Source, variability, and transformation of nitrate in a regional karst aquifer: Edwards aquifer, central Texas

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HIGHLIGHTS

- Groundwater nitrate is increasing regionally in the karstic Edwards aquifer.
- Sources of increased nitrate loading are likely anthropogenic.
- Groundwater nitrate concentrations are elevated relative to surface water recharge.
- Nitrification within the aquifer is potential source of nitrate in groundwater.

GRAPHICAL ABSTRACT
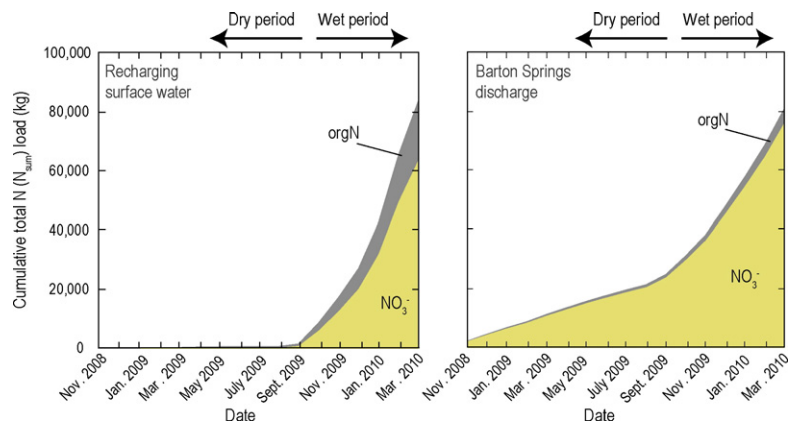
ABSTRACT

Many karst regions are undergoing rapid population growth and expansion of urban land accompanied by increases in wastewater generation and changing patterns of nitrate (NO_3^-) loading to surface and groundwater. We investigate variability and sources of NO_3^- in a regional karst aquifer system, the Edwards aquifer of central Texas. Samples from streams recharging the aquifer, groundwater wells, and springs were collected during 2008–12 from the Barton Springs and San Antonio segments of the Edwards aquifer and analyzed for nitrogen (N) species concentrations and NO_3^- stable isotopes ($\delta^{15}N$ and $\delta^{18}O$). These data were augmented by historical data collected from 1937 to 2007. NO_3^- concentrations and discharge data indicate that short-term variability (days to months) in groundwater NO_3^- concentrations in the Barton Springs segment is controlled by occurrence of individual storms and multi-annual wet-dry cycles, whereas the lack of short-term variability in groundwater in the San Antonio segment indicates the dominance of transport along regional flow paths. In both segments, longer-term increases (years to decades) in NO_3^- concentrations cannot be attributed to hydrologic conditions; rather, isotopic ratios and land-use change indicate that septic systems and land application of treated wastewater might be the source of increased loading of NO_3^-. These results highlight the vulnerability of karst aquifers to NO_3^- contamination from urban wastewater. An analysis of N-species loading in recharge and discharge for the Barton Springs segment during 2008–10 indicates an overall mass balance in total N, but recharge contains

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higher concentrations of organic N and lower concentrations of NO_3^- than does discharge, consistent with nitrification of organic N within the aquifer and consumption of dissolved oxygen. This study demonstrates that subaqueous nitrification of organic N in the aquifer, as opposed to in soils, might be a previously unrecognized source of NO_3^- to karst groundwater or other oxic groundwater systems.

1. Introduction

Anthropogenic activity has transformed the global nitrogen (N) cycle, and increases in nutrient concentrations in water resources are of worldwide concern (Galloway et al., 2008). In particular, elevated nitrate (NO_3^-) concentrations in surface water and groundwater resources are of growing concern for resource management. NO_3^- occurs naturally in many aquatic environments, but concentrations can be augmented by anthropogenic sources, including agricultural and urban fertilizers, sewage effluent, and animal waste. In conjunction with concentrations, the isotopic composition of NO_3^- ($\delta^{15}N$ and $\delta^{18}O$) provides insight into NO_3^- sources and aids in identifying processes affecting N dynamics (Kendall et al., 2008).

Karst aquifers are important groundwater resources, providing drinking water for roughly 25% of the global population (Ford and Williams, 2007) and habitat for a rich diversity of fauna (Humphreys, 2011). Karst aquifers also are extremely vulnerable to contamination because of the rapid transport of recharge through voids and conduits (Ford and Williams, 2007). Although karst groundwater in undeveloped areas commonly has low concentrations of NO_3^-, many karst aquifers are characterized by elevated NO_3^- concentrations from anthropogenic activities (e.g., Boyer and Pasquarell, 1996; Croll and Hayes, 1988; Katz, 2004). Understanding aquifer vulnerability and pollution risk to NO_3^- is important for resource management and protection, especially in karst aquifers. There are a variety of methods available to assess aquifer vulnerability, some with specific applicability to karst (e.g., Marin et al., 2015). Aquifers are not equally vulnerable to contamination, even where similar sources might exist, and an understanding of aquifer hydrogeology and geochemical processes is needed to inform vulnerability assessments, particularly for karst aquifers.

The Edwards (Balcones Fault Zone) aquifer in central Texas is a highly productive karst aquifer; the aquifer and its springs provide habitat for a number of endemic and threatened or endangered species (U.S. Fish and Wildlife Service, 2015). Central Texas is rapidly urbanizing (U.S. Census Bureau, 2014) and the Edwards aquifer is among the top ten endangered karst ecosystems (Belson, 1999). Numerous studies have demonstrated that the aquifer is vulnerable to anthropogenic contamination (e.g., Mahler and Massei, 2007), but historical trends and the effects of accelerated urbanization on NO_3^- in the aquifer are not well documented. Previous studies have demonstrated that NO_3^- concentrations in recharging streams are lower than in Edwards aquifer groundwater (Mahler and Garner, 2009), but a plausible explanation for this discrepancy is lacking.

While nitrification in the soil and vadose zone is a well-recognized process (Kendall et al., 2008), nitrification might occur subaqueously if reduced forms of N reach the saturated zone with conditions such as rapid recharge, shallow depths to the water table, or high organic carbon loads (DeSimone and Howes, 1998). Karst aquifers are characterized by rapid recharge via karst features and extensive surface water-groundwater interaction — features conducive to multiple forms of N reaching the water table. The formation of NO_3^- in the soil zone by nitrification as a source of NO_3^- to karst aquifers has been documented in prior studies (e.g. Einsiedl and Mayer, 2006) and is likely a primary source of nitrate to the Edwards aquifer; however, the possibility of nitrification occurring within an aquifer (subaqueously) might be an important

potential pathway for NO_3^- production in karst and other oxic groundwater systems.

Here we use recent (2008–12) and historical (1937–2007) N-species concentrations and NO_3^- isotopic compositions from streams that recharge the aquifer, groundwater wells, and major springs to investigate N dynamics for the San Antonio segment (SAS) and the Barton Springs segment (BSS) of the Edwards aquifer (Fig. 1). We characterize potential impacts of urbanization in the context of long-term (decadal) trends in NO_3^- concentrations, and quantitatively account for differences between NO_3^- concentrations in recharging streams and groundwater. The results provide a regional-scale perspective on N dynamics in an urbanizing karst aquifer.
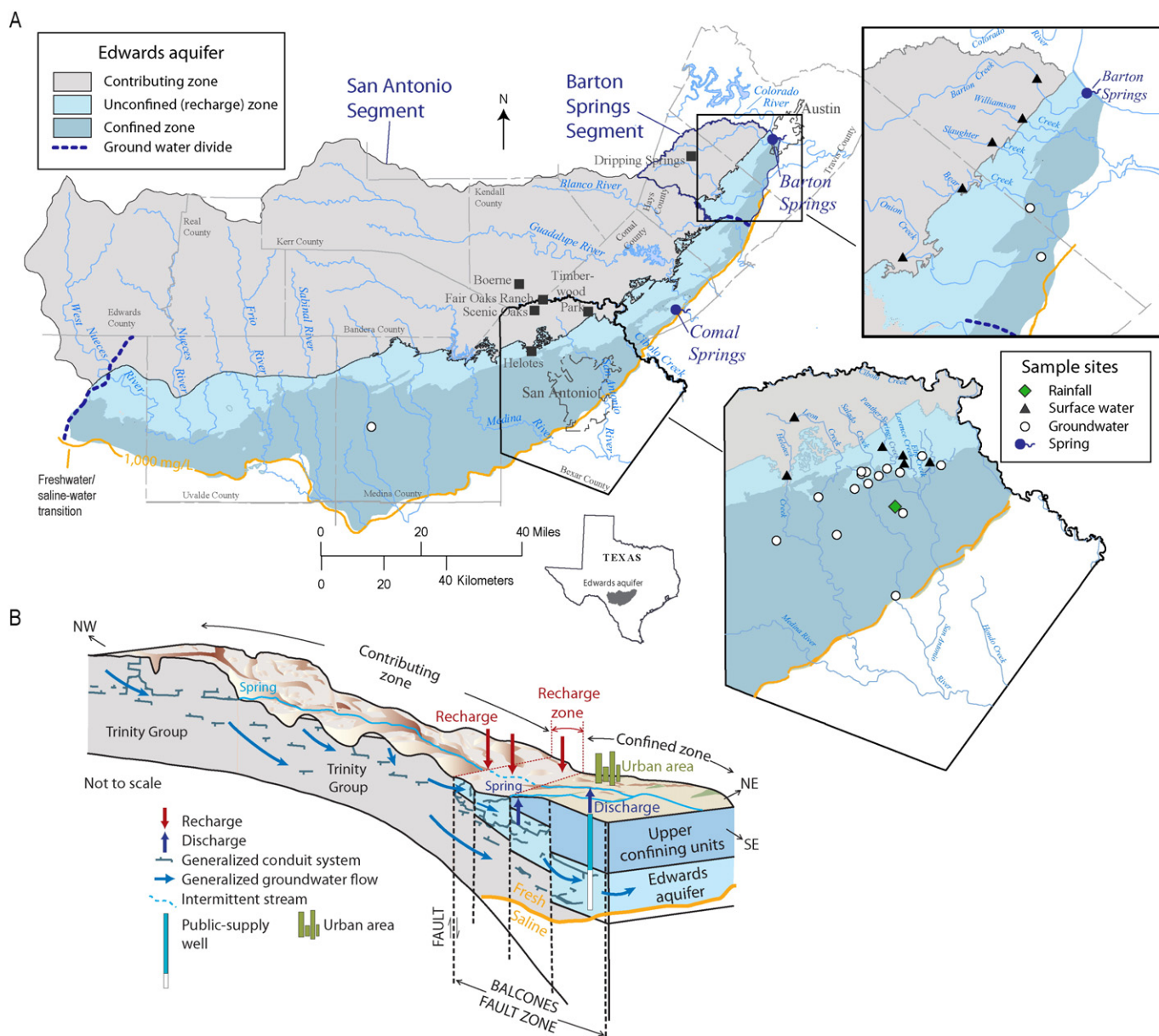
2. Hydrogeologic setting

2.1. Regional hydrogeology

The Edwards aquifer is developed in extensively karstified Cretaceous-age carbonate rocks (Maclay and Small, 1983). Late Cenozoic faulting formed a series of faults with down-to-the-coast displacement, resulting in blocks of Edwards aquifer rocks that are partially to completely offset. The highly transmissive aquifer is present in a narrow band along the Balcones Fault Zone (Fig. 1), along which aquifer rocks dip steeply to the south/southeast. As a result, the rocks containing the Edwards aquifer outcrop to the north and west (the recharge zone), but are overlain by the younger Del Rio Clay to the south and east (the confined zone) (Maclay and Small, 1983). Overlying confining units are of low permeability and a barrier to vertical flow (Lindgren et al., 2004). Upland streams flow to the south and east, and their watersheds define the Edwards aquifer contributing zone (Fig. 1). Losses as the streams cross the recharge zone have been estimated to contribute as much as 85% of aquifer recharge (Slade et al., 1986; Lindgren et al., 2004). Recharge also occurs through karst features in interstream areas (Lindgren et al., 2004). Recharge as distributed infiltration is not well documented, but is likely minor; together, direct and distributed recharge in interstream areas have been estimated to contribute from 15% (Slade et al., 1986) to about 35% of total recharge (Hauwert, 2009). Regional groundwater flow in the aquifer is to the east and northeast with natural discharge occurring at large springs, such as Comal and Barton Springs (Fig. 1). The discharge and geochemistry of these large springs provide an indicator of regional hydrologic conditions, reflecting water levels and integrated flow path geochemistry. Discharge also occurs through smaller springs and groundwater-supply wells.

2.2. Climate setting

Central Texas climate is characterized as subhumid to semiarid, with hot summers and mild winters (Larkin and Bomar, 1983). The region is prone to climatic and hydrologic extremes, cycling between wet and dry conditions (Griffiths and Strauss, 1985). Mean annual rainfall increases from west to east across the region, averaging 73.9 cm/yr (29.1 in/yr) (1871–2013) in San Antonio and 84.6 cm/yr (33.4 in/yr) (1856–2013) in Austin; mean annual rainfall has ranged from about 25 to >150 cm/yr (National Oceanic and Atmospheric Administration, 2014). Like many karst aquifers, the Edwards aquifer responds rapidly to changes in climatic and hydrologic conditions (Mahler and Massei,

Fig. 1. The Edwards aquifer (San Antonio segment and Barton Springs segment), showing: (A) hydrogeologic setting and sample sites. Map base modified from USGS 1:2,000,000-scale and 1:24,000-scale digital data. Aquifer boundaries from Ashworth and Hopkins (1995) and Texas Water Development Board (2006), from 1:250:000-scale digital data. Universal Transverse Mercator, zone 14 N projection North American Datum of 1983. Freshwater/saline-water transition (1000 mg/L total dissolved solids concentration) from Schultz (1994); and (B) schematic conceptual north-northwest to south-southeast aquifer cross section and conceptual model (modified from Musgrove et al., 2014).

2007; Musgrove and Crow, 2012; Wong et al., 2012). Groundwater levels can rise within hours in response to rainfall and corresponding recharge, accompanied by increases in springflow; during periods of low rainfall and recharge, water levels and springflow decrease.

2.3. Characteristics of the Barton Springs and San Antonio segments

The SAS and BSS are parts of a large continuous aquifer system. A groundwater divide in Hays County separates the two segments and

Table 1
Summary of sample collection date, sites, and frequency.

Aquifer segment	Sampling dates	Sampling sites[a]	Sampling frequency
Barton Springs	Nov. 2008-Mar. 2010	Major recharging streams (n = 5)	Every 3–4 weeks, flow permitting[b]
	Nov. 2008-Mar. 2010	Groundwater wells (n = 2)	Every 3–4 weeks[b]
	Nov. 2008-Mar. 2010	Barton Springs (Main Spring orifice)	Every 3–4 weeks[b]
San Antonio	Jan. 2012	Rainfall	2x
	Oct. 2011 and Mar. 2012	Small ephemeral streams (n = 5)	2x
	Jan.-July 2012	Groundwater wells (n = 16)	1x
	Mar. 2012	Comal Springs (Comal 1 orifice)	1x

[a] Sample sites detailed in Table S1.
[b] Some samples also collected in response to recharge-generating storm events.

Table 2
Summary statistics for selected geochemical and isotopic constituents.

Sites[b]	Detail	Number of samples (unless noted)	Geochemical and isotopic constituents[a]							
			Specific Conductance, μs/cm		Dissolved Oxygen, mg/L		NO₃-N, mg/L[d]		NH₃-N, mg/L[d,e,f]	
			Median	(range)	Median	(range)	Median	(range)	Median	(range)
Barton Springs segment										
SW	Streams (n = 5)	104	656 (n = 116)	116–927	9.1 (n = 51)	4.4–14.6	0.60	<0.02–2.01	<0.02 (n = 90)	<0.02–0.146[c]
	Barton Creek	30	683 (n = 33)	604–736	8.4 (n = 20)	6.1–11.3	0.22	E0.01–0.87	<0.02 (n = 27)	<0.02–0.04[c]
	Onion Creek	14	603 (n = 16)	444–729	10.0. (n = 6)	7.4–10.6	1.06	0.84–2.01	<0.02 (n = 12)	<0.02–E0.015
	Bear Creek	24	695 (n = 25)	216–924	8.0 (n = 11)	4.4–10.8	0.90	<0.02–1.72	<0.02 (n = 21)	<0.02–E0.015
	Slaughter Creek	13	814 (n = 14)	628–927	10.2 (n = 6)	7.8–11.2	0.75	0.34–1.22	<0.02 (n = 12)	<0.02
	Williamson Creek	23	395 (n = 24)	116–840	10.8 (n = 8)	7.4–14.6	0.46	0.22–1.08	<0.02 (n = 18)	<0.02–0.146[c]
GW	Wells (n = 2)	42	606 (n = 44)	569–618	5.7 (n = 43)	4.3–8.2	1.19	1.05–2.44	<0.020	<0.020–0.036
Spring	Barton Springs	56	687 (n = 65)	564–735	4.9 (n = 65)	3.9–7.8	1.54	1.30–1.80	<0.020 (n = 53)	<0.020–E0.010
San Antonio segment										
Rainfall	USGS South TX office	2	23	19–27	–	–	0.33	0.20–0.47	0.562	0.388–0.735
SW	Bexar County streams (n = 5)	10	219 (n = 7)	110–439	8.2 (n = 5)	7.2–9.3	0.43	0.22–1.05	0.033	<0.010–0.053
GW	Wells (n = 16)	16	603	465–839	6.4	1.2–8.2	1.86	0.84–3.98	<0.010	<0.010–0.011
Spring	Comal Springs (Comal 1)	1	577	–	5.7	–	2.01	–	0.01	–

[a] Analyses are for filtered samples unless otherwise indicated; uf = unfiltered.
[b] SW = surface water; GW = groundwater; wells include monitoring, domestic, and public-supply wells.
[c] Detections occurred in samples collected during the dry period.
[d] Quantifiable concentrations less than the laboratory reporting level (LRL) were flagged as estimated (E).
[e] NH₃ measurement includes both the ammonium ion (NH₄) and unionized NH₃; at the measured pH values, reported concentrations of NH₃ predominantly represent NH₄.
[f] For most samples the measurement of organic N + NH₃ consists mostly of organic N, and are referred to as orgN in text (detection limits for organic N were often higher than for organic N + NH₃). NH₃ concentrations were generally small relative to organic N (excluding rainfall, for which concentrations of NH₃ were > organic N) and NH₃ analyses had much lower detection limits.

limits mixing between them under most hydrologic conditions (Fig. 1) (Smith et al., 2012). The SAS is the primary water source for the City of San Antonio, which has a population >1.3 million (U.S. Census Bureau, 2014). The SAS recharge zone covers about 3160 km² over 9 major watersheds. Major discharging springs in the SAS include Comal Springs, which is hydrologically isolated from large sources of local recharge (Musgrove and Crow, 2012). Long-term daily median discharge for Comal Springs is 8.58 m³/s (303 ft³/s) (1927–2013; U.S. Geological Survey, 2014). The BSS covers about 400 km² and is the primary water supply for about 60,000 people. Barton Springs is the main discharge point for the BSS, with a long-term daily median discharge of 1.73 m³/s (61 ft³/s) (1978–2013) (U.S. Geological Survey, 2014). As much as 85% of recharge to the BSS is provided by streamflow losses from 5 major streams: Barton, Williamson, Slaughter, Bear, and Onion Creeks (Slade et al., 1986; Hauwert, 2009) (Fig. 1). As demonstrated for the BSS (Wong et al., 2012) and consistent with streamflow losses that are the dominant recharge source, the geochemistry of recharging streams has a strong control on the geochemistry of the aquifer and its springs. Comal Springs and Barton Springs both are habitat for endemic and federally listed endangered species (U.S. Fish and Wildlife Service, 2015).

2.4. Potential nitrate sources to the Edwards aquifer

The numerous natural and anthropogenic sources of N species to groundwater include wet and dry atmospheric deposition; organic and synthetic fertilizers; human and (or) animal waste; decaying soil organic matter and soil mineralization processes; runoff from fertilized residential lawns, golf courses, and construction sites; and vehicle exhaust (Dubrovsky et al., 2010). Atmospheric sources include atmospheric N₂ converted to NO₃⁻ by lightning, compounds released to the atmosphere during plant decay, industrial emissions, and fossil-fuel combustion.

Fertilizers and human and (or) animal waste are the primary potential sources of anthropogenic NO₃⁻ to the Edwards aquifer. There is little agriculture in central Texas (land use detailed in Fig. S1) and what little agricultural land there is has decreased in recent years (detailed in Supplementary information). Because there is

little cultivated cropland in the contributing or recharge zone of either aquifer segment (Fig. S1, Table S1), agriculture is unlikely to be a notable source of fertilizer NO₃⁻. Land cover in the region is largely undeveloped forest and rangeland (grassland and scrub) and the developed large urban areas of Austin and San Antonio (Fig. 1 and Fig. S1). Fertilizers are used commonly in urban and residential landscapes, the extent of which is increasing as population grows. Between 2000 and 2010, the population of Travis and Hays counties combined increased by 30% and the population of Bexar County increased by 23%; the more rural western counties of the SAS that are upgradient of regional aquifer flow paths (Medina, Kinney, and Uvalde counties) had a combined population increase of 11% (U.S. Census Bureau, 2014). A recent analysis of residential and commercial fertilizer application over the BSS recharge zone estimated an annual load of up to about 42,500 kg of N (Turner, 2012).

Direct discharge of treated wastewater to streams that recharge the Edwards aquifer has not been permitted historically. Treated wastewater commonly is disposed of by spray irrigation or subsurface drip irrigation by Texas Land Application Permit (TLAP) facilities (Texas Commission on Environmental Quality, 2014), which are located primarily in the contributing zone (Ross, 2011). The estimated total TLAP daily flow of treated wastewater for 70 permitted facilities across the region (in 2011) is 0.25 and 0.14 m³/s (5.75 and 3.18 mgd) for the BSS and SAS, respectively, with the smaller BSS having a higher density of TLAPs (Ross, 2011). Septic systems (onsite sewage facilities; OSSFs) also are used for wastewater disposal. In the BSS, there are an estimated 10,000 OSSFs, which each treat as much as 19,000 L/day (5000 gal/day) of sewage (Herrington et al., 2010). In the SAS, permits for OSSFs are issued by a range of entities, and numbers there thus are difficult to estimate. Nonetheless, many of the communities in the San Antonio area that have undergone marked population growth are located on the city's north side and in the aquifer's contributing or recharge zone; for example, while San Antonio population grew by 16% between 2000 and 2010, growth in several north-side communities (Scenic Oaks, Fair Oaks Ranch, Helotes, Timberwood Park, and Boerne) (Fig. 1) exceeded 50% between 2000 and 2010 (U.S. Census Bureau, 2014). This population growth has been accompanied by increased amounts of wastewater requiring treatment by TLAPs and OSSFs; for example,

Organic N mg/L (uf)[d,f]		Organic N + NH$_3$ (uf), mg/L[d,e,f]		N$_{sum}$ (NO$_3^-$ and Organic N + NH$_3$), mg/L[e,f]		δ^{15}N (NO$_3$), ‰		δ^{18}O (NO$_3$), ‰	
Median	(range)	Median	(range)	Median	(range)	Median	(range)	Median	(range)
<0.39	<0.07–1.2	0.27	E0.07–1.90	1.02	0.07–2.71	10.65 (n = 47)	2.90–29.28	9.22 (n = 47)	6.27–35.96
<0.27	<0.13–0.31	0.24	0.13–0.58	0.56	0.19–1.10	10.92 (n = 10)	8.43–29.28	9.64 (n = 10)	7.52–19.90
<0.25	<0.12–E0.54	0.23	0.12–0.56	1.38	1.07–2.20	9.03 (n = 10)	7.56–10.65	7.19 (n = 10)	6.27–8.27
<0.58	<0.07–1	0.23	E0.07–1.90	1.35	0.07–2.71	10.56 (n = 8)	3.02–11.46	8.98 (n = 8)	7.88–24.14
<0.33	<0.15–0.44	0.33	0.15–0.57	1.06	0.73–1.55	13.75 (n = 9)	11.42–14.82	9.99 (n = 9)	8.77–10.61
<0.48	<0.12–1.2	0.45	0.12–1.60	0.96	0.58–2.06	9.45 (n = 10)	2.90–14.92	12.61 (n = 10)	8.50–35.96
<0.10 (n = 41)	<0.02–<0.21	<0.10 (n = 40)	<0.10–0.21	1.22 (40)	1.12–2.57	6.69 (n = 7)	5.05–7.01	5.7 (n = 7)	3.63–6.44
<0.10 (n = 52)	<0.05–<0.18	E0.08 (n = 53)	<0.10–0.18	1.62 (n = 53)	1.38–1.90	7.51 (n = 41)	5.34–8.84	5.45 (n = 41)	4.04–6.72
0.05	0.03–0.07	0.62	0.42–0.81	0.95	0.62–1.28	−0.35	−2.63–1.94	63.50	57.94–69.05
0.62 (n = 9)	<0.35–1.3	0.65	0.46–1.80	1.06	0.78–2.44	3.15	1.31–6.34	14.14	9.39–24.41
<0.07	<0.06–<0.1	<0.07	<0.07–0.10	1.86	0.94–3.98	7.77	5.46–10.21	5.41	3.69–6.91
<0.06	–	<0.07	–	2.08	–	6.8	–	5.66	–

about 45% of Fair Oaks Ranch residential properties use OSSFs for wastewater disposal (City of Fair Oaks Ranch, 2015).

3. Methods

3.1. Sample collection

Samples were collected during 2008–10 (BSS) and 2011–12 (SAS) from streams that recharge the aquifer, groundwater wells, and major discharging springs (Fig. 1, Tables 1, S1). In the BSS, samples were collected routinely (every 3–4 weeks at most sites, flow permitting) and in response to recharge-generating storm events. In the SAS, sites were sampled only 1–2 times, but a larger number of wells were sampled (16) than in the BSS (2). Most sites in the SAS were located in the urban San Antonio area. Rainfall samples associated with two winter storms in January 2012 were collected in the SAS. Stream samples were collected by both grab and autosampler methods in the BSS and by grab methods in the SAS. All of the streams sampled are ephemeral, so flow generally indicates recent rainfall, and samples are assumed to represent the quality of aquifer recharge. Watersheds in both aquifer segments span a large range of development (from <5 to >50%) (Table S1). The sampled spring orifices (Tables 1, S1) are referred to herein as Barton Springs (in the BSS) and Comal Springs (in the SAS), respectively.

3.2. Historical data

Available U.S. Geological Survey (USGS) historical data was compiled to augment data collected from 2008 to 2012. Multidecadal historical water-quality data, including NO$_3^-$ concentration, are available for Barton Springs and Comal Springs as far back as the 1930s (U.S. Geological Survey, 2014). Stream and spring discharge data (daily mean) for Barton and Comal Springs, the five recharging BSS streams, and one of the recharging SAS streams (Helotes Creek) were obtained from the USGS National Water Information System (NWIS; U.S. Geological Survey, 2014). Available historical (1983–1994) isotopic data for NO$_3^-$ (δ^{15}N) were also considered (Kreitler and Browning, 1983; City of Austin, 2010).

3.3. Sampling and analytical methods

Sample collection followed USGS guidelines and samples were analyzed by USGS laboratories using established analytical methods, summarized below; more detail is provided in Supplementary information. All analyses are for filtered samples unless otherwise indicated. Nutrient concentrations were analyzed at the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado. Measures of N-species are reported and discussed in units of mg/L as N. The NWQL reports either NO$_3^-$ or NO$_3^-$ + NO$_2^-$ (nitrate plus nitrite [NO$_2^-$]) (Patton and Kryskalla, 2011) and NO$_2$; NO$_3^-$ was computed as the difference between NO$_3^-$ + NO$_2^-$ and NO$_2^-$ where data were available. In general, NO$_2^-$ concentrations were negligible (<3% of NO$_3^-$ + NO$_2^-$ measurements) and NO$_3^-$ + NO$_2^-$ concentrations are discussed as NO$_3^-$. Organic N plus ammonia (orgN + NH$_3$, unfiltered) was measured by a Kjeldahl digestion method and an automated photometric finish (Patton and Truitt, 2000). NH$_3$ concentrations, as reported by the NWQL, comprise the ammonium ion (NH$_4^+$) and unionized NH$_3$. At the measured pH values, NH$_3$ is expected to be a minor component, and reported concentrations of NH$_3$ predominantly represent NH$_4^+$. For most samples, NH$_3$ concentrations were generally small relative to orgN (excluding rainfall, for which concentrations of NH$_3$ were > orgN) and NH$_3$ analyses had much lower laboratory reporting levels (LRLs) than orgN (Table 2); as a result, for most samples, excluding rainfall, the measurement of orgN + NH$_3$ predominantly represents orgN, and is referred to as such hereafter. Quantifiable concentrations less than the LRL were flagged as estimated ("E") by the NWQL and are considered herein at the estimated concentration. Isotopes of NO$_3^-$ (δ^{15}N and δ^{18}O) were measured by the USGS Stable Isotope Laboratory in Reston, Virginia, by continuous-flow isotope-ratio mass spectrometry of N$_2$O produced from NO$_3^-$ by bacterial reduction (Sigman et al., 2001; Casciotti et al., 2002; Coplen et al., 2004) using calibration data from Böhlke and Coplen (1995) and Böhlke et al. (2003). NO$_3^-$ isotopes were analyzed for selected samples from the BSS and for all samples from the SAS (Table 2). Quality-control results for blanks and replicates were considered acceptable and are discussed in Supplementary information.

3.4. Numerical and statistical methods

Nonparametric statistical tests were used for most data analysis (Helsel and Hirsch, 1992). Spearman's rho (R), a rank-based statistical test, was used to test for correlation. The Mann-Whitney *U* test was used to test for differences. Statistical results with p < 0.05 were considered significant and only results with p < 0.05 are reported.

The model LOADEST (Runkel et al., 2004) was used for a mass-balance of total N loads by estimating mean monthly loads of N species in BSS streams and Barton Springs discharge. LOADEST uses a time series of streamflow and measured constituent concentrations to develop a regression model for the estimation of constituents loads. A simple regression model with a single explanatory variable, log discharge, was used. Measured concentrations of NO_3^- and estimated rates of recharge for each stream and of measured discharge for Barton Springs were used for model calibration. Recharge rates were estimated using measured streamflow as described by Barrett and Charbeneau (1996), and do not account for other possible recharge sources, such as upland or interstream recharge. The application of LOADEST for the BSS is detailed in Mahler et al. (2011a).

4. Results

4.1. Hydrologic conditions

Climatic and hydrologic conditions in the BSS transitioned from exceptional drought (as defined by the U.S. Drought Monitor, 2011) to an extended period of wetter-than-normal during the BSS sampling period (Fig. 2). Extended dry conditions preceded the onset of sampling in November 2008, and discharge at Barton Springs had been below the long-term median for 8 months. From November 2008 to August 2009 (hereafter, the dry period), flow was absent or intermittent in the 5 recharging streams and daily mean discharge at Barton Springs averaged 0.51 m³/s (18 ft³/s) (U.S. Geological Survey, 2014). From September 2009 to March 2010 (hereafter, the wet period), conditions became increasingly wetter and discharge at Barton Springs increased to >2.55 m³/s (>90 ft³/s). The five recharging streams had continuous flow from within a month of the onset of wet conditions to the end of the sampling period.

Climatic and hydrologic conditions in the SAS were relatively dry during the SAS sampling period (October 2011–July 2012), but were not characterized by exceptional drought. Daily mean discharge at Comal Springs had been less than the long-term median for 7 months preceding the onset of sampling (Fig. 2). During the sampling period, Comal Springs discharge increased gradually from 5.27 to 8.13 m³/s (186 to 287 ft³/s) in early April 2012, then gradually decreased to about 5.38 m³/s (190 ft³/s).

4.2. Geochemical compositions

Results for concentrations of N species for samples from streams, wells, and springs for the two aquifer segments reflect consistent and systematic differences between aquifer recharge (samples from streams) and groundwater (samples from wells and springs) (Table 2). While NO_3^- concentrations in the streams (<0.02 to 2.01 mg/L) varied with hydrologic condition, they were generally lower than groundwater (0.84 to 3.98 mg/L). In contrast, concentrations of orgN were generally higher in streams (E0.07 to 1.90 mg/L) than in groundwater (<0.07 to 0.21 mg/L). Rainfall NO_3^- (median = 0.33 mg/L) and orgN (median = 0.62 mg/L) concentrations were similar to those for streams. Given that only two rainfall samples were collected, and that N species concentrations differed by 100% or more between them, these samples likely do not reflect the full variability of atmospheric N. NO_3^- concentrations were significantly higher in groundwater (wells and springs) from the SAS (median = 1.91 mg/L) relative to the BSS (median = 1.46 mg/L). OrgN groundwater concentrations for both aquifer segments
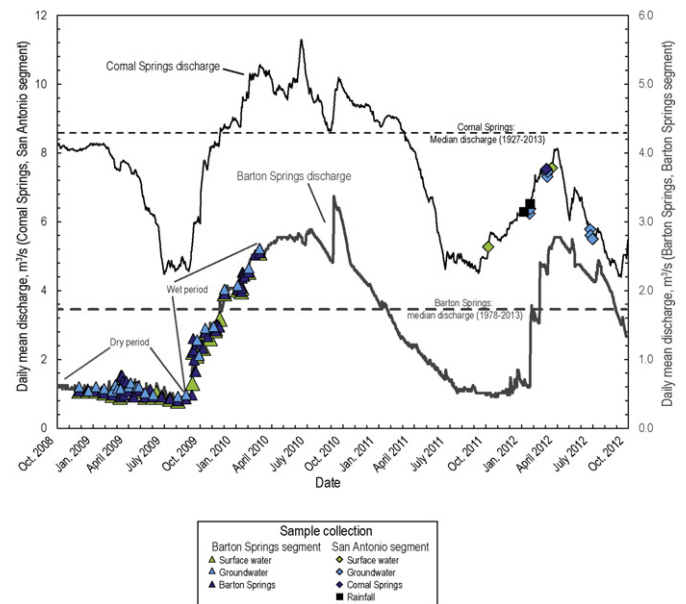
Fig. 2. Timeseries of discharge for Barton Springs (Barton Springs segment) and Comal Springs (San Antonio segment) showing hydrologic conditions and timing of sample collection. Discharge data available from the National Water Information System (NWIS) (U.S. Geological Survey, 2014) for USGS stations 08,155,500 (Barton Springs at Austin, Texas) and 08,168,710 (Comal Springs at New Braunfels, Texas).

frequently were less than the LRL (Table 2), however, the detection frequency was substantially higher for the BSS (67%) than for the SAS (6%).

Results for isotopes of NO_3^- are interpreted here relative to characteristic isotopic signatures or fields of values for common NO_3^- sources (Fig. 3) (Kendall et al., 2008). Rainfall samples were isotopically distinct from stream and groundwater samples, with low $\delta^{15}N$ values and elevated $\delta^{18}O$ values (median = −0.35‰ and 63.50‰, respectively). Stream samples, particularly from the BSS, had a large range of isotopic values. Samples with the highest $\delta^{15}N$ values were collected during the dry period from Barton Creek (Fig. 1). Samples with the highest $\delta^{18}O$ values (>20‰) also were collected during the dry period, mostly from Williamson Creek, in response to storm events (outside of response to several storm events, Williamson Creek did not flow during the dry period). Onion and Slaughter Creeks did not flow during the dry period. Isotopic ratios for groundwater and spring samples were less variable than stream samples. In the SAS, isotope results for stream samples generally were intermediate between rainfall and groundwater samples. Groundwater and spring samples in the SAS had isotopic compositions similar to those in the BSS, but included higher $\delta^{15}N$ values.

5. Discussion

5.1. Temporal trends in nitrate concentration

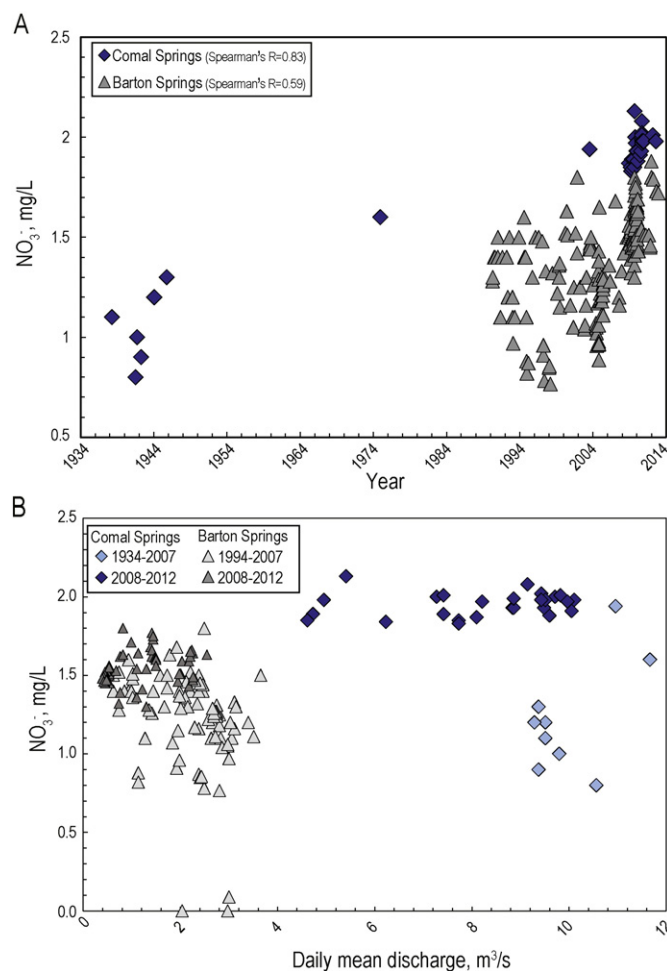NO_3^- concentrations at Barton Springs and Comal Springs (U.S. Geological Survey, 2014) have increased significantly over the period of record (Fig. 4a). The median NO_3^- concentration at Comal Springs in the late 1930s and 1940s was 1.1 mg/L (n = 7). This concentration is similar to the national background concentration of 1 mg/L estimated for groundwater (Dubrovsky et al., 2010), and likely reflects the concentration for the Edwards aquifer in the absence of anthropogenic influences. The NO_3^- concentration for Comal Springs discharge has since about doubled, with recent (2000 − 12) values around 2 mg/L (median = 2.0 mg/L, n = 27). The only measurement for NO_3^- for Comal Springs from 1950 through 2000 was 1.6 mg/L in 1974, so the data are insufficient to determine whether the increase was gradual or whether it occurred over a discrete period. At Barton Springs, in contrast, NO_3^- concentrations underwent a step increase in about 2008 (Mahler et
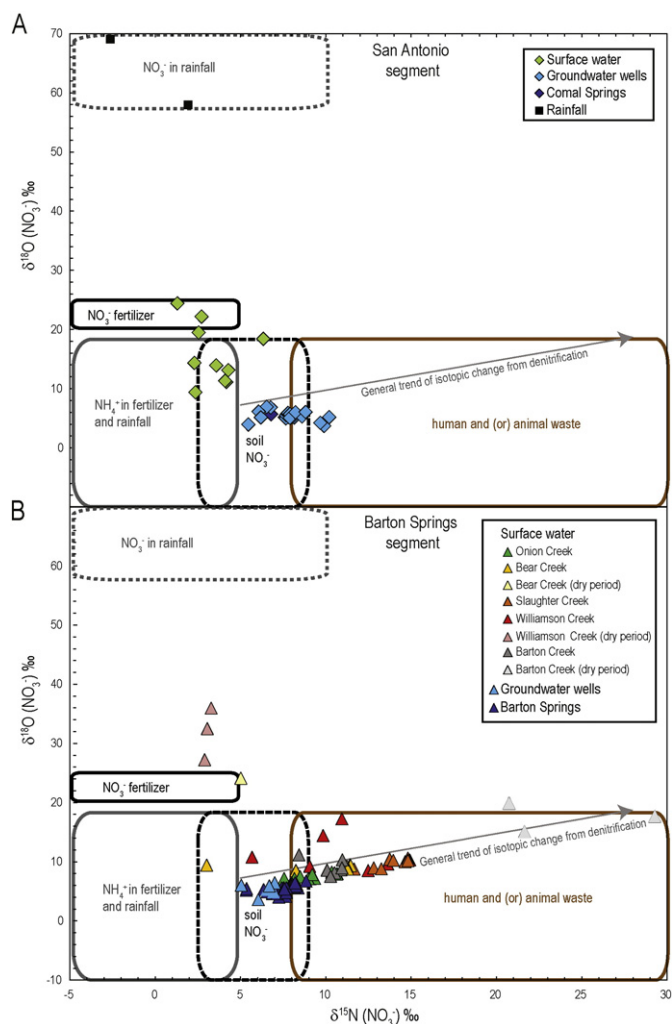
Fig. 3. The isotopic composition of NO_3^- for samples collected from A) the San Antonio segment and B) the Barton Springs segment. Boxes show typical ranges in composition of NO_3^- sources, modified from Kendall et al. (2008). Ranges do not reflect specific central Texas sources but a compilation of measured values from numerous studies. Ranges for some sources overlap.

Fig. 4. Historical and recent NO_3^- concentrations for discharge from Barton and Comal Springs relative to A) year and B) spring discharge. Discharge values are daily mean values for USGS stations 08,155,500 (Barton Springs at Austin, Texas) and 08,168,710 (Comal Springs at New Braunfels, Texas).

al., 2011b). NO_3^- at Barton Springs has been measured by the USGS since 1990, and concentrations have increased from a median of 1.30 mg/L for 1990–95 (n = 28) to 1.54 for 2008–12 (n = 68) (Fig. 4a). Prior to 2006, NO_3^- concentrations often approached but rarely exceeded 1.5 mg/L (Mahler and Garner, 2009); in contrast, NO_3^- concentrations from 2008 to 2012 exceeded 1.50 mg/L in 63% of samples. Although these concentrations are relatively low from the perspective of human health (the U.S. Federal standard for NO_3^- in drinking water is 10 mg/L; U.S. Environmental Protection Agency, 2016), NO_3^- concentrations >2.5 mg/L adversely affect some amphibians (Rouse et al., 1999), and a continued increase in NO_3^- concentrations in the Edwards aquifer might be of concern for endemic species.

NO_3^- concentrations at Barton Springs vary more over short time-scales than at Comal Springs, likely because the BSS is smaller than the SAS. The geochemistry of Barton Springs discharge is more directly affected by changes in hydrologic conditions, from individual storms (Mahler and Massei, 2007) to multiannual wet-dry cycles (Wong et al., 2012), than is the geochemistry of Comal Springs (Musgrove and Crow, 2012). During 2008–10, increases in NO_3^- in the BSS following the onset of wet conditions (Mahler et al., 2011a) are consistent with reports that rewetting of soils following a drought likely results in an enhancement of mineralization and nitrification processes (Lucey and Goolsby, 1993; Reynolds and Edwards, 1995). In contrast, even with

large changes in discharge over wet-dry cycles (such as 2008–2012) (Fig. 2), the geochemistry of Comal Springs remains relatively stable (Fig. 4b), which likely reflects the dominance of discharge supplied by regional flow paths (Musgrove and Crow, 2012). Historical increases in NO_3^- concentrations for both Barton and Comal Springs, however, have occurred for a range of spring discharges (Fig. 4b), indicating that variations in hydrologic conditions and flushing of rewetted soils cannot account for the observed increases in NO_3^-.

In the BSS, the increase in NO_3^- concentrations at Barton Springs corresponds to an increase in NO_3^- in recharging streams. NO_3^- concentrations in samples from the five major recharging streams have increased in recent years (2008–12) across a range of flow conditions relative to historical values from 1993 to 2007 by as much as a factor of 8 (Fig. S2). There are insufficient historical data for the SAS streams to evaluate changes in NO_3^- concentrations for recharging streams, although a recent study of five streams in the San Antonio area reported median NO_3^- concentrations between 0.29 and 0.59 mg/L (1997–2012) (Opsahl, 2012), which are within the range of recent (2008–12) median values for BSS streams.

In spite of recent increases in NO_3^- concentration in many BSS streams, median stream NO_3^- concentrations for both aquifer segments remain less than groundwater concentrations (Table 2). A comparison between recent and historical values, however, indicates that the concentration difference between recharging streams and groundwater has decreased, in turn decreasing the degree of dilution of NO_3^- in groundwater by stream recharge. At Barton Springs, prior to 2008

NO_3^- concentrations decreased with increasing spring discharge, but in 2008 this relation reversed, indicating that recharge no longer substantially dilutes the NO_3^- concentration in groundwater (Mahler et al., 2011b).

5.2. Sources of nitrate to groundwater

5.2.1. Isotopic indicators of nitrate source

NO_3^- concentration and isotopic composition of rainfall, stream, and groundwater samples provide insight into sources that contribute N species to the Edwards aquifer. Rainfall might contribute a substantial proportion of the total NO_3^- in central Texas streams and groundwater. Measured rainfall NO_3^- concentrations (median = 0.33 mg/L) were only moderately lower than in streams (median = 0.60 and 0.43 mg/L for the BSS and SAS, respectively; Table 2). There are few additional available data for rainfall NO_3^- concentrations in the region and, to our knowledge, no previous measurement of NO_3^- isotopes in Texas rainfall. The isotopic composition of NO_3^- in precipitation can be highly variable, both seasonally and during individual storm events (Buda and DeWalle, 2009), but is generally distinguished by high $\delta^{18}O$ and relatively low $\delta^{15}N$ values (Kendall et al., 2008). Rainfall samples collected for this study are within the range of typical isotopic values (Fig. 3) and also within the range of NO_3^- concentrations in precipitation from sites (n = 14) (1991–2010) across the country (0.1 to 1.6 mg/L; Lajtha and Jones, 2013).

A large range of isotopic values were measured in streams, which likely integrate a variety of NO_3^- sources (Fig. 3b). The isotopic composition of SAS surface-water samples is in the range typical of fertilizer and soil NO_3^-. Most BSS stream samples collected during the wet period trended from the boundary between soil NO_3^- and human and (or) animal waste into the human and (or) animal waste NO_3^- field, which indicates contributions from both sources. The general relation between $\delta^{15}N$ and $\delta^{18}O$ values for many of these samples follows the trend line for denitrification (i.e., the microbially mediated process of NO_3^- reduction; Kendall et al., 2008) and stream samples with higher isotopic values had low NO_3^- concentrations, which also is indicative of denitrification (Fig. 5). Denitrification, however, requires anoxic conditions (Knowles, 1982), whereas measured stream dissolved oxygen (DO) concentrations ranged from 4.4 to 14.6 mg/L. A component of stream water might nonetheless be affected by denitrification, which can occur intermittently in anoxic microsites within an otherwise oxygenated soil, sediment, or water body (Koba et al., 1997). Samples with the highest $\delta^{15}N$ values (>20‰) were collected from Barton Creek during the dry period and were not associated with rainfall events. Return flow from wastewater irrigation of a golf course upstream from the sampling site might be the source of much of the low but sustained dry-period flow in this stream, and the corresponding isotopic signature might result from a combination of wastewater and denitrification that occurred during wastewater treatment of irrigation water or in holding ponds with anoxic conditions.

Groundwater from both aquifer segments had a narrower range of isotopic compositions than the streams providing aquifer recharge (Fig. 3). Because relatively little aquifer recharge occurs during dry hydrologic conditions when stream flows are low to nonexistent, the isotopically distinct composition of stream water during the dry period had little effect on the groundwater isotopic composition. Within a month of the onset of wet conditions in 2009, the majority of Barton Springs discharge was composed of stream recharge (Wong et al., 2012). Onion Creek was estimated to account for 40% of stream recharge during the wet period (Mahler et al., 2011a), and the isotopic composition of BSS groundwater is similar to that of Onion Creek samples; both are within the range of soil NO_3^- and trend into the range of human and (or) animal waste NO_3^-.

A shift in $\delta^{15}N$ toward higher values in both aquifer segments for recent samples indicates an increased contribution of NO_3^- from human and (or) animal waste. Values of $\delta^{15}N$ measured during 1994–2000 for
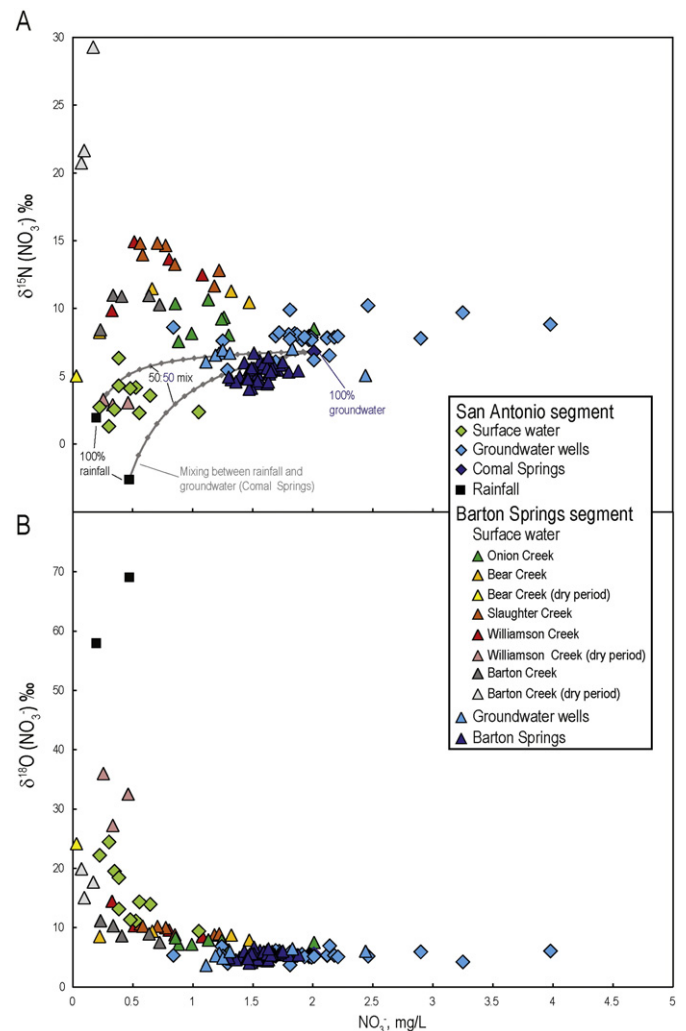

Fig. 5. Relation between NO_3^- concentration and N isotope composition—A) NO_3^- and $\delta^{15}N$ (NO_3^-), and B) NO_3^- and $\delta^{18}O$ (NO_3^-). Model curves in A illustrate mixing between samples of rainfall and Comal Springs discharge; model calculations follow Mariotti et al. (1988).

groundwater samples from wells and springs in the BSS range from 0.3 to 10.7‰ with a median value of 4.0‰ (n = 30) (City of Austin, 2010). $\delta^{15}N$ values measured for BSS groundwater during 2008–10 are significantly higher (5.05–8.84‰, median of 7.40‰ [n = 48]); if only samples from Barton Springs are considered, recent values also are significantly higher than historical values (Fig. S3). Recent (2011 − 12) $\delta^{15}N$ values for SAS groundwater also are significantly higher than historical $\delta^{15}N$ values (Kreitler and Browning, 1983), regardless of whether all samples across the regional aquifer are considered or only samples from Bexar County (Fig.S3). Although these comparisons are subject to uncertainty because of variations in analytical methods and, with the exception of Barton Springs, because the same sites were not sampled, the similar shift to higher $\delta^{15}N$ values for both aquifer segments is consistent with an increased NO_3^- contribution from human and(or) animal waste.

5.2.2. Land-use change and nitrate sources

Is urban development contributing to recent increases in NO_3^- in the aquifer (Fig. 4) and recharging streams (Fig. S2)? Here we consider potential anthropogenic sources of NO_3^- in the BSS (there are fewer data are available to quantify potential anthropogenic sources of NO_3^- in the SAS, but similarities with the BSS in population increases and waste disposal methods indicate that sources are likely similar). The notable increase in NO_3^- concentrations in the BSS indicates that urban

development, in particular in the contributing zone, might be a contributing factor. Given that isotopic ratios for N and O isotopes of NO_3^- point to a human and (or) animal waste source, we considered potential sources of human and (or) animal waste: pet waste (domesticated dogs and cats), septic systems (OSSFs), and land application of treated wastewater (TLAPs). Although leaking infrastructure for centralized sewage treatment is a potential source of N species to surface and groundwater, centralized sewage treatment in the BSS contributing zone is limited to the Williamson Creek watershed, where it was installed relatively recently (Mahler et al., 2011b). The amount of leakage from this type of infrastructure, therefore, is assumed to be small and unlikely to be the source of the increase in NO_3^- concentrations. Additionally, although animal wastes from livestock are a potential source of NO_3^- to surface water and groundwater, as urban development has increased in the BSS, agricultural land use, including livestock, has decreased (detailed in Supplementary information). Livestock operations therefore also are unlikely to be the source of the increase in NO_3^- concentrations. An increase in population is accompanied by an increase in dogs and cats, whose feces are a potential source of NO_3^- (Tota-Maharaj and Scholz, 2010). A detailed evaluation of the numbers of domesticated dogs and cats and the timing of their population changes as a potential NO_3^- source concluded that pet waste is not a major factor driving the increase in NO_3^- concentrations in the BSS (Herrington et al., 2010; Mahler et al., 2011b).

Currently (2016), all wastewater disposal in the BSS contributing zone, with the exception of the Williamson Creek watershed, is by OSSFs or TLAPs (although one permit has been granted for wastewater discharge into a contributing zone stream, no direct discharges have yet occurred (as of early 2016). Although OSSFs and land application of treated wastewater do not involve intentional discharge to surface water, overloaded OSSF drain fields will flood discharging sewage to the ground surface (U.S. Environmental Protection Agency, 2005), and runoff can occur from land application facilities if the infiltration capacity of the soil is exceeded (Alberta Environment, 2000). Additionally, infiltration from both septic systems and land application can affect groundwater, which in turn can discharge into streams during base flow conditions (Stelzer et al., 2010). For example, a study of sprayfield irrigation with treated municipal wastewater in the karstic Upper-Floridan aquifer documented increased NO_3^- concentrations at a downgradient spring (Katz et al., 2009). Additionally, discharges to streams or the ground surface may result from wastewater spills, which are more likely to occur in urban areas, or overloaded OSSF drainfields. For example, more than 3 million liters of wastewater were spilled in the recharge zone in the SAS during 2004–12, primarily in Bexar County (Geotex Environmental Solutions, 2012). Septic-system density for the BSS was determined on the basis of OSSF permits (Herrington et al., 2010) in the BSS and its contributing zone. Septic-system density has increased markedly in the BSS (Fig. 6) and is a likely source of the increase in NO_3^- concentrations. TLAP facilities use either surface (spray) irrigation or subsurface drip irrigation of treated effluent. The density of TLAP facilities has also increased markedly in the BSS (Fig. 6), and the timing of the permitted irrigation rate (volume per unit area per time) of treated wastewater effluent is consistent with the increase in NO_3^- concentrations in the Barton, Onion, and Bear Creek watersheds (Mahler et al., 2011b). Increases in both OSSFs and TLAPs indicate that urban development on the contributing zone is a likely source of NO_3^- in the BSS.

5.2.3. Isotopic indicators of land-use change

The isotopic composition of rainfall, stream, and groundwater samples also provides insight into the contribution of urban development to changes in N dynamics in the Edwards aquifer. We hypothesize that the NO_3^- concentration and isotopic composition of most stream samples is controlled by mixing of NO_3^- from rainfall and soils and by effects of land use. Some stream-water sample compositions were intermediate between rainfall and groundwater, with low $\delta^{15}N$ values but

relatively high $\delta^{18}O$ values. These include samples collected from BSS streams in response to storms during the dry period, particularly those from Williamson Creek (Fig. 3b), and samples from the SAS streams, which also were collected in response to storms (Fig. 3a). These compositions reflect mixing of NO_3^- from rainfall and from soils contributed to streams via runoff. A mass-balance mixing model of rainfall and groundwater samples, representing atmospheric deposition and soil NO_3^-, accounts for the composition of these stream samples (Fig. 5a). The Williamson Creek watershed is the most developed (Table S1) and has the highest median $\delta^{18}O$ value of BSS streams. The median $\delta^{18}O$ value for BSS streams is correlated (Spearman's $R = 0.90$) with the percentage of developed land in the stream watersheds (Fig. S4). In the SAS, the watershed with the highest proportion of developed land (Lorence Creek, Fig. 1, Table S1) had the highest median $\delta^{18}O$ value but the relation for sampled SAS streams was not statistically significant (Fig. S4). These streams were sampled only twice, however, so the lack of correlation might reflect the small number of samples. Several other studies have reported that urban streams have elevated $\delta^{18}O$ values, particularly in response to storms, reflecting an increased component of atmospheric NO_3^-, likely because high amounts of impervious cover limit contributions of NO_3^- from other sources (Chang et al., 2002; Divers et al., 2014); this effect has been previously noted in central Texas streams (Silva et al., 2002). Runoff from impervious cover also would likely have relatively low concentrations of NO_3^-. Stream samples with the highest $\delta^{18}O$ values, all sampled in response to storms, had relatively low NO_3^- concentrations (Fig. 5b), consistent with a relatively large component of lower-NO_3^- rainfall and runoff from impervious cover.

5.2.4. Shallow groundwater

For groundwater samples from wells in the SAS, both NO_3^- concentration and $\delta^{15}N$ values generally decrease with depth (Fig. 7). Although a decrease in NO_3^- concentration with depth or along flow paths commonly is attributed to denitrification, denitrification results in a corresponding increase in $\delta^{15}N$ values (Mariotti et al., 1988), which is not observed in the oxic SAS. We propose, similar to Musgrove et al. (2014) that shallower and younger groundwater affected by anthropogenic N sources accounts for observed changes with depth in NO_3^- and $\delta^{15}N$ values. The deepest groundwater wells (>1000 ft) have NO_3^- concentrations that are similar to historical values at Comal Springs from the early 1900s (Fig. 4) and to the national background value of 1 mg/L (Dubrovsky et al., 2010); $\delta^{15}N$ compositions in samples from these deep wells are in the range of a natural soil source (Fig. 3). These results highlight the observed temporal trends in both NO_3^- concentration and isotopic composition, which has predominantly affected shallower and younger groundwater; recent increased NO_3^- loading has, to date, had little effect on deeper and likely older groundwater.

5.3. Nitrogen speciation

5.3.1. Nitrification

Many studies of NO_3^- and its isotopes have documented decreases in aquifer NO_3^- with depth or along flow paths as a result of denitrification (Korom, 1992), but within the Edwards aquifer, oxic groundwater conditions preclude denitrification as an important process. Conversely, nitrification—the multistep, microbially mediated conversion of orgN to NO_3^-—occurs in oxic conditions and is an important transformation process for soils and aquatic systems, providing a NO_3^- source. Higher concentrations of orgN in streams that recharge the aquifer than in groundwater (Table 2) indicate that nitrification might be occurring within the aquifer. OrgN was detected in all stream samples in both aquifer segments at concentrations as high as 1.90 mg/L, but was detected in only about one-half of groundwater samples from the BSS, and in only one groundwater sample from the SAS (0.10 mg/L) (Table 2).The inverse relation between orgN and NO_3^- (Spearman's R = −0.58) also is consistent with nitrification (Fig. 8). Nitrification is a recognized
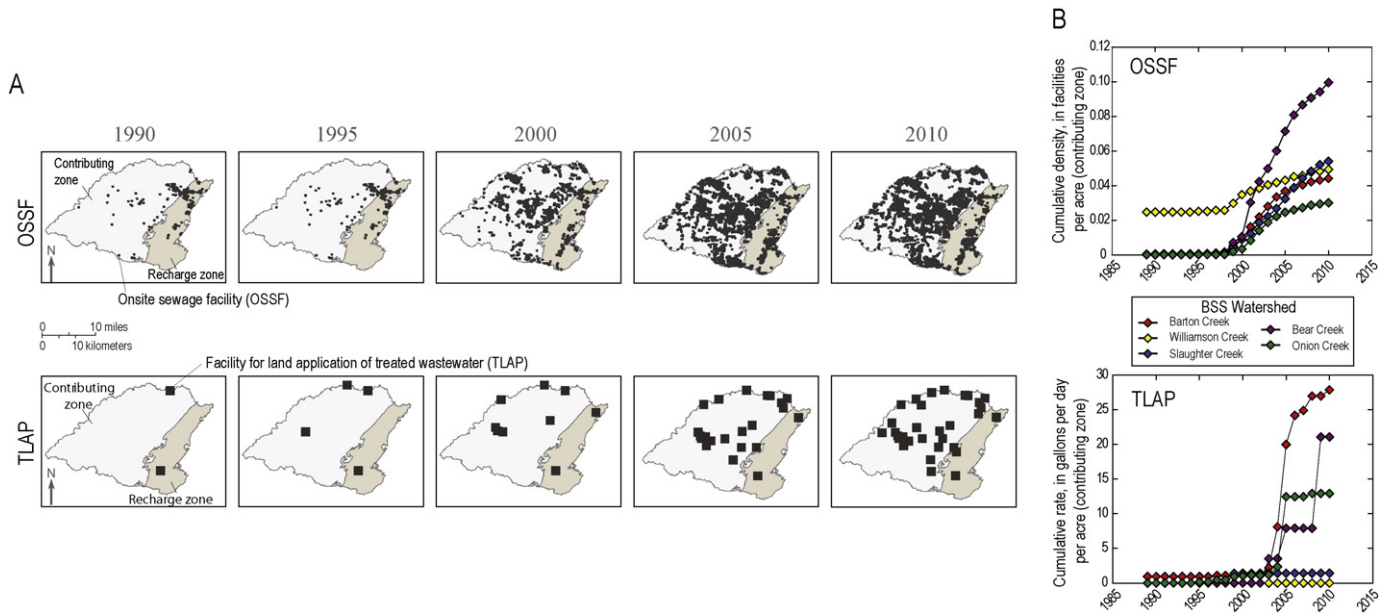
Fig. 6. A) Changes in the number of onsite sewage facilities (OSSFs) (septic systems) and permitted facilities for land application of treated wastewater (Texas Land Application [TLAP] Permit system) in the contributing and recharge zones of the Barton Springs segment (Mahler et al. (2011b). B) Changes by watershed in the contributing zone (Mahler et al., 2011b).

source of NO_3^- in soils (De Boer and Kowalchuk, 2001; Stoewer et al., 2015), streams (Mayer et al., 2002; Burns et al., 2009), and marine water (Wankel et al., 2007), but is not generally invoked as a process occurring within a groundwater system to provide a source of NO_3^-. Yet nitrification of orgN in the Edwards aquifer can account for both the elevated concentration of orgN in streams relative to groundwater and the dominant occurrence of N as NO_3^- in groundwater.

Because recharge is dominantly rapid and occurs primarily by focused streamflow losses to the Edwards aquifer, nitrification likely occurs within the aquifer rather than in overlying soils. Nonetheless, some interstream and diffuse recharge contributes to the aquifer (estimates of recharge outside of the major recharging streams, which includes focused recharge through interstream sinkholes and recharge features in tributaries as well as diffuse recharge, range from ~15–35%; Slade et al., 1986; Hauwert, 2009), which might contribute NO_3^- from soils directly. From a mass-balance perspective, diffuse recharge

would require a NO_3^- concentration ranging from about 3 to >7 mg/L), markedly higher than that measured in streams, to account for measured groundwater NO_3^- concentrations. The relatively few data available indicate that NO_3^- concentrations in upland recharge and vadose groundwater are not substantially higher than those in stream recharge or in phreatic groundwater. For example, Wong et al. (2012) reported the median NO_3^- concentration of upland recharge to be 0.75 mg/L (n = 26). Data for vadose groundwater (cave dripwaters) from a cave in the contributing zone in Comal county are similar, with a median NO_3^- concentration < 1 mg/L (n = 103) (Guilfoyle, 2006).

Concentrations of DO in streams and groundwater support the hypothesis that nitrification is occurring within the aquifer. Edwards aquifer groundwater DO concentrations are generally lower than in recharging streams by several mg/L (Table 2), consistent with consumption of DO by nitrification. The DO concentration has been reported to have decreased historically (1975–2014) at Barton Springs (Porras, 2014), although no trend was reported for a shorter period (2006–12) (Mahler and Bourgeais, 2013). One mole of NO_3^- produced
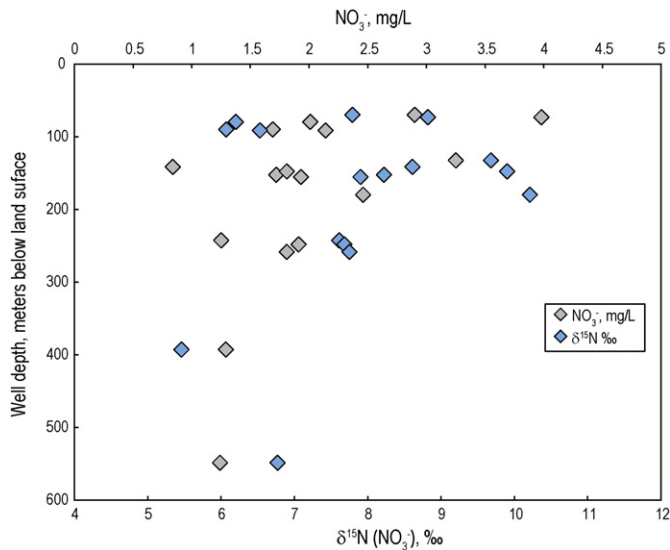
Fig. 7. Relation between well depth and NO_3^- concentration and $\delta^{15}N$ values for samples from groundwater wells in the San Antonio segment.
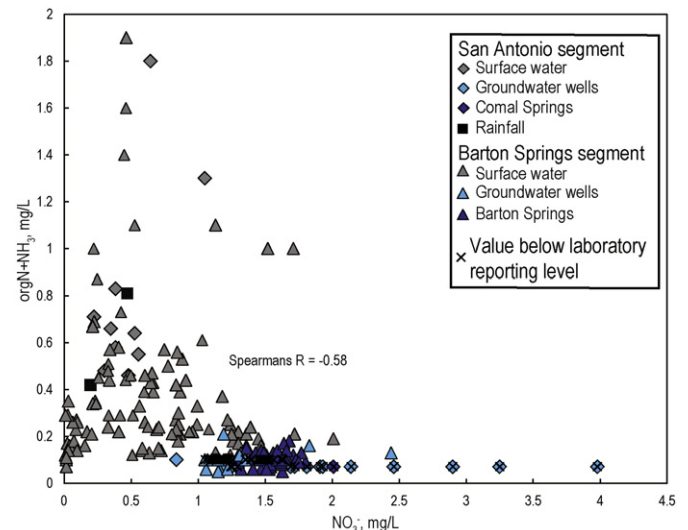
Fig. 8. Relation between orgN (orgN + NH_3) and NO_3^- concentrations.

by nitrification would consume 2 mol of DO; the ~1 mg/L increase in median NO_3^- concentrations from BSS streams to Barton Springs, if attributed to nitrification, would consume ~1 mg/L DO (as O_2). Other factors might also affect groundwater DO concentrations and contribute to lower concentrations in groundwater relative to surface water. Oxidation of orgN presumably would co-occur with oxidation of organic carbon, which also would consume DO. Available data for total organic carbon (TOC) for the Edwards aquifer indicate that TOC concentrations are higher in recharging streams (median for BS streams from 2008 to 12 = 5.6 mg/L [n = 28]) than in groundwater (median for BS discharge from 200812 = 0.7 mg/L [n = 13]), consistent with this hypothesis.

5.3.2. Nitrogen balance

We investigated the hypothesis that the sum (N_{sum}) of NO_3^- (NO_3^- + NO_2^-) and orgN (orgN + NH_3) in recharge from streams might account for the majority of aquifer N for the BSS and that OrgN is being nitrified to NO_3^- in the aquifer with LOADEST, a statistical load estimation model. Model results indicate that the cumulative load during 2008–10 of N_{sum} in BSS recharging streams (162 kg/d) and in Barton Springs discharge (157 kg/d) were similar, but that the timing of loading and N-species loads differed (Fig. 9). During the dry period, when recharge was low, the N_{sum} load in stream recharge was negligible, and N_{sum} loads in Barton Springs discharge indicate the release of N stored in the aquifer. During the wet period, N_{sum} loads from stream recharge exceeded those in Barton Springs discharge, accompanied by increases in aquifer water levels, indicating storage of recharge and the N it contained within the aquifer. Further, OrgN makes up about 24% of cumulative N loading in recharge during the wet period, but only 7% of N loading in discharge. We hypothesize that the balance between modeled N loads in aquifer recharge and discharge in the BSS and the conversion of OrgN to NO_3^- over the dry and wet periods comprised by the study period is an example of a long-term pattern given that alternation of drought and wet conditions is typical of the region (Griffiths and Strauss, 1985; Wong et al., 2012). While there are uncertainties not accounted for by the LOADEST model, including quantity of recharge (e.g., interstream recharge sources) and discharge (e.g., other springs and withdrawals from wells), the balance between modeled N loading in recharge and discharge (within 3%) supports the hypothesis that the majority of N_{sum} in discharge is accounted for by recharge from streams, and that nitrification is occurring within the aquifer. Although sufficient time-series data are not available for the SAS to estimate monthly loads for streams or spring discharge, similar processes likely are occurring in the SAS, given that N-species concentrations for recharging streams and groundwater are similar in the two aquifer segments.

6. Conclusions and implications

An analysis of NO_3^- concentrations and isotopic compositions for two adjoining segments of the Edwards aquifer in south-central Texas provides regional-scale insights on sources, variability, and transformation of N species in this productive karst resource. NO_3^- concentrations in the Edwards aquifer are increasing, likely in response to increased loading of N species from anthropogenic sources. The rate of change, however, is different in the two aquifer segments. NO_3^- concentrations in the BSS have increased by about 20% since the 1990s, with the most notable increase occurring in the 2000s. The increase in NO_3^- concentrations for recharging streams in the BSS is coincident with increases in the density of OSSFs and TLAPs, whereas changes in the SAS have occurred over a longer period (NO_3^- concentrations in the SAS have doubled since the 1930s, though the timing of the increase is uncertain because of a lack of data between 1950 and 2000). The increase in NO_3^- concentrations in the Edwards aquifer cannot be accounted for by changes in hydrologic conditions. Although NO_3^- concentrations remain low relative to the U.S. federal drinking water standard (10 mg/L; U.S. Environmental Protection Agency, 2016), there might be implications for endangered species associated with the aquifer, in particular regarding potential associated decreases in DO. Many aquatic species are sensitive to concentrations of DO, and Woods et al. (2010) demonstrated that an endangered salamander (*Eurycea sosorum*) endemic to Barton Springs is adversely affected by concentrations of DO less than 4.4 mg/L. A study of small central Texas streams receiving wastewater effluent documented that streams receiving effluent had relatively high amounts of nutrients and low DO (Mabe, 2007). While effects from high NO_3^- concentrations have not been specifically documented for endangered species associated with the Edwards aquifer, concentrations >2.5 mg/L adversely affect some amphibians (Rouse et al., 1999). The area contributing recharge to the BSS is undergoing rapid growth accompanied by increased generation of wastewater, which is a source of N to the aquifer. In the San Antonio area, population growth and urbanization also are likely contributing to increased NO_3^- concentrations in the SAS. The isotopic signature of NO_3^- and comparison with historical $\delta^{15}N$ values in both aquifer segments indicates that human and (or) animal waste is a source of some of the NO_3^- in groundwater. Consistently lower NO_3^- concentrations associated with deeper groundwater wells in the urban San Antonio area of the SAS indicate that young, recently recharged groundwater is particularly vulnerable to NO_3^- contamination. A mass-balance of NO_3^- loads for the BSS indicates that stream recharge can account for the majority of N in the aquifer, and supports the hypothesis that nitrification within the aquifer of orgN from aquifer recharge might be a source of NO_3^- to Edwards aquifer

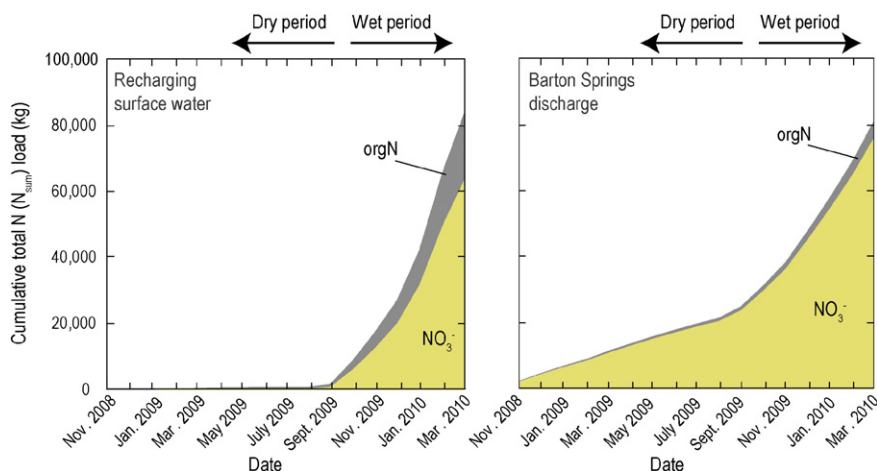**Fig. 9.** Cumulative N load (the sum of orgN + NH_3 and NO_3^- + NO_2^-) in stream recharge in the Barton Springs segment and Barton Springs discharge, estimated with LOADEST (Runkel et al., 2004).

groundwater. Additional future approaches to further substantiate the hypothesis of subaqueous nitrification include documenting the presence of nitrifying bacteria in the aquifer using molecular techniques, demonstrating active microbial decomposition of dissolved organic matter in laboratory studies, and focusing new field methods on the interstitial zones of the soil-aquifer interface. Results of this study have implications for other oxic groundwater systems, and particularly for karst aquifers with direct recharge through karst features, where orgN in aquifer recharge may be an unrecognized but potential source of NO_3^-.

Supplementary information as noted in the text contains additional figures and information including details regarding land use, sampling and analytical methods, and quality-control results. Supplementary data associated with this article can be found in the online version, at [doi:10.1016/j.scitotenv.2016.05.201].

Acknowledgements

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