute to the prevailing assumption that denitrification is negligible in this system (Katz, 2004).

$NO_3^-$ concentrations in Florida springs have risen dramatically over the past half-century, and springs discharge accounts for a large proportion of the N load to estuarine and coastal waters (Pittman et al., 1997). Despite the perceived vulnerability of the UFA to nutrient enrichment, significant imbalances between inputs to North Florida landscapes and riverine export remain poorly understood (Katz et al., 2009). Landscape-scale mass balance generally suggests inorganic fertilizer as the primary source of N enrichment (Katz et al., 2009), but isotopic studies (that assumed negligible denitrification) have indicated a greater role of organic N from animal or human wastewater (Katz et al., 2001).

## 2.2 Sample collection and analysis

Between June and September 2010, we sampled 33 Floridan Aquifer springs that varied in size, surficial hydrogeology, and $NO_3^-$ and $O_2$ concentrations. At each spring, we measured $O_2$, temperature, specific conductance, and pH from spring vents using a YSI 556 sonde equipped with an optical or Clark probe. Water samples for laboratory analyses were collected using a peristaltic pump with a 5 m weighted intake tube placed as near as possible to the spring vent. We collected 3 replicate samples for nutrient and isotopic analyses in acid-washed pre-rinsed polyethylene bottles. During the synoptic survey, we collected 5 replicate field samples for dissolved gas analysis by flushing 300 ml BOD bottles 3 times, sealing with glass stopper, and capping with water-filled plastic caps to minimize exchange with atmosphere and to prevent stoppers from becoming dislodged during transport. Dissolved gas samples were stored under ice water until analysis within 36 hours; water samples were frozen until analysis.

We measured dissolved $N_2$ and Ar using a Membrane Inlet Mass Spectrometer (MIMS: Kana et al., 1994) within 36 hours of collection, over which period our storage protocol exhibited negligible atmospheric contamination. The membrane inlet mass spectrometer was equipped with a copper reduction column heated to 600 °C to remove $O_2$ and reduce interference with $N_2$ measurements (Eyre et al., 2002). Standards for $N_2$ and Ar concentration consisted of atmosphere-equilibrated deionized water in 1 L spherical vessels incubated and stirred in high-precision water baths (± 0.01 °C) at their respective temperatures (10, 15, and 20 °C) for at least 24 hours prior to analysis. Gas concentrations in each standard were calculated using temperature-solubility formulas without salinity correction (Hamme and Emerson, 2004). Signal strength for samples and standards was determined as the mean value of the 1st minute following signal stabilization. To account for instrument drift, we ran complete standard curves every 6-8 samples and applied interpolated parameter values from adjacent standard curves ($r^2$ range: 0.997-1.00; mean $r^2$ = 0.9997) to estimate gas concentrations in each sample. A fourth standard equilibrated with pure $N_2$ gas served as an external source QC. Coefficients of variation for field replicates ranged from 0.22-2.27% (mean: 0.80%; median: 0.49%).

We measured nitrate concentrations (expressed in this paper in mg N $L^{-1}$) in samples from the synoptic survey and Ichetucknee River springs time~~s~~ series using second-derivative UV spectroscopy (APHA et al., 2005) using an Aquamate UV-Vis spectrometer. Isotopic composition of nitrate ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$) was measured using the bacterial denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) in the Department of Geological Sciences at the University of Florida (2007-2009) or the UC-Riverside Facility for Isotope Ratio Mass Spectrometry (2010).

Previously~~-~~collected data both increased spatial coverage and in many cases provided repeated measurements of springs included in our synoptic survey (Fig. 1). Measurements of Ne, Ar, $N_2$, $O_2$, $NO_3^-$, and nitrate isotopes ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$) spanning from 1997 to 2008 were obtained from published articles (Katz, 2004; Katz et al., 2001, 2004; Toth and Katz, 2006; Knowles et al., 2010) and agency reports (Katz et al., 1999; Phelps, 2004; Phelps et al., 2006; Chasar et al., 2005), or directly from researchers when dissolved gas concentration or other data were not reported directly. Thirty~~-~~six archival observations (from 31 springs) included Ne in addition to Ar, $O_2$, and $N_2$ (and in 23 cases $\delta^{15}N_{NO3}$). In all, we assembled 112 observations of dissolved gas concentrations ($O_2$, Ar, $N_2$) from 62 distinct spring vents, of which 58 included both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, and 34 others included $\delta^{15}N_{NO3}$ but not $\delta^{18}O_{NO3}$. Excluding the repeated measurements of the Ichetucknee River springs in 2008-2009, our data included 166 observations of $\delta^{15}N_{NO3}$ and $O_2$ and 204 total observations of $\delta^{15}N_{NO3}$. Of the 113 springs represented in the isotope data set, 14 had 4 or more instances of concurrent measurements of both $O_2$ and $\delta^{15}N_{NO3}$. Observations were drawn from springs in each major drainage in North and Central Florida (Fig. 1), and with discharges ranging from <0.01 $m^3 s^{-1}$ to 11 $m^3 s^{-1}$.

All measurements of Ne from prior studies were determined by mass spectrometry at the Lamont-Doherty Earth Observatory Noble Gas Laboratory at Columbia University. $NO_3^-$ was generally measured using the cadmium reduction method (Wood et al., 1967). $N_2$ and Ar from previously published studies were measured using gas chromatography. Recent (2007 and later) measurements of $\delta^{15}N_{NO3}$ were generally conducted using the bacterial denitrifier method and included $\delta^{18}O_{NO3}$ (Sigman et al., 2001; Casciotti et al., 2002). For data prior to 2007, $\delta^{15}N_{NO3}$ was measured via combustion and mass spectrometry (Kendall and Grim, 1990).

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## 2.3 Springshed characterization

To determine hydrogeologic predictors of excess air entrainment and recharge temperature, we characterized each spring by latitude, long-term mean discharge, and springshed hydrogeology as measured by aquifer vulnerability to surface contamination (Arthur et al., 2007). We collected discharge records for each spring from online databases of the United States Geological Survey National Water Information System (http://waterdata.usgs.gov/nwis), Southwest Florida Water Management District (http://www.swfwmd.state.fl.us/data/), and the St. John's River Water Management District (http://www.sjrwmd.com/toolsGISdata/index.html) where available, since these records were generally the most complete. Where continuous records were unavailable, we used the mean of discrete measurements from published studies and agency reports as our estimate of mean long-term discharge. Since discharge variability of Floridan Aquifer springs is extremely low, use of these more limited data to quantify long-term mean discharge is unlikely to have introduced significant error in subsequent analyses.

Where available, we used previously delineated boundaries (http://www.dep.state.fl.us/geology/programs/hydrogeology/hydro_resources.htm) to characterize springshed hydrogeology, and to estimate springshed size and location for un-delineated springs. The relationship between discharge and springshed area was determined for those springs with previously delineated springsheds ($A = Q \times 134.9$, where $A$ is springshed area in $km^2$ and $Q$ is discharge in $m^3\ s^{-1}$; $n = 14$, $r = +0.79$, $p < 0.001$). For springs without a delineated springshed, we estimated the contributing area based on their period-of-record discharge. We assumed each springshed was circular, and estimated the springshed orientation based on the regional drainage network such that the springshed was located with one edge at the spring vent, and the rest up-gradient from the closest spring-fed river.

We used the Floridan Aquifer Vulnerability Assessment (FAVA) as a metric of springshed hydrogeologic characteristics (Arthur et al., 2007). This measure quantifies the intrinsic contamination risk of the Upper Florida Aquifer (UFA) based on local hydrogeologic conditions. Point observations of aquifer properties diagnostic of rapid recharge rates (e.g., nutrient and major element chemistry, high $O_2$ concentrations) comprise a data set (n = 148) on which aquifer risk was trained using a weights-of-evidence approach based on a variety of spatially extensive data layers. These included surface soil permeability, surface elevation, subsurface stratigraphy, presence of karst features (e.g., sinkholes) at the surface, thickness of a the intermediate aquifer system that regulates hydraulic confinement of the UFA, and the potentiometric head difference between the surface and UFA interpolated from a regional well network. Posterior contamination probabilities were classified as “less vulnerable”, “vulnerable” and “most vulnerable”. None of the springsheds in this study contained more than 3% of their area in the less vulnerable category, so we used the fraction of each springshed area delineated as most vulnerable, typically more than 75% of the area, as a predictor in our model of excess air entrainment.

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## 2.4 Estimation of excess air, recharge temperature, and excess $N_2$

We used Ne and Ar concentrations to determine recharge temperature ($T_{rec}$ ) and excess air ($A_{ex}$) in the subset ($n$ = 36) of springs for which measurements of both gases were available. We estimated these parameters for each observation air by simultaneously solving the following equations using the Solver function in Microsoft Excel:

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$$[Ne]_{obs} = k_{Ne} \cdot [Ne]_{Trec} + A_{ex} \cdot P_{Ne} \qquad (1)$$

$$[Ar]_{obs} = k_{Ar} \cdot [Ar]_{Trec} + A_{ex} \cdot P_{Ar} \qquad (2)$$

where $k_{Ne}$ and $k_{Ar}$ are coefficients for unit conversion of Ne and Ar from nmol $kg^{-1}$ (for Ne, $k$ = 0.02) or µmol $kg^{-1}$ (for Ar, $k$ = 0.04; for $N_2$, $k$ = 0.028) to mg $L^{-1}$; $P_{Ne}$ and $P_{Ar}$ are the mass proportion of Ne ($1.818 \cdot 10^{-5}$) and Ar ($9.34 \cdot 10^{-3}$) in the atmosphere; and $[Ne]_{Trec}$ and $[Ar]_{Trec}$ are the equilibrium concentrations (Hamme and Emerson, 2004) of those gases at the recharge temperature as determined by:

$$\ln([Ne]_{Trec}) = A_0 + A_1T_S + A_2T_S^2 + A_3T_S^3 \quad (3)$$

$$\ln([Ar]_{Trec}) = A_0 + A_1T_S + A_2T_S^2 + A_3T_S^3 \quad (4)$$

where $T_S$ is determined as:

$$T_S = \ln\left(\frac{298.15 - T_{rec}}{273.15 + T_{rec}}\right) \quad (5)$$

and $A_0$-$A_3$ are compound specific solubility constants (Table 1).

Among this set of springs with Ne data, estimated $T_{rec}$ ranged from 15-22°C, was overwhelmingly determined by Ar rather than Ne, and varied significantly as a function of latitude (Fig. 2a-c). The observed latitudinal variation in $T_{rec}$ is much greater than variation in mean annual air temperature, potentially reflecting regional variation in timing of precipitation and thus temperature of infiltrating water (Schmidt et al., 2001). ?

$A_{ex}$ ranged from 1.0 to-2.7 ml $L^{-1}$ and was overwhelmingly determined by Ne rather than Ar (Fig. 2a,b). Based on multiple regression analysis, mean discharge over the period of record ($Q_{POR}$) and springshed vulnerability were strong predictors of excess air (Fig. 2d). Palm spring, whose springshed had no land in the 'most vulnerable' category and was the only value less than 50%, was excluded from this analysis. We used this statistical relationship to estimate $A_{ex}$ in springs for which Ne data were not available, then solved for recharge temperature in those springs using Eq. 1.

We used estimates of $T_{rec}$ and $A_{ex}$, whether direct or modeled statistically, to determine $N_2$ concentrations that would be observed based only on those physical processes ($[N_2]_{phys}$), without any biological $N_2$ production. Specifically, we calculated expected $N_2$ concentrations using the same temperature-solubility relations described in Eq. 1-5, but parameterized for $N_2$:

$$[N_2]_{phys} = k_{N2} \cdot [N_2]_{Trec} + A_{ex} \cdot P_{N2} \quad (6)$$

where $k_{N2}$ is 0.028; $P_{N2}$ is 0.78084 and $[N_2]_{Trec}$ is the concentration of $N_2$ at recharge temperature as determined by:

$$\ln([N_2]_{Trec}) = A_0 + A_1 T_S + A_2 T_S^2 + A_3 T_S^3 \tag{7}$$

where $T_S$ is given in Eq. 5 and $N_2$-specific values for the $A_0$-$A_3$ are given in Table 1. We estimate the magnitude of denitrification ($[N_2]_{den}$) for each sampling date and time as the difference between observed $N_2$ concentrations and concentration predicted by physical processes: meaning?

$$[N_2]_{den} = [N_2]_{obs} - [N_2]_{phys} \tag{8}$$

To test the hypothesis that $[N_2]_{den}$ reflects the magnitude of denitrification, we used regression analyses to evaluate the relationship between $[N_2]_{den}$ and dissolved $O_2$. We used both linear and logarithmic forms to predict $[N_2]_{den}$ from $O_2$, for the entire data set and for the subset of observations in which $A_{ex}$ was calculated from Ne data, rather than estimated statistically. We also analyzed the relationship between mean dissolved $O_2$ and mean $[N_2]_{den}$ from the subset of springs for which 3 or more observations were available.

To evaluate the relative precision and accuracy of $[N_2]_{den}$ estimates based on Ne and statistically modeled excess air, we calculated the mean and standard deviation of $[N_2]_{den}$ estimates for all springs with $O_2$ greater than 2 mg $L^{-1}$. Bias in estimates would cause divergence of the mean from zero, assuming that denitrification is negligible in these oxic springs (Bohlke et al., 2002; Green et al., 2008).

## 2.5 Denitrification progression and isotopic fractionation

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We indirectly evaluated the relationship between denitrification progression and $\delta^{15}N_{NO3}$ via analysis of relationships between dissolved $O_2$ and $\delta^{15}N_{NO3}$ both within and across springs, reasoning that springs with lower dissolved $O_2$ would have greater depletion of $NO_3^-$ pools by denitrification than springs with higher $O_2$. We used both linear and logarithmic regression equations ① to evaluate dissolved $O_2$ as a predictor of $\delta^{15}N_{NO3}$ across all observations and excluding observations from the Ichetucknee time series collected between July 2007 and November 2009. Inclusion of the entire Ichetucknee data set had a minimal influence on regression parameters, so only the results from the complete (global) data set are reported here. In addition to this global analysis, we used linear regression ② to evaluate relationships between dissolved $O_2$ and $\delta^{15}N_{NO3}$ within springs for which 3 or more observations were available. We then used regression analysis to evaluate how the strength (as measured by the correlation coefficient [*r*]) and slope of these within-spring relationships varied as a function of mean dissolved $O_2$. This analysis allowed us to evaluate the contribution of variation within and among springs to patterns seen across all observations.

We directly evaluated the relationship between denitrification progression and isotopic composition of $NO_3^-$ by determining the fractionation coefficient ($^{15}\varepsilon$) for $\delta^{15}N_{NO3}$ from a cross-system analysis that included springs with dissolved gases from both our synoptic survey and previously reported data, and a separate analysis from the Ichetucknee Springs time series (of which most dates did not include dissolved gas measurements). These analyses required estimates of initial $NO_3^-$ concentration ($[NO_3^-]_{init}$ ) at the time of recharge, which we estimated using different approaches for springs with dissolved gas data and for the Ichetucknee Springs time series. For analysis of data from the synoptic survey and previous observations that included dissolved gases, we calculated $[NO_3^-]_{init}$ as the sum of $[NO_3^-]_{obs}$ and $[N_2]_{den}$ (all in units of mg N $L^{-1}$). This estimate would include nitrate derived from nitrification in the vadose zone or UFA as part of $[NO_3^-]_{init}$, and assumes that denitrification is the only sink for $NO_3^-$ (i.e., that assimilation, dissimilatory nitrate reduction to ammonium [DNRA], etc. are negligible) as indicated by concentrations of ammonium and particulate and dissolved organic nitrogen that are typically below detection limits at spring vents. Effects of these processes on $\delta^{15}N_{NO3}$ are also assumed to be zero.

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Estimates of $[NO_3^-]_{init}$ allow determination of the progression of denitrification. For each observation, we calculated the proportion of nitrate remaining from the original pool $[NO_3^-]_R$ as:

$$[NO_3^-]_R = \frac{[NO_3^-]_{obs}}{[NO_3^-]_{init}} \qquad (9)$$

where $[NO_3^-]_{obs}$ is measured concentration, and $[NO_3^-]_{init}$ is the initial concentration. We used linear regression to determine the isotopic enrichment factor ($^{15}\varepsilon$) for $\delta^{15}N_{NO3}$ (assuming Rayleigh distillation kinetics) as the slope of the relationship between $\delta^{15}N_{NO3}$ and $\ln([NO_3^-]_R)$ (Mariotti, 1986; Bohlke et al., 2002; Green et al., 2008). We excluded springs with $NO_3^-$ concentrations below 0.1 mg $L^{-1}$ (Juniper, Silver Glen, and Alexander Springs) from this analysis due to the high variability of $[N_2]_{den}$ estimates relative to these lower concentrations.

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how can it be estimated
in another way

For the Ichetucknee Springs time~~s~~ series, we calculated $[NO_3^-]_R$ for each spring and sampling date by assuming that $[NO_3^-]_{init}$ for all springs was equal to $[NO_3^-]_{obs}$ in the Ichetucknee Headspring on the same date. The first assumption implicit in this analysis is that denitrification rates in the Ichetucknee Headspring are negligible. High $O_2$ concentrations (mean ± SD: 4.1 ± 0.2 mg $O_2$ $L^{-1}$), low values of $[N_2]_{den}$ (which averaged 0.32 mg N $L^{-1}$ and represented minimal (<30%) depletion of the estimated original nitrate pool), and the low and temporally stable $\delta^{15}N_{NO3}$ (mean ± SD: 3.6±0.3, $n$ = 16) observed in the Ichetucknee Headspring all support this assumption. The second implicit assumption, that springsheds of the Ichetucknee springs receive equivalent N loads from sources with identical isotopic signatures, is based on the relative homogeneity of land use in the Ichetucknee springshed, and the predominance of fertilizer application to improved pasture as a source of N to the watershed (Katz et al., 2009). The third assumption, that variation among springs of the Ichetucknee is driven by denitrification, is supported by strong correlations between dissolved $O_2$ and $NO_3^-$ within and across these systems (Fig. 3**a**).

inf. area

To further evaluate the latter two assumptions, we used $[NO_3^-]_{init}$ and $[NO_3^-]_R$ values for the springs of the Ichetucknee River on three dates when $[N_2]_{den}$ estimates were available. First, we compared $[NO_3^-]_{init}$ from the Ichetucknee Headspring with the mean value of other springs on the same date. The similarity and covariation of these values (Table 2) is consistent with the assumption that all springs in the Ichetucknee System receive similar N loads. In addition, we assessed the correlation between alternative estimates of $[NO_3^-]_R$, namely estimates calculated from $[N_2]_{den}$ and $NO_3^-$ from reach spring and those estimated from the differences in $NO_3^-$ concentration between each spring and the Ichetucknee Headspring (Fig. 3a). The relationship between these estimates (Fig. 3b) suggests that the $NO_3^-$ difference approach used in the analysis of the Ichetucknee Springs time series provides a more conservative estimate of the progression of denitrification than those determined using $[N_2]_{den}$, which is to be expected if denitrification has also reduced $NO_3^-$ concentrations to a small degree in the Ichetucknee Headspring. More importantly, the correlation between these estimates is consistent with the assumption that variation in $NO_3^-$ concentration both within and among the springs of the Ichetucknee River is driven at least in part by differences in the progression of denitrification along the flowpaths that contribute to these springs.

## 3 Results

For the vast majority of our observations, $N_2$ concentrations exceeded values predicted from recharge temperature and excess air (51 of 61 springs; 94 of 112 observations). $[N_2]_{den}$ ranged from -0.7 to 3.5 mg $N_2$ $L^{-1}$ (median: 0.67 mg $N_2$ $L^{-1}$; mean ± SD: 0.82 ± 0.83 mg $N_2$ $L^{-1}$), and was inversely correlated with $O_2$ (Fig. 4a). Among springs with Ne data, this relationship exhibited a sharp break at ca. 2 mg $O_2$ $L^{-1}$, above which $[N_2]_{den}$ averaged 0.003 mg $N_2$ $L^{-1}$ (± 0.32; 2 SE); below 2 mg $O_2$ $L^{-1}$, $[N_2]_{den}$ averaged 1.5 mg $N_2$ $L^{-1}$ (±0.33; 2 SE). Among all springs, this threshold was less distinct, and a linear relationships was a better fit than a logarithmic relationship.

Among the 16 springs with 3 or more observations (max = 5) of $[N_2]_{den}$ ~~in our data set,~~ over 90% of the total variation in $[N_2]_{den}$ occurred among rather than within springs (ANOVA; Figure 5a, Table 3). Standard deviations within springs for $[N_2]_{den}$ ranged from 0.05 to 0.65 mg $N_2$ $L^{-1}$ and averaged 0.31 mg $L^{-1}$. Among the same set of springs, over 88% of total variation in $[NO_3^-]_R$ occurred among springs (ANOVA; Fig. 5b, Table 3). Standard deviations within springs for $[NO_3^-]_R$ ranged from <1% to 29% and averaged 10.4%. For both variables, variation among springs was strongly correlated with mean dissolved $O_2$ from the same set of observations. However, variation in $[N_2]_{den}$ and $[NO_3^-]_R$ within springs was not correlated with variation within springs in dissolved $O_2$, presumably due to low sample size

Fluxes of $[N_2]_{den}$ from UFA springs were comparable to but uncorrelated with those of $NO_3^-$, and the proportion of $NO_3^-$ removed by denitrification varied among springs from 0 to as high as 97% (mean ± 2 SE: 34 ± 9%) among springs. Denitrification removed more than 75% of N inputs in 8 of 61 springs, and more than 50% in 20 of 61. Compared to this spatial heterogeneity, temporal variation in $[N_2]_{den}$ among springs was low (Fig. 5, Table 3). We estimate that denitrification reduced total flow-weighted $NO_3^-$ flux from sampled UFA springs by 32%, with uncertainty in this estimate primarily driven by the representativeness of our sample of springs. Volumetric denitrification rates calculated from $N_{2den}$ and apparent He water age (Green et al. 2008) averaged of 2.75 μmol $m^{-3}$ $d^{-1}$. Aggregate (i.e. flow-weighted) areal denitrification for all springsheds was 1.22 kg $ha^{-1}$ $y^{-1}$, with ca. 20% of springsheds exceeding the estimated global average for groundwater denitrification (3.49 kg $ha^{-1}$ $y^{-1}$).

Across 292 observations from 103 springs, $\delta^{15}N_{NO3}$ ranged from -0.3 to 23.9‰, was inversely correlated with $O_2$, and varied more among low-$O_2$ (<2 mg $L^{-1}$) than high-$O_2$ springs (Fig. 4b). Among springs with 3 or more observations (max = 18) of $\delta^{15}N_{NO3}$, 86% of the total variation in $\delta^{15}N_{NO3}$ in our data set was accounted for by variation among springs (ANOVA; Fig. 6, Table 3). Standard deviations of $\delta^{15}N_{NO3}$ within individual springs ranged from 0.1 to 5.2‰ and averaged 1.4‰. Unlike $[N_2]_{den}$ and $[NO_3^-]_R$, variation within springs in $\delta^{15}N_{NO3}$ was also correlated with variation in dissolved $O_2$, particularly for low $O_2$ springs, which had strong, steeply negative relationships between temporal variation in $\delta^{15}N_{NO3}$ and dissolved $O_2$ (Fig. 7).

The progression of denitrification, as indicated by $[NO_3^-]_R$, was a strong predictor of $\delta^{15}N_{NO3}$ in both the synoptic survey and Ichetucknee River time series (Fig. 8a,b). Estimated fractionation coefficients were similar between the Ichetucknee River time series and the synoptic survey, particularly when only springs with Ne data were considered. Both data sets also exhibited strong relationships between nitrate $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$; however, the slope of this relationship differed substantially between the synoptic cross-system survey (1.8:1) and the Ichetucknee River time series (1:1; Fig. 8c,d). For the Ichetucknee River springs, relationships between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ within sampling dates were consistently near unity (Table 4).

ratio different

?

Estimates of source $\delta^{15}N_{NO3}$ from denitrification progression and observed $\delta^{15}N_{NO3}$ values suggest that denitrification alters $\delta^{15}N_{NO3}$ at the regional scale. Among springs with estimates of $N_{2\text{-}den}$, nearly 20% of observed $\delta^{15}N_{NO3}$ values were greater than 9‰, and more than 50% were greater than 6‰ (Fig. 9~~b,~~c), values used in Florida and elsewhere to delineate inorganic and organic sources and mixtures thereof (Katz 2004, Bohlke 2002). Estimated $\delta^{15}N_{NO3}$ of source N (Fig. 9d) were much lower, with only 5.5% of observations estimated to have original source $\delta^{15}N_{NO3}$ greater than 9‰, and 26% greater than 6‰. Within the Ichetucknee River time series, differences between the distribution of observed and estimated source $\delta^{15}N_{NO3}$ were even greater (Fig. 9e,f). $\delta^{15}N_{NO3}$ values greater than 6‰ were rare among springs with DO greater than 3 mg $L^{-1}$ (Fig. 9a,b). In all three data sets, estimated source $\delta^{15}N_{NO3}$ was both lower on average and much less variable than spring water.

||?

# 4 Discussion

## 4.1 Evidence for Denitrification in the Upper Floridan Aquifer

Relationships among denitrification-derived $N_2$ ($[N_2]_{den}$), $O_2$, and $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ all support the widespread occurrence and significance of denitrification in the Upper Floridan Aquifer. In springs with low dissolved $O_2$, $N_2$ concentrations exceeded those predicted by physical processes (as measured by noble gas tracers), but closely matched those predictions in high $O_2$ springs (Fig. 4a). The negative relationship between $O_2$ concentrations and $[N_2]_{den}$ provides clear evidence of both the accuracy and precision of our approach and the occurrence of denitrification in hypoxic portions of the UFA. Like $[N_2]_{den}$, $\delta^{15}N_{NO3}$ was inversely related to dissolved $O_2$, with high values observed almost exclusively below 2 mg $O_2$ $L^{-1}$. $\delta^{15}N_{NO3}$ values for springs with $O_2$ greater than 2 mg $L^{-1}$ were generally consistent with nitrogen derived from predominantly but not exclusively inorganic sources (Fig. 4b). Relationships between the progression of denitrification and $\delta^{15}N_{NO3}$ (Fig. 8a,c) support both the inference of denitrification in the Floridan aquifer and the hypothesis that variation in isotopic signatures is primarily driven by denitrification rather differential contribution from organic and inorganic sources. Fractionation coefficients ($^{15}\varepsilon$) derived from both the synoptic survey and the Ichetucknee River springs are within the lower range of values reported for other aquifers (Mariotti, 1986; Bohlke et al., 2002; Green et al., 2008), and other marine and freshwater environments (Sigman et al., 2005; Granger et al., 2008), but lower than some recent experimental values (Knoller et al., 2011). These relatively low values may indicate diffusion-constrained $NO_3^-$ limitation of denitrification in the UFA (Sebilo et al., 2003).

Covariation between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ confirms the inference of denitrification in the UFA and its influence on isotopic composition of $NO_3^-$ at the regional scale. Across springs and over time within the springs of the Ichetucknee River, we observed strong relationships between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, although the slopes of these relationships differed between the synoptic survey and the temporally intensive study of the Ichetucknee system. The 1:1.7 relationship across sites (Fig. 8b) is consistent with theoretical and empirical studies showing 1:2 enrichment by denitrification (Lehmann et al. 2003, Aravena and Robertson 1998). In contrast, covariation within the springs of the Ichetucknee River exhibited slopes near 1:1 on each date and across all dates (Fig. 8d, Table 4). A recent study of isotope dynamics within the Ichetucknee River itself yielded similar 1:1 fractionation ratio for $^{18}O$:$^{15}N$ associated with denitrification (Cohen et al., in revision). These relationships, both from studies with independently-constrained, direct estimates of denitrification, are similar to fractionation ratios obtained from laboratory experiments (Granger et al., 2008) and marine systems (Sigman et al., 2005). The divergence of fractionation during denitrification between freshwater and marine ecosystems has been attributed to taxonomic or environmental differences between these systems, but our observations suggest factors varying among watersheds can alter these relationships. Regardless of these differences, strong covariation among $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ provide additional evidence that denitrification drives variation in nitrate isotopic composition in the Floridan Aquifer.

Temporal patterns within individual springs provide a final line of support for denitrification as a driver of $NO_3^-$ concentrations and isotopic composition. Within the springs of the Ichetucknee River, $O_2$ concentrations were positively correlated with $NO_3^-$ concentrations (Fig. 3a). Across all springs, $O_2$ concentrations were negatively correlated with $\delta^{15}N_{NO3}$, and negative relationships were also observed over time within low-$O_2$ springs. The apparent absence of $O_2$-driven variation within springs for $[N_2]_{den}$ and $[NO_3^-]_R$ is most likely attributable to low power in our data set for those variables and lower precision in estimates of $[N_2]_{den}$ (and thus $[NO_3^-]_R$) than for $\delta^{15}N_{NO3}$. Given the strength of observed relationships across springs between denitrification progression and $\delta^{15}N_{NO3}$, the most likely explanation for within-spring $\delta^{15}N_{NO3}$-$O_2$ relationships (Fig. 7) is that variation in both $O_2$ and $\delta^{15}N_{NO3}$ reflect differential contributions of young, oxic groundwater, the isotopic signatures of which are unlikely to have been altered by denitrification, and older, anoxic groundwater, for which deplet~~ion~~ed nitrate by denitrification has produced elevated values of $\delta^{15}N_{NO3}$. A plausible alternative hypothesis is that $O_2$-$\delta^{15}N_{NO3}$ relationships reflect the concurrent influence of human- or animal-derived effluent on dissolved $O_2$ (via increased BOD) and $\delta^{15}N_{NO3}$. However, if coincident BOD and $\delta^{15}N$-enriched $NO_3^-$ inputs were responsible for these patterns, then high-$O_2$ springs would exhibit covariation between $O_2$ and $\delta^{15}N_{NO3}$, as is observed in low-$O_2$ springs. Thus, while alternative explanations might plausibly explain some of the pairwise correlations observed in this study (e.g. organic waste inputs as a driver of both $O_2$ and $\delta^{15}N_{NO3}$), the convergence of multiple lines of evidence clearly indicates that denitrification is a significant process in the UFA, as both a sink for N inputs and as a driver of variation in isotope signatures.

## 4.2 Magnitude and mechanisms of nitrate reduction

An important feature of this study is that springs integrate upgradient N transformations over considerable spatial domains and over the entire duration of water residence in the subsurface. In combination with the large spatial extent of our study springs, this feature of springs enables relatively simple scaling of groundwater denitrification to springshed and regional scales. Studies of aquifer denitrification generally address denitrification along individual flowpaths with relatively small spatial footprints, and as such may not reflect the distribution of rates or residence times within the larger aquifer.

At the scale of the UFA, denitrification appears to be a significant sink for N leaching to the Upper Floridan Aquifer, removing approximately 32% of the total (flow-weighted) N discharging from sampled springs. Average volumetric rates derived from $[N_2]_{den}$ were toward the low end of values obtained from direct measurement of $N_2$, but were within the range reported for agriculturally enriched aquifers (Green et al., 2008). Nonethless, aggregate areal rates of denitrification (122 kg $km^2$ $y^{-1}$) are comparable to the estimated global average for aquifer denitrification (Seitzinger et al., 2006). These averages, however, integrate a high degree of variation among springs. Average areal rates for individual springs ranged from less than 0 to as high as 5300 kg $km^2$ $y^{-1}$, and the depletion of $NO_3^-$ load from 0 to more than 97%. Thus within the Floridan Aquifer we observe variation in denitrification comparable to that observed globally (Seitzinger et al., 2006). Significant spatial heterogeneity of denitrification has been observed in other aquifers, but none to our knowledge have measured denitrification directly over the spatial and temporal extent found in this study. Our results suggest that measurements along individual groundwater flowpaths are unlikely to be applicable to entire regional aquifer systems.

too general

The occurrrence and significance of denitrification in the organic-matter-poor Floridan Aquifer is superficially suprising, given the importance of organic matter supply as a constraint on denitrification across diverse ecosystem types. However, several recent studies have found isotopic evidence for denitrification in karstic aquifers, including those in southwestern Illinois, USA (Panno et al., 2001), southern Germany (Einsiedl and Mayer, 2006), and the Floridan Aquifer (Albertin et al., 2011). One feature of karst aquifers that may facilitate denitrification is large difference in hydraulic conductivity, and thus water age and chemistry, between karstic aquifer matrices and conduits. McCallum et al. (2008) showed that mixing of groundwaters of different ages, $NO_3^-$ concentrations, and redox potentials can promote aquifer denitrification, and such oxic-anoxic interfaces are widely recognized as locations of significant biogeochemical reactivity in surface waters (Dahm et al., 1998). Similar phenomena might be occurring throughout the considerable surface area of the conduit-matrix interface in karstic systems, and its occurrence and distribution might vary sufficiently to generate the observed differences in denitrification among UFA springs.

The source and character of electron donors that fuel nitrate reduction in the UFA are not known. Low DOC concentrations in UFA springs imply that denitrification is fueled by some other source, but availability of labile DOC is likely to be higher in water entering the aquifer than in water discharging from springs. Runoff entering sinkholes provide one pathway for relatively labile carbon to enter karstic aquifers, in either dissolved or particulate form. In some cases backflow from C-rich surface waters influences the chemistry of springs discharge (Gulley et al., 2011), and anthropogenic carbon from septic, municipal, or agricultural waste might also provide labile C to the UFA. The importance of electron donors other than organic C for denitrification in the UFA remains unclear, but has been demonstrated through correlational and experimental studies in other aquifers, including some karstic systems. Although the carbonate Floridan Aquifer matrix itself is generally poor in minerals that might serve as terminal electron donors, Florida springs exhibit significant variation in mineral chemistry (Scott et al., 2004), and many springsheds include the clay-rich Hawthorn Formation (Wicks and Herman 1994). If such alternative electron donors drive variation in denitrification among UFA springs, then concentrations of solutes such as Fe or $SO_4^-$ should be correlated with denitrification. The role of different nitrate reduction pathways, as well as broader drivers such as hydrogeology, geochemistry, and land use as factors that influence denitrification in the UFA, are beyond the scope of this paper, but clearly worthy of further investigation.

## 4.3 Uncertainty in estimates of denitrification

Use of dual noble gas tracers (Ne, Ar) to estimate recharge temperature and excess air produced estimates that were more precise and more accurate than those derived from statistical that high

modeling of excess air. Among springs with $O_2$ greater than 2 mg $L^{-1}$ and thus presumably negligible denitrification, $[N_2]_{den}$ estimates based on dual tracers averaged 0.003 mg N $L^{-1}$ (0.1 μmol $N_2$ $L^{-1}$) with a standard deviation of 0.32 mg N $L^{-1}$ (11.6 μmol $N_2$ $L^{-1}$). For observations from high $O_2$ springs where Ne data were unavailable, $[N_2]_{den}$ estimates based on statistically modeled excess air averaged 0.32 mg $N_2$ $L^{-1}$ (11.6 μmol $N_2$ $L^{-1}$) with a standard deviation of 16.8 μmol $N_2$ $L^{-1}$. For $[N_2]_{den}$, $[NO_3^-]_R$, and $\delta^{15}N_{NO3}$, spatial variation among springs was large compared to temporal variation within springs.

The stronger relationship between denitrification progression and $\delta^{15}N_{NO3}$ for springs with Ne data vs. all springs provides additional evidence for greater precision of these estimates. Greater bias and lower precision of these estimates most likely reflects the variability of excess air entrainment over time among springs, but may also reflect introduction of excess air during sampling, an artifact for which our statistical approach does not account. Nonetheless, uncertainty of our direct and statistically-derived estimates of denitrification compare favorably with bias (5 μmol $N_2$ $L^{-1}$) and precision (SD = 22 μmol $N_2$ $L^{-1}$) in a previous study of denitrification in agricultural aquifers, in which limited spatial extent permitted assumptions of constant recharge temperature within regions, and calculation of excess air from Ar concentrations (Green et al., 2008). The relatively high precision and minimal bias of $[N_2]_{den}$ estimates in this study illustrate both the value of dual isotope tracers and the utility of statistical modeling of physical processes where direct measurements are unavailable. Similar approaches will likely be necessary and useful in evaluating the spatial heterogeneity of denitrification in other aquifers.

One important implication of the low within-spring variance in $[N_2]_{den}$ and $[NO_3^-]_R$ (Fig. 5) is that uncertainty in our regional estimate of the magnitude of denitrification and its effect on N loads delivered to surface waters is largely influenced by whether or not sampled springs are representative, rather than by uncertainty of estimates within sampled springs. Our population of springs almost certainly overrepresents large springs, since we include more than half of the first magnitude springs in northern Florida. It is unclear whether our study oversampled N rich or N poor springs, since the distribution of $NO_3^-$ concentrations in small springs is not known. A second source of uncertainty is the magnitude of diffuse groundwater discharge from the UFA, and the comparability of the chemistry of this discharge to that of springs. On an areal basis, we have almost certainly underestimated denitrification in the UFA because estimates only include

but big sprgs big contribution

discharge from one spring over the area of its springshed. Incorporation of excess $N_2$ fluxes from springs with overlapping springshed and from diffuse groundwater efflux would increase areal estimates of denitrification, but the magnitude of this bias is not known. It is also unclear whether these unmeasured hydrologic flowpaths have excess $N_2$ concentrations comparable to those of the measured springs. In light of these uncertainties, our estimate of denitrification in the Floridan Aquifer should be viewed as a first approximation.

vague only individual no overall

## 4.4 Implications for N source inference

Concurrent measurements of dissolved gases, nitrate concentrations and isotopes enabled direct estimation of the absolute magnitude and relative progression of denitrification, and their relationship to $\delta^{15}N_{NO3}$ enrichment (Fig. 8). The strong negative relationships between the size of residual nitrate pools and $\delta^{15}N_{NO3}$ not only provide evidence for the occurrence of denitrification, but also clearly indicate that denitrification exerts a significant influence on nitrate isotopic composition in the UFA (particularly where dissolved $O_2$ concentrations are < 2 mg $L^{-1}$). These relationships, in turn, allowed us to estimate the isotopic composition of the original source $NO_3^-$ for each spring. These estimates suggest that $\delta^{15}N_{NO3}$ of nitrate discharging from UFA springs may in some cases differ substantially from the isotopic signature of the original N source.

summary

Observed variation in $\delta^{15}N_{NO3}$ among UFA springs was considerable, ranging from values near zero to more than 20‰. $\delta^{15}N_{NO3}$ distributions for springs with denitrification estimates and within the Ichetucknee River springs were similar to that of all springs, but the former was biased towards heavier $\delta^{15}N_{NO3}$ values, and the latter had a smaller range of values and relatively fewer high values. Nonetheless, the similarity of these distributions suggests that interpretation of our source estimates should be applicable to the broader population of springs. In all three data sets, the largest subset of springs had $\delta^{15}N_{NO3}$ less than 6%, but in each case more than one-third of springs had $\delta^{15}N_{NO3}$ greater than 6‰. Observed values in springs with $N_{2\text{-den}}$ estimates were higher than the larger data set that included all springs; more than 50% of $N_{2\text{-den}}$ springs had $\delta^{15}N_{NO3}$ values greater than 6‰.

Estimated source $\delta^{15}N_{NO3}$ signatures from springs with dissolved gas data and from the Ichetucknee River springs time series had distributions that differed from observed values in two important respects. First, mean and median values for estimated sources were lower by ca. 2‰ and 1.5‰, respectively. Second, the frequency of extremely high $\delta^{15}N_{NO3}$ values was much lower. Significantly, these estimated source values had distributions similar to those of all springs with $O_2 > 3$ mg $L^{-1}$, where denitrification is presumably negligible. Together, these observations strongly suggest that most $\delta^{15}N_{NO3}$ values in UFA springs somewhat overestimate the contribution of organic sources, and in particular that very high values overwhelmingly reflect fractionation resulting from nitrate removal by denitrification, rather than large contributions from organic N sources.

To date, denitrification has largely been assum~~t~~ed to be negligible in the Upper Floridan Aquifer, and as a result, elevated $\delta^{15}N_{NO3}$ values have been interpreted as indicating a significant contribution from organic sources. Despite mass balance studies indicating fertilizer application as the dominant N source to springsheds, policy and management efforts have largely focused on reducing N inputs from septic tanks, and agricultural and municipal waste (Loper et al. 2005, Dederkorkut 2005, Mattson et al. 2006), largely on the basis of enriched $\delta^{15}N_{NO3}$ signatures. Our data suggest that interpretation of $\delta^{15}N_{NO3}$ values must account for fractionating N transformations within the aquifer.

As one example of the effects of fractionation, Wekiwa springs, near Orlando FL, has a heavily urbanized catchment and consistently elevated $\delta^{15}N_{NO3}$ values (mean of 4 observations from 2001-2010: 11.5 ± 3.1‰), which would initially suggest that elevated $NO_3^-$ concentrations (wult in that system are primarily derived from organic sources. However, $\delta^{18}O_{NO3}$ are also highly enriched (12.4‰), and measurements of $N_{2\text{-den}}$ are consistently high (mean of 4 observations from 2001-2010: 3.1 ± 0.4 mg $N_2$ $L^{-1}$). We estimate that ~~that~~ dentrification within the aquifer typically removes ca. 75% of $NO_3^-$ before discharge from Wekiwa springs, and that the original source of nitrate in Wekiwa Springs had a $\delta^{15}N_{NO3}$ value ca. 6.3‰. While individual estimates of source $\delta^{15}N_{NO3}$ should be viewed with caution, it seems likely that despite its urban setting, N enrichment of Wekiwa springs is due primarily to inorganic fertilizers, with contributions from organic sources. In constrast, Wakulla Springs, near Tallahassee, FL, has somewhat enriched $\delta^{15}N_{NO3}$ (6.4-7.9‰) but no excess $N_2$. Moreover, hydrologic tracer studies have demonstrated direct connections between the Tallahassee municipal waste sprayfield and Wakulla Springs (Kincaid et al. 2005). Thus, it seems safe to conclude that elevated $\delta^{15}N_{NO3}$ values in Wakulla springs do in fact reflect the isotopic signature of N sources, which include significant contributions from organic sources. However, our observations suggest that this is the exception rather than the rule. Absent direct evidence for substantial organic sources for a specific spring, efforts to reduce N loading to the UFA should focus on fertilizer inputs.

## 5 Conclusions

The ~~surprising~~ importance of denitrification for N fluxes and isotopic composition in the UFA has important implications both for management of North Florida landscapes and for broader understanding of groundwater denitrification. Methodologically, this study illustrates the value of multiple lines of inference for assessing denitrification, which are strengthened by direct estimates of the physical processes that influence $N_2$ concentration using multiple tracers. Significant spatial and temporal variability of denitrification within the UFA suggests that improving regional and global estimates of denitrification will require more extensive measurements in other aquifers. The variability of denitrification in the Floridan Aquifer has implications not only for regional estimates of N removal, but also for values and variability of isotopic signatures of residual nitrate pools at the regional scale. Accurate assessments of the contribution of various sources of N enrichment, in North Florida and elsewhere, must account for the influence of denitrification on N isotope ratios.

Upper

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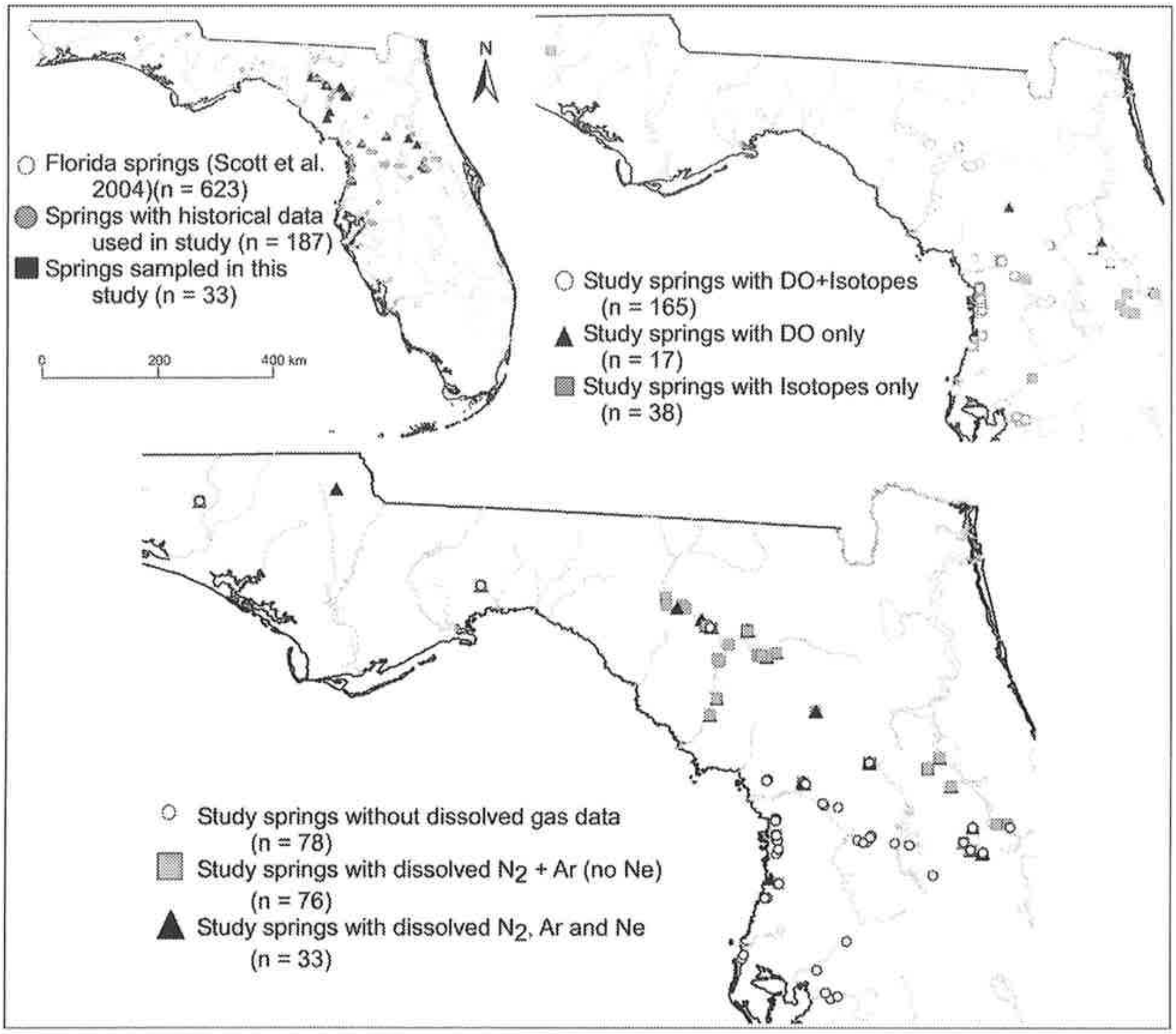
Figure 7. Parameters of within-spring relationships between dissolved $O_2$ and $\delta^{15}N_{NO3}$ as a function of spring mean dissolved oxygen. Large negative values of both (a) correlation coefficient and (b) slope in low-$O_2$ springs, and their absence in higher-$O_2$ systems, suggest that isotopically-enriched nitrate pools are associated with old, deeply anoxic flowpaths where denitrification would be most likely to occur. Open symbols indicate springs with 3 observations of $O_2$ and $\delta^{15}N_{NO3}$. Closed symbols indicated springs with 4 or more observations.

Figure 8. Effects of denitrification on isotopic composition of nitrate in Florida springs. Variation in $\delta^{15}N_{NO3}$ was strongly correlated with denitrification progression (a) as estimated from excess $N_2$ and observed nitrate concentrations across 61 springs, and (b) as estimated from differences between the Ichetucknee Headsprings and other springs in the Ichetucknee River. Positive correlation between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (c) across springs and (d) over time within the Ichetucknee system are also consistent with denitrification rather than variation in source as a driver of $\delta^{15}N_{NO3}$.

Figure 9. Implications of fractionation by denitrification for inference of N sources to Florida springs. The distribution of $\delta^{15}N_{NO3}$ across all observations (A), among springs sampled for dissolved gases in this study (C) and from the Ichetucknee time series (E) all suggest meaningful contributions of organic sources (one third to one half of springs). However, $\delta^{15}N_{NO3}$ values in high DO springs (B) and source $\delta^{15}N_{NO3}$ as back-calculated from isotopic enrichment factor and denitrification progression (D, F) suggest inorganic fertilizers and soil N (from mineralized OM) as the predominant source in the overwhelming majority of springs.

Figure 1


N
○ Florida springs (Scott et al. 2004)(n = 623)
● Springs with historical data used in study (n = 187)
■ Springs sampled in this study (n = 33)
0
200
400 km
○ Study springs with DO+Isotopes (n = 165)
▲ Study springs with DO only (n = 17)
▨ Study springs with Isotopes only (n = 38)
○ Study springs without dissolved gas data (n = 78)
▨ Study springs with dissolved N2 + Ar (no Ne) (n = 76)
▲ Study springs with dissolved N2, Ar and Ne (n = 33)