



Determining sources of nitrate in the semi-arid Rio Grande using nitrogen and oxygen isotopes



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ABSTRACT

The Rio Grande, a semi-arid river in the American Southwest, is a major source of surface water for agriculture and municipal purposes in New Mexico and western Texas. In addition to increasing salinity, considerable increases in nitrate (NO_3^-) concentrations have been observed in the semi-arid portion of the Rio Grande. It is possible that elevated water salinity inhibits denitrification on irrigated fields and, thus fails to mediate the excess nutrient load from anthropogenic activities. Therefore, the two major goals of this study were to i) characterize and quantify major NO_3^- sources, and ii) assess whether elevated water salinity affects microbial denitrification in the watershed. In fall 2014 and summer 2015, the Rio Grande surface water, irrigation drains, precipitation (urban runoff), and municipal waste effluents were sampled between Elephant Butte, New Mexico and Tornillo, Texas (~260 km distance) for chemical and stable isotope analyses. The highest NO_3^- concentrations, up to ~70–140 mg/L, were observed in waste effluents and agricultural drains irrigated with the reclaimed city water. Conversely, NO_3^- concentrations in the river and agricultural drains were significantly lower (<1–10 mg/L) in the areas farther away from urban centers. Two major NO_3^- sources were identified using isotope tracers: fertilizers, with low $\delta^{15}\text{N}$ and high $\delta^{18}\text{O}$ (average + 0.6 and + 18.3‰, respectively), and waste water effluents from cities, with high $\delta^{15}\text{N}$ and low $\delta^{18}\text{O}$ (average + 10.5 and –5.1‰, respectively). According to nitrogen isotope mass balance constraint, the contribution of waste effluent-derived NO_3^- was the smallest in upstream locations, between Elephant Butte and Las Cruces, and accounted for up to 0–25% ($\pm 10\%$) compared to the fertilizer-derived NO_3^- . Further downstream near big urban centers, the effluent contributions increased and accounted for up to 70–100% between Las Cruces and El Paso. The highest effluent-derived NO_3^- contributions of 90–100% were measured in the agricultural district located below El Paso where the reclaimed city water is commonly used for irrigation. Elevated salinity does not appear to limit microbial denitrification. Locally, the strongest isotopic evidence of microbial denitrification was observed in a couple of water samples showing elevated salinity (EC 2.9–4.2 mS/cm). The results of this study suggest that urban centers are important NO_3^- contributors into the aquatic system of the semi-arid Rio Grande watershed, and that microbial processes (e.g., denitrification) do not appear to significantly reduce NO_3^- loads from anthropogenic sources.

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1. Introduction

The Rio Grande is an important source of water for agriculture and urban centers in the semi-arid American Southwest (Fig. 1; Ellis et al., 1993). Several factors such as increasing population, overuse of water resources for irrigation, and climate change affect water quality in the Rio Grande by increasing its salinity and nitrate (NO_3^-) concentrations (Oelsner et al., 2007; Hogan et al., 2007; Borrok and

Engle, 2014; Szykiewicz et al., 2015). This is a concern as it threatens water quality and availability for agricultural and municipal users. In New Mexico and west Texas, the main sources of NO_3^- in the semi-arid Rio Grande are likely agricultural fertilizers and wastewater effluents from large cities in Albuquerque, Las Cruces and El Paso (Oelsner et al., 2007; Szykiewicz et al., 2015). Minor amounts of NO_3^- might be also delivered through atmospheric deposition (Elliott et al., 2007). Despite this general understanding, there are no detailed quantitative studies characterizing major NO_3^- sources into the Rio Grande and its irrigation network of canals and drains.

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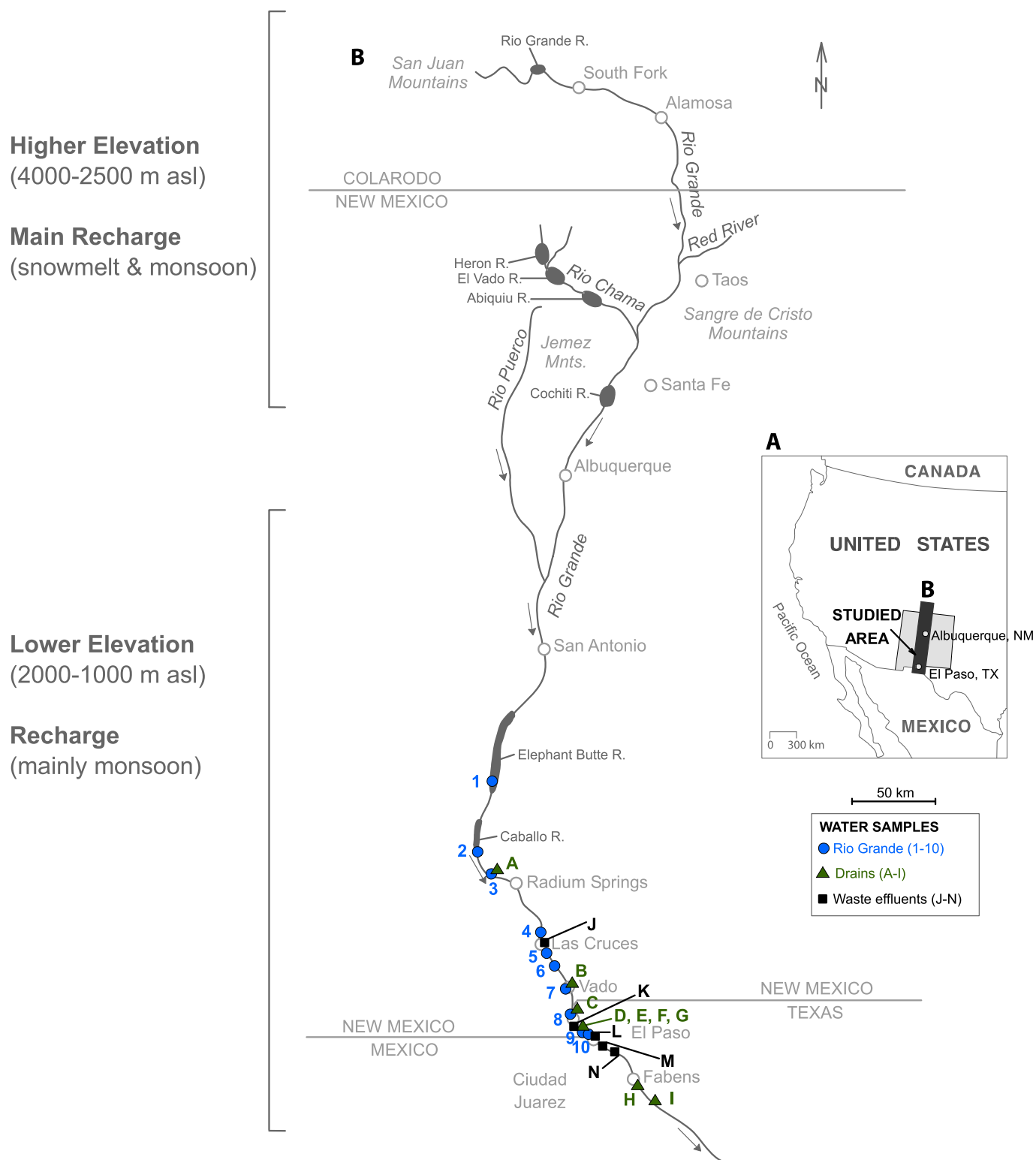


Fig. 1. Location map of water sampling sites along the Rio Grande in South New Mexico and West Texas.

NO_3^- fluxes from human activities might be naturally attenuated via denitrification (Carlson and Ingraham, 1983). This process is a type of microbial respiration which leads to reduction of NO_3^- and NO_2^- to gaseous forms of nitrogen (NO , N_2O and N_2) by a diverse group of microorganisms including bacteria, archaea, and fungi (Zumft, 1997). Denitrification occurs in places where oxygen is

scarce, for example in poorly oxygenated groundwater, and in manmade systems such as artificial freshwater reservoirs and retention ponds (e.g., McClain et al., 2003; Davidson and Seitzinger, 2006). Denitrification is believed to be important in global nitrogen (N) cycle and is considered to be a major NO_3^- sink in groundwater (Trudell et al., 1986; Smith et al., 1991). However, little is known

about how this process can be inhibited by elevated salinity, which is a common problem in the semi-arid Rio Grande. High salinity has been shown to decrease activity rates of microbial denitrification in marine sediments (e.g., Seo et al., 2008). In the studied portion of the Rio Grande watershed, lower NO_3^- concentrations ($<1\text{--}2\text{ mg/L}$) have been previously observed in agricultural drains with less saline water ($\text{EC} < 1\text{--}2\text{ mS/cm}$) compared to higher concentrations ($3\text{--}12\text{ mg/L}$) in drains with higher salinity ($\text{EC } 2\text{--}4\text{ mS/cm}$) (Szynkiewicz et al., 2015). Nevertheless, in this area higher NO_3^- concentrations might be also a result of increasing nutrient loading from waste effluents in growing urban centers. For example, the highest population density is mainly observed downstream in southern New Mexico and western Texas, which is followed by significant increases of NO_3^- concentrations in the Rio Grande (up to 50 mg/L ; Syznkiewicz et al., 2015). In order to address this problem, more quantitative studies are needed to determine anthropogenic NO_3^- contributions and to assess the role of microbial processes in NO_3^- attenuation.

Previous studies in the Rio Grande watershed mainly relied on the measurements of NO_3^- concentrations with limited use of isotope tracers (e.g., Oelsner et al., 2007; Syznkiewicz et al., 2015). Generally, nitrogen (N) and oxygen (O) isotope compositions of NO_3^- ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively) are good indicators of point (urban) and non-point (agricultural) source pollution because of their distinctive isotope compositions (e.g., Mayer et al., 2002; Kendall et al., 2007; Stadler et al., 2008; Chen et al., 2009; Kaushal et al., 2011; Fenech et al., 2012). Further, microbial denitrification involves significant isotope fractionation which enriches NO_3^- in heavier N and O isotopes (e.g., Kendall et al., 2007; Groffman, 2012). Therefore, the main goal of this study was to i) characterize major NO_3^- sources, and ii) assess the role of water salinity on microbial denitrification by using multiple chemical and isotope tracers. These included analysis of water chemistry, and the isotope compositions of water, dissolved NO_3^- and sulfate (SO_4^{2-}). The waste water effluents, fertilizers, precipitation (urban runoff), the Rio Grande surface water and its associated drain network were sampled in the non-irrigation and irrigation seasons of 2014 and 2015, respectively. The sampling took place after a multi-year drought, which significantly reduced stream flows in the Rio Grande and led to increases of groundwater pumping and municipal discharges. This, in turn, allowed for better assessment of major factors controlling NO_3^- fluxes under dry conditions.

2. Environmental setting

The Rio Grande watershed is a desert shrubland and rangeland with an arid to semi-arid climate with average precipitation of $\sim 250\text{ mm}$ per year. Temperatures range from average 7°C during the winter to average 28°C in the summer (Oelsner et al., 2007). In the study area (Fig. 1), surface water and groundwater are critical resources for economic growth (Ellis et al., 1993) but they are limited to municipal, industrial and agricultural users because of the dry climate and increasing salinization (e.g., Phillips et al., 2003; Hogan et al., 2007; Sheng, 2013; Borrok and Engle, 2014; Syznkiewicz et al., 2015). The Rio Grande is mainly fed by spring snowmelt from the San Juan Mountains in southern Colorado and high mountain ranges of Sangre de Cristo and Jemez Mountains in northern New Mexico (Phillips et al., 2003). Summer monsoon rainfall contributes less recharge to the Rio Grande but it is an important source of precipitation in the semi-arid locations of southern New Mexico and western Texas. Additionally, municipal wastewater treatment plants (WWTPs) in Albuquerque, Las Cruces and El Paso make significant water contributions to the Rio Grande, constituting a greater percentage of stream flows during dry seasons between October and March (e.g., non-irrigation periods). It

has been suggested that the WWTP effluents account for approximately 20% and 38% of the river discharge in wet and dry years, respectively (Oelsner et al., 2007). Other significant water contributions come from irrigation drains that are present along the Rio Grande Valley and make up approximately 66% and 33% of the river flows in dry and wet years, respectively (Oelsner et al., 2007). Over 85% of water withdrawals from the Rio Grande are used for flood irrigation of cotton, alfalfa, pecans and pasture for livestock production. Previous studies in central New Mexico showed that the WWTPs in big cities (e.g., Albuquerque) are potentially the largest sources of NO_3^- to the Rio Grande (Oelsner et al., 2007). In contrast, the agricultural districts appear to be nitrogen sinks. Nevertheless, further downstream in southern New Mexico and western Texas (e.g., Las Cruces, El Paso), the NO_3^- concentrations are relatively high in both the Rio Grande and agricultural drains (Szynkiewicz et al., 2015) suggesting significant regional differences in nitrate loads into surface waters.

From its headwaters in southern Colorado to Fort Quitman, Texas, over 120,000 ha of farmland depend on the Rio Grande surface water for irrigation (Bureau of Reclamation, 2007). However, during droughts the aquifer groundwater is commonly used to substitute for the Rio Grande surface water. The semi-arid climate affects the quality of surface water in the area, mainly by increasing its salinity (Borrok and Engle, 2014; Syznkiewicz et al., 2015). This, in turn, decreases crop productivity in the Rio Grande irrigation districts. It has been proposed that major sources of salinity in the Rio Grande include natural inflows of deep brines to the surface (e.g., Hogan et al., 2007; Williams et al., 2013) and anthropogenically-induced factors such as high evapotranspiration rates during flood irrigation (Phillips et al., 2003; Syznkiewicz et al., 2015) and/or fertilizer use (Szynkiewicz et al., 2011). It is predicted that the Rio Grande watershed will experience a drier climate in the future because of global warming (Gutzler and Robbins, 2011). Consequently, this will increase land desertification and water salinity due to less snowpack accumulation in the high mountain recharge areas and higher evapotranspiration rates due to rising surface temperatures (e.g., Phillips et al., 2003; Borrok and Engle, 2014). Additionally, it is possible that the surface waters of the Rio Grande watershed will experience a shift toward being more Na-Cl- SO_4 -rich because of climate change (Borrok and Engle, 2014; Syznkiewicz et al., 2015). This could be a result of several processes such as localized upwelling of brines (Witcher et al., 2004; Hogan et al., 2007) and intensive pumping of saline groundwater for irrigation (Sheng, 2013). Note that both basinal brines and aquifer groundwater show elevated concentrations of Na, Cl and SO_4 . Additionally, evolution of water chemistry toward Na-Cl- SO_4 -rich is controlled by secondary calcite formation (removal of Ca and $\text{HCO}_3^-/\text{CO}_2$) as water partially evaporates on (near) the surface (Szynkiewicz et al., 2015).

3. Methods

3.1. Field sampling

The surface water samples were collected along a $\sim 250\text{ km}$ stretch of the Rio Grande from the Elephant Butte reservoir, New Mexico to Tornillo, Texas (Fig. 1). In this area, the three major urban centers are Las Cruces in New Mexico, El Paso in Texas, and Ciudad Juarez in Mexico with total (combined) population of ~ 3 million people. The collected samples included surface water from the river, major agricultural drains, rain precipitation, and wastewater treatment plant (WWTP) effluents (Fig. 1). In El Paso, the Rio Grande becomes an international border of the United States and Mexico and various security measures precluded us from the Rio Grande sampling along the border (e.g., south of El Paso, below

Location 10 on Fig. 1). In order to characterize seasonal processes affecting stream chemistry and isotope compositions, the water samples were collected during one non-irrigation season in October 2014 and one irrigation season in July 2015. Individual sampling usually took place within a three-day period, which aided in avoiding episodic effects such as dilution by surface water releases from local dams. Because the rain events are rare and relatively short in the semi-arid Rio Grande watershed, rainwater was sampled during two large rain events of 2014 monsoon season. The rain samples comprised of urban runoff were collected from storm drains and house roofs in the city of El Paso.

Dissolved oxygen (DO) was measured in situ using a YSI 556 Multiparameter System. The dissolved concentrations of nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) were measured in situ using a Hach DR 900 Colorimeter and the concentration of dissolved bicarbonate (HCO_3^-) using a LaMotte field titration kit.

For cation analysis, water was filtered through a 0.20 μm filter with a syringe into 125 mL plastic bottles and acidified in situ with concentrated nitric acid to a pH of <2. Water sampled for anion analysis was obtained in the same manner except no acidification was performed. The un-acidified samples were also used for determination of O isotope composition of water. Water samples for analysis of N and O isotope composition of NO_3^- were filtered with a syringe through a 0.20 μm filter into 30 mL plastic bottles. Water samples for analysis of sulfur (S) and O isotope composition of SO_4^{2-} were filtered in situ into a plastic bottle using a Nalgene filtration device with a glass microfiber filter and a hand-operated vacuum pump. After returning to the laboratory, the dissolved SO_4^{2-} was precipitated to barium sulfate (BaSO_4). All bottles with the sampled water were kept cold on ice in a cooler for the duration of the sampling campaign and during the transport to the laboratory. Afterward, they were stored in a refrigerator for cation/anion and S-O isotope analyses, and in the freezer at -20°C for N-O isotope analysis.

3.2. Chemical analysis

Chemical analysis of major cations and anions were performed using a Dionex ICS 2000 (IC) in the Department of Earth and Planetary Sciences at University of Tennessee in Knoxville (EPS-UTK). For cation measurements, a CS16 column at 40°C with a flow rate of 1 mL/min of MSA was used, and for anion measurements, an AS11 column at 30°C with a flow rate of 1.5 mL/min of 26 mM KOH. Precision of these analyses was ± 0.1 mg/L.

3.3. N and O isotope analysis

The N and O isotope analyses of NO_3^- were performed on downstream samples with NO_3^- concentration >0.3 mg/L (e.g., Locations 4–10 along the Rio Grande; Fig. 1). Conversely, in upstream locations no. 1 through 3 (Rio Grande), as well as A and E (drains), the NO_3^- concentrations were too small (<0.3 mg/L) for N and O isotope analysis.

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- were analyzed using a bacterial denitrifier method (Casciotti et al., 2002; Sigman et al., 2001) in the Stable Isotope Laboratory of EPS-UTK. In summary, the N and O isotope compositions of NO_3^- were determined using the denitrifying bacteria *Pseudomonas Aureofaciens*. These bacteria lack the N_2O reductase and therefore convert all NO_3^- dissolved in water to N_2O gas, which is consequently analyzed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ by an isotope ratio mass spectrometer (see more details in on-line Supplementary Materials). In this study, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of N_2O were measured using a GasBench II coupled with a Thermo Finnigan Delta Plus XL mass spectrometer. Two in-house KNO_3 standards with a range of isotopic values were utilized to calibrate

the method. Solutions of 50 μM KNO_3 were made for each standard and measured in the same fashion as samples with 1 mL of standard solution being used for each analysis. A set of two standards was run with every 12 samples. One standard deviation for $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ was 0.8‰ and 0.7‰, respectively. The measured isotope values are presented relative to an Air- N_2 standard for $\delta^{15}\text{N}$ and a VSMOW standard for $\delta^{18}\text{O}$.

3.4. S and O isotope analysis of sulfate and water

After returning from the field, the water samples were transferred to glass beakers and acidified to a pH of 2 using 20% HCL. Afterward, 10 mL of 10% BaCl_2 was used to precipitate a dissolved SO_4^{2-} to BaSO_4 . The precipitate was left to settle overnight to the bottom of the beaker. Afterward, excess water was siphoned out and the precipitate was rinsed with DI water; this process was repeated three times. Subsequently, the precipitate was dried in an oven overnight and stored in a plastic vial.

For $\delta^{34}\text{S}$ analysis, 0.4 mg of BaSO_4 was enclosed in a tin capsule with 1–2 mg of V_2O_5 for complete combustion of the sample to SO_2 prior to ionization in the mass spectrometer. The tin capsules with BaSO_4 precipitate were combusted using a Costech elemental analyzer (EA) following the quartz-buffering method by Fry et al. (2002). An additional modification was made to replace the separation column by a 15 cm piece of capillary tubing (0.22 mm in diameter). Consequently, the standard deviation for $\delta^{34}\text{S}$ results was 0.1‰ as a result of removal of the Gas Chromatography (GC) column from the EA. For $\delta^{18}\text{O}$ analysis, 0.3 mg of BaSO_4 was enclosed in silver capsules. The silver capsules were analyzed using a Conflow system coupled to a Thermo Scientific high-temperature conversion elemental analyzer (TC/EA). Both of these systems were interfaced with a Finnigan Delta Plus XL mass spectrometer. One standard deviation for $\delta^{18}\text{O}$ was 0.3‰. The measured isotope values are presented relative to a Vienna Cañon Diablo Troilite standard (VCDT) for $\delta^{34}\text{S}$ and a VSMOW standard for $\delta^{18}\text{O}$.

The $\delta^{18}\text{O}$ of water samples were measured using a Laser Water Isotope Analyzer from Los Gatos Research. For each sample, 1.5 mL of water was added to a glass vial with a septum cap on the day of analysis. An autosampler withdrew 0.8 μL of sample and was injected through a heated septum, and allowed to evaporate in the measuring cell of the isotope analyzer. A tunable laser was projected through the measuring cell and the time taken for the intensity of light to decay was measured. Based on the isotope composition of water vapor in the measuring cell, decay times differed. In-house standards were analyzed every three samples. These included the KNOW1 and SLDW1 standards previously calibrated to the VSMOW and SLAP scale. Standard deviation was 0.2‰.

4. Results and discussion

All field in-situ measurements, water chemistry, and isotope compositions of the Rio Grande, agricultural drains, waste effluents and urban runoff are presented in Supplementary Tables 1, 2, 3, 4 and 5. These tables summarize seasonal variations of all measured parameters relative to a distance from the Elephant Butte reservoir during the non-irrigation season of fall 2014 and irrigation season of summer 2015. The obtained chemical and isotope results were used to discuss major NO_3^- sources and make an assessment whether elevated salinity is a limiting factor for microbial denitrification in the studied portion of the semi-arid Rio Grande.

4.1. Water quality & main N forms

A Piper Diagram (Suppl. Fig. 1) was used to determine water

type(s), but none were found to be distinctive in the studied portion of the Rio Grande watershed during the fall 2014 (non-irrigation) and summer 2015 (irrigation). Nevertheless, many of the non-irrigation season water samples appeared to be more enriched in Na^+ , Cl^- , and SO_4^{2-} compared to the irrigation season showing greater enrichment in Ca^{2+} , Mg^{2+} , and HCO_3^- ions. This is consistent with previous studies showing significant increases in Na-Cl-SO_4 concentrations during dry periods (Phillips et al., 2003; Hogan et al., 2007; Borrok and Engle, 2014; Szykiewicz et al., 2015). Only one waste effluent sample from a small WWTP plant in Location K showed more distinct water chemistry indicative of a bicarbonate type (Suppl. Fig. 1). This plant serves a small community in the City of Sunland Park on the border of New Mexico, Texas and Mexico. Most likely, in this location the main municipal water supplies come from groundwater because its $\delta^{34}\text{S}$ of SO_4^{2-} was similar (+8‰) to the shallow HCO_3^- -rich groundwater of this area (>8–12‰; Witcher et al., 2004; Szykiewicz et al., 2011). Conversely, the Rio Grande surface water used by larger cities (e.g., Las Cruces, west El Paso) has significantly lower $\delta^{34}\text{S}$ of –2 to +2‰ due to higher inputs of sulfide-derived SO_4 with negative $\delta^{34}\text{S}$ in upstream locations (Szykiewicz et al., 2015).

Previous studies suggested that human activities such as agriculture, groundwater pumping and urbanization significantly affect water quality and NO_3^- concentrations in the Rio Grande (Ellis et al., 1993; Passell et al., 2005; Oelsner et al., 2007; Szykiewicz et al., 2015). While arid climate and flood irrigation enhances evaporation rates leading to both salt increases (Ellis et al., 1993; Phillips et al., 2003) and leaching of N-bearing compounds from fertilizers (Szykiewicz et al., 2015), waste effluents from big cities account for significant NO_3^- loads to the Rio Grande (Oelsner et al., 2007; Szykiewicz et al., 2015). However, the magnitude and extent of NO_3^- contamination due to agriculture and urbanization could not be previously quantified using NO_3^- concentrations alone. This was also problematic in part due to more focus on the localized areas (e.g., separated topographic basins), limited seasonal monitoring, and poor spatial characterization of major NO_3^- endmembers using isotope tracers (Ellis et al., 1993; Passell et al., 2005; Oelsner et al., 2007; Hale et al., 2014; Szykiewicz et al., 2015).

In this study, variations of NO_3^- , NO_2^- , and NH_4^+ concentrations in major endmembers (e.g., effluent, drain water) were used to characterize contributions of main N forms to the Rio Grande relative to a distance from the Elephant Butte reservoir. Generally, the NO_2^- and NH_4^+ concentrations in these endmembers were significantly smaller (0.1–2.6 mg/L in drains and 0.1–2.8 mg/L in effluents) compared to NO_3^- concentrations (0.1–72 mg/L in drains and 44–140 mg/L in effluents) (Suppl. Tab. 2; Fig. 2). This implies that most of the reduced N inputs are readily oxidized to NO_3^- . Accordingly, this is consistent with high concentrations of dissolved O_2 measured in the drains, waste effluents and the Rio Grande during investigated seasons (5.3–11.1 mg/L; Suppl. Tab. 1). The Rio Grande surface water had similar concentrations of NO_2^- and NH_4^+ (0.1–1.6 mg/L) to the drains and effluents (Fig. 2). However, the concentrations of NO_3^- were significantly lower (0.1–2.72 mg/L) in the Rio Grande compared to these endmembers (1–140 mg/L), suggesting important dilution by upstream water from Elephant Butte reservoir which is significantly depleted in NO_3^- (<0.1 mg/L). The dilution effect was likely most prominent in the upstream Rio Grande. For example, in Locations 2 and 3 (located in a close proximity to the Elephant Butte; Fig. 1) the lowest concentrations of NO_3^- were measured during both investigated seasons (<0.1 mg/L).

The irrigation drains and waste effluents from large cities in Las Cruces and El Paso become important tributaries to the Rio Grande due to increasing aridity, limited precipitation, and reduced stream flows (e.g., Anderholm, 2002; Szykiewicz et al., 2015). This is particularly a problem during non-irrigation season (fall and

winter) when little surface water is released from the Elephant Butte reservoir. Accordingly, the downstream increases of NO_3^- concentrations in the Rio Grande (<0.1–2.72 mg/L) and agricultural drains (0.36–71 mg/L) are likely a result of high NO_3^- concentrations in urban waste effluents (44–140 mg/L; Fig. 2). In particular, this was the case in the fall 2014 (non-irrigation) when the drain water at Fabens (Location H) showed similarly high NO_3^- concentrations (72 mg/L) as the waste effluents (66–73 mg/L) from three WWTPs in El Paso (Locations M, N). These WWTPs are an important source of the reclaimed city water, which is conveyed by the American Canal to the irrigation districts located south of El Paso. The American Canal is a stone/concrete canal transporting a portion of the Rio Grande surface water (from Location L; Fig. 1) and effluent water along the international border and is governed by the International Boundary and Water Commission regulating surface water allocation between the United States and Mexico. In this portion of the Rio Grande watershed, precipitation mainly occurs during summer monsoon and the stone/concrete lining of American Canal reduces mixing with shallow groundwater. Therefore, the dilution of waste effluents from the city of El Paso is limited, particularly during non-irrigation seasons, and likely contributed to high NO_3^- loads into the southern irrigation districts near Fabens in fall 2014 (Locations H and I on Fig. 2).

4.2. Main nitrate sources & their isotope composition

Several studies have shown that N and O isotope tracers can be used to pinpoint major sources of NO_3^- in aqueous systems (e.g., Kreitler and Jones, 1975; Gormley and Spalding, 1979; Heaton, 1986; Mayer et al., 2002; Kendall et al., 2007). In this study, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of NO_3^- were determined in three major endmembers: waste effluent from five WWTPs, local fertilizers, and precipitation (urban runoff). Similar to previous studies, these endmembers have shown distinctive isotopic compositions, with highest $\delta^{15}\text{N}$ and lowest $\delta^{18}\text{O}$ in the waste effluents (+7.4 to +11.6‰ and –10 to –4‰ respectively), lower $\delta^{15}\text{N}$ and higher $\delta^{18}\text{O}$ in liquid fertilizers (–0.1 to +3‰ and +15.8 to +19.9‰, respectively), and lowest $\delta^{15}\text{N}$ and highest $\delta^{18}\text{O}$ in precipitation (–4.7 to –1.6 and +36.5 to +44.9‰, respectively) (Fig. 3). Generally, a distinctive mixing trend can be delineated between these three endmembers using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- (Fig. 3). Except for three samples affected by microbial denitrification (see section 4.4 for more details), the Rio Grande and drain samples mainly plotted between the fertilizer and waste effluent endmembers, suggesting they are the main NO_3^- sources. In contrast, local precipitation appears to contribute rather minor amounts of NO_3^- since none of the collected water samples plotted close to this endmember (Fig. 3). This is in good agreement with rare precipitation in the studied area, mainly limited to the short rain events during summer monsoon. Note that there was no rain during sampling campaigns in fall 2014 and summer 2015. Nevertheless, the measured NO_3^- concentrations in the rain samples were relatively high (1.4–8.1 mg/L; Suppl. Tab. 4) during the 2014 summer monsoon in El Paso. This implies that precipitation might be seasonally an important NO_3^- source to the Rio Grande during single storm events. Alternatively, the measured elevated NO_3^- concentrations in precipitation resulted from rapid leaching of atmospheric N accumulated on the surface over longer periods of time since these samples were collected from the city storm drains and house roofs. We infer that seasonal atmospheric NO_3^- inputs to the Rio Grande might be significant, thus future studies should be accompanied by sampling shortly after main rain events to better quantify this source and evaluate its significance in regional scale.

Assuming two-endmember model, the $\delta^{15}\text{N}$ of the effluent- and fertilizer- NO_3^- can be used to calculate their contributions to the Rio

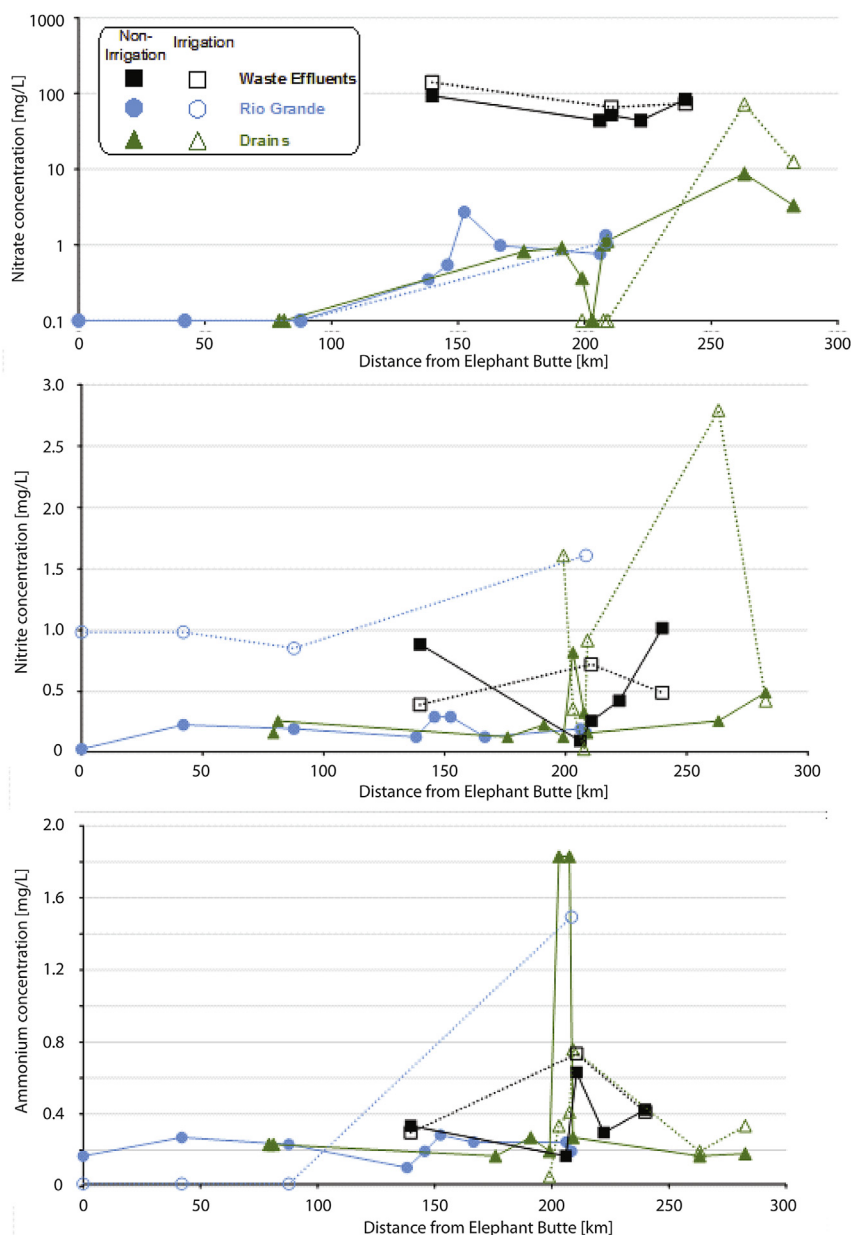


Fig. 2. Variations of NO_3^- , NO_2^- , and NH_4^+ concentration versus distance in the Rio Grande watershed. Note that the lines connecting drain and effluent samples are mainly for depicting the increasing distance from the Elephant Butte Reservoir; there is no physical connection between individual drains and waste effluents analyzed in this study.

Grande and agricultural drains during dry seasons (e.g., no main rain events) using the following isotope mass balance equation:

$$\delta^{15}\text{N}_{\text{Sample}} = x \cdot \delta^{15}\text{N}_{\text{WWTP}} + (1-x) \cdot \delta^{15}\text{N}_{\text{Fertilizers}} \quad (1)$$

where x is the proportion of total dissolved NO_3^- coming from the WWTP effluents. In this calculation, the average measured $\delta^{15}\text{N}$ of NO_3^- in the liquid fertilizers (+0.6‰) and the WWTP effluents (irrigation season: +10.1‰, non-irrigation season: +9.0‰) were used. Given the ~2–3‰ variation in each endmember, this calculation is accompanied by ±10–20% error. While similar isotope mass balance could be done for $\delta^{18}\text{O}$ of NO_3^- , we did not consider it because of plausible exchange of O isotopes between water and NO_3^- (e.g., Andersson and Hooper, 1983; Casciotti et al., 2002), which could affect quantitative estimations.

The results of N isotope mass balance are presented on Figs. 4–5

and in Suppl. Tab. 6. It appears that the contributions of the WWTP effluent-derived NO_3^- varied in a wide range (0–100%) in both the Rio Grande and agricultural drains when compared to the fertilizer-derived NO_3^- . Higher contributions of the fertilizer-derived NO_3^- were usually observed upstream in Locations 4 and 5 (near Las Cruces) with fewer inhabitants and lack of large cities. Conversely, greater contributions of the effluent-derived NO_3^- were more typical in the downstream Locations 7 through 10 near large urban centers (e.g., El Paso area), and in the agricultural drains south of El Paso (Locations H and I) where there is a significant effort of reusing the reclaimed city water (e.g., treated WWTP effluent). Consequently, the isotope results are in good agreement with the observed higher NO_3^- concentrations in waste effluents (44–140 mg/L) compared to the smaller NO_3^- concentrations in other endmembers (1–72 mg/L in drain water; 1–8 mg/L in precipitation) (Fig. 2). Because of very low NO_3^- concentrations

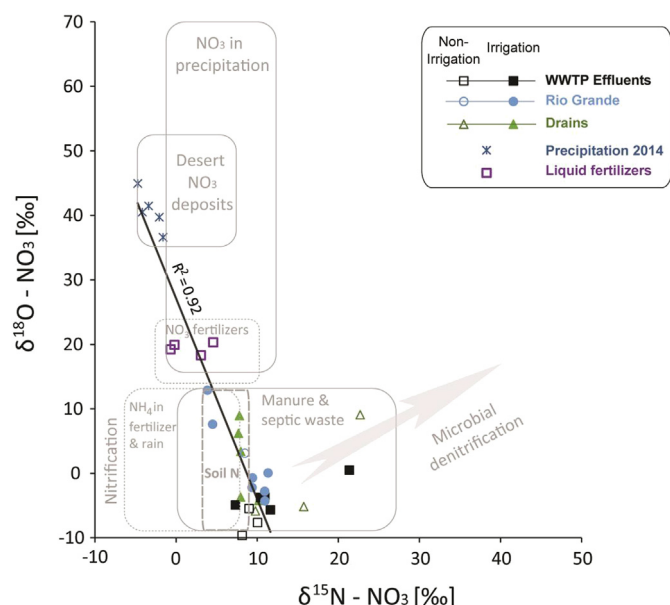


Fig. 3. Variations of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved NO_3^- in the Rio Grande watershed. Closed and open symbols represent irrigation and non-irrigation seasons, respectively. Rounded boxes delineate isotope compositions of NO_3^- derived from different sources (after Casciotti et al., 2002). Except for three outliers showing a shift toward positive values indicative of microbial denitrification, the regression line ($R^2 = 0.92$) is presented for the analyzed samples of precipitation, liquid fertilizers, Rio Grande surface water, agricultural drains and WWTP effluents.

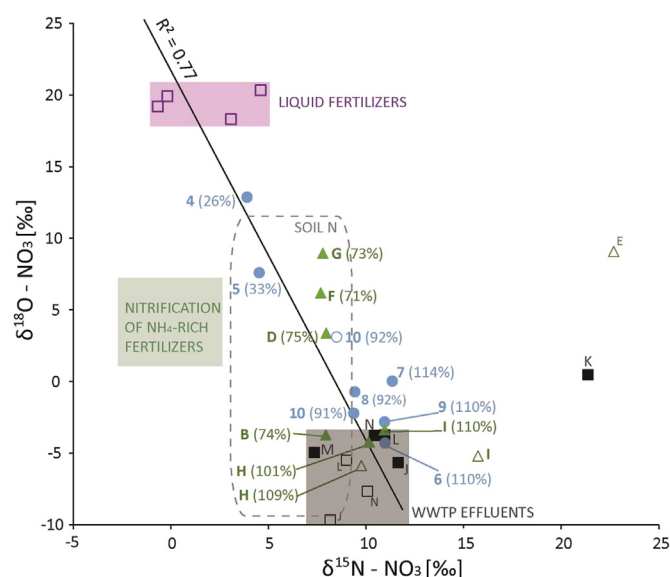


Fig. 4. Estimation of effluent-derived NO_3^- into the drain and Rio Grande surface water compared to the fertilizer-derived NO_3^- using the N isotope mass balance constraint. The % contributions of effluent-derived NO_3^- are presented in brackets. Three outliers in Locations E, I, K are the most affected by microbial denitrifications and have been excluded from isotope mass balance constraint. In the isotope mass balance, the average $\delta^{15}\text{N}$ of +10.1 and +9.1‰ were used for the effluent-derived NO_3^- in irrigation and non-irrigation seasons, respectively, and average $\delta^{15}\text{N}$ of +1.7‰ for liquid fertilizers. The green field indicates the theoretical $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in NO_3^- estimated for the nitrification of local ammonium-rich fertilizers and a light gray dotted line field from the nitrification in soil (after Kendall et al., 2007). Numbers and letters indicate site locations presented on Fig. 1 and symbols are same as on Fig. 3. The regression line ($R^2 = 0.77$) is presented for the analyzed samples of liquid fertilizers, Rio Grande surface water, agricultural drains and WWTP effluents. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(<0.1 mg/L) in the Elephant Butte reservoir (Location 1) and near Caballo/Radium Springs (Locations 2–3), we infer that these upstream locations were the least, if any, affected by NO_3^- inputs from fertilizers and waste effluents. This is likely a result of smaller population centers (without WWTP facilities), less agricultural irrigation, and bigger dilution effect by NO_3^- -depleted water released from Elephant Butte.

Mixing between effluent- and fertilizer-derived NO_3^- can be also identified using a comparison of $\delta^{15}\text{N}$ values versus NO_3^- concentrations (Fig. 6). Note that the ratio of $1/\text{NO}_3^-$ instead of NO_3^- concentrations was used in this comparison because of the wide range of NO_3^- concentrations measured in the waste effluents (44–140 mg/L), Rio Grande (0.1–2.7 mg/L) and drain water (4.5–15.7 mg/L). The top insert on Fig. 6 shows the expected changes of $\delta^{15}\text{N}$ in the effluent endmember due to dilution by NO_3^- -depleted water and mixing with irrigation water carrying NO_3^- -derived from microbial processes (e.g., nitrification and denitrification in soil) and fertilizers. While mixing with the endmembers having lower concentrations of NO_3^- than the effluent increases the ratio of $1/\text{NO}_3^-$ (decreases NO_3^- concentration), the $\delta^{15}\text{N}$ change differently because of distinctive N isotope compositions. Generally, dilution does not involve any significant N isotope fractionation, thus it would only decrease NO_3^- concentrations in the studied area. In contrast, microbial denitrification significantly increases $\delta^{15}\text{N}$ because of a preferential ^{14}N uptake by microbes (e.g., Kendall et al., 2007). Additionally, this process increases the ratio of $1/\text{NO}_3^-$ due to removal of NO_3^- by microbes. Since local fertilizers contribute NO_3^- with lower $\delta^{15}\text{N}$ and lower concentrations due to dilution by the NO_3^- -depleted Rio Grande surface water used for irrigation, the mixing of waste effluent with irrigation water would decrease $\delta^{15}\text{N}$ and considerably increase the $1/\text{NO}_3^-$ ratio. It should be noted that irrigation return flows might also carry additional NO_3^- from soil nitrification and/or precipitation with lower $\delta^{15}\text{N}$ (Figs. 3–4; Panno et al., 2001; Kendall et al., 2007). We assume that NO_3^- concentration from these two sources would be also significantly lower in irrigation return flows compared to waste effluents, thus $\delta^{15}\text{N}$ would decrease and $1/\text{NO}_3^-$ increase (Fig. 6).

As presented on Fig. 6, the Rio Grande surface water showed decreasing $\delta^{15}\text{N}$ of NO_3^- with increasing $1/\text{NO}_3^-$ ($R^2 = 0.75$) suggesting mixing between the effluent (high $\delta^{15}\text{N}$ and NO_3^- concentrations) and irrigation water with low $\delta^{15}\text{N}$ and NO_3^- concentrations from either dissolution of local fertilizers, precipitation, and/or soil processes (e.g., nitrification). A similar correlation, but less significant, was observed for drain water ($R^2 = 0.40$) with a slight shift toward dilution (Fig. 6). Our sampling campaigns took place after a few years long drought when local farmers had increased the use of aquifer groundwater to substitute the shortages of the Rio Grande surface water. This groundwater is depleted in NO_3^- (usually <1 mg/L; Witcher et al., 2004), thus it explains higher dilution effect observed in the drains (Fig. 6).

Mixing with deeper aquifer groundwater is also inferred using the $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ of dissolved SO_4^{2-} . Three major sources of SO_4^{2-} (e.g., fertilizers, waste water, evaporites in bedrock) significantly differ in their isotope composition in the Rio Grande watershed (Fig. 7; Szykiewicz et al., 2011, 2015). Most of the analyzed drain samples showed higher $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values (>4–6‰) compared to the Rio Grande and waste effluents (<4‰), and were shifted toward the isotope composition of Paleozoic bedrock evaporites (8–12‰) common in this area (Fig. 7). The evaporite-derived SO_4^{2-} significantly controls isotope composition of groundwater in south New Mexico (Witcher et al., 2004) and west Texas (Szykiewicz et al., 2015). Because of dry condition and increases in pumping of aquifer groundwater for irrigation, higher $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ of SO_4^{2-} in several drains were observed during the investigated seasons.

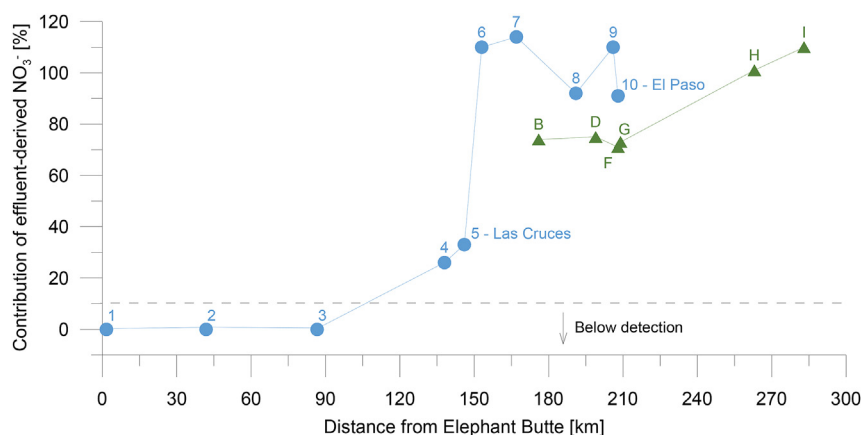


Fig. 5. Spatial changes of effluent-derived NO_3^- in the Rio Grande (blue dots) and agricultural drains (green triangles) when compared to the fertilizer-derived NO_3^- contribution (see chapter 4.2 for more details). Numbers and letters indicate site locations presented on Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

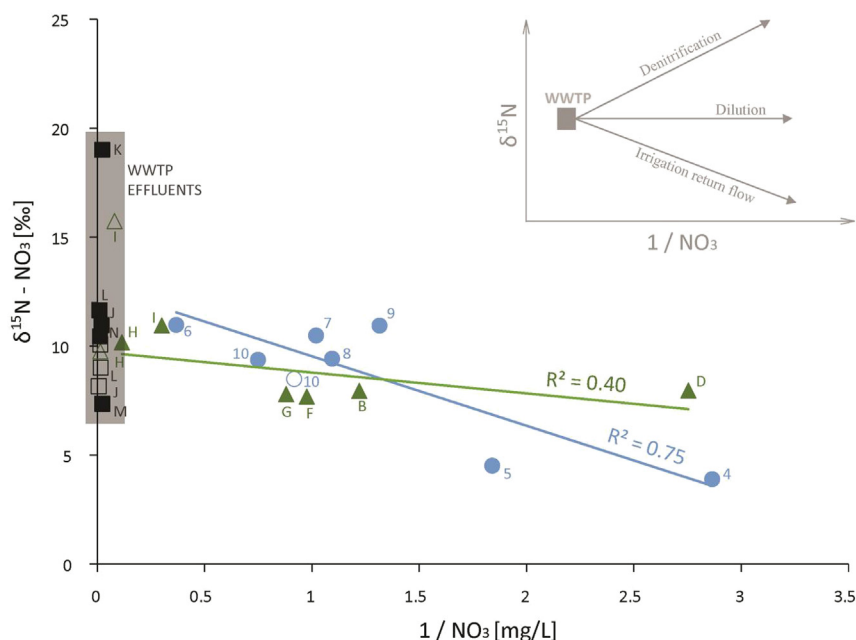


Fig. 6. Variations of $\delta^{15}\text{N}$ compared to $1/\text{NO}_3$ ratio in the Rio Grande watershed. Regression lines are presented in blue for the Rio Grande surface water and in green for the drain surface water during irrigation season 2015. Closed and open symbols represent irrigation and non-irrigation seasons, respectively. Numbers and letters indicate site locations presented on Fig. 1 and symbols are same as on Fig. 3. The top right insert shows the expected trends resulted from dilution and mixing between the WWTP effluent- and the return irrigation flow- and waters carrying microbial denitrification/nitrification-derived NO_3^- . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conversely, the drains affected by irrigation with the Rio Grande surface water had lower $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values, reflecting different proportions of SO_4^{2-} from the Elephant Butte reservoir, fertilizers and WWTP effluents.

4.3. Alternative nitrate sources

In addition to liquid fertilizers, some of the solid fertilizers in the Rio Grande watershed are enriched in ammonium. This type of inorganic fertilizers usually has distinctive low $\delta^{15}\text{N}$ values (−4 to +4‰) because of the manufacturing process that uses atmospheric N_2 gas with low $\delta^{15}\text{N}$ of ~0‰ (Kendall et al., 2007). Accordingly, in our study the ammonium sulfate-based fertilizers showed relatively low $\delta^{15}\text{N}$, from −2.3 to +2.8‰ (average −0.5‰),

and were similar to $\delta^{15}\text{N}$ of liquid fertilizers (−0.6 to +3.0‰) (Suppl. Tab. 7). It can be expected that some quantities of solid ammonium-rich fertilizers undergoing dissolution on agricultural fields are subsequently oxidized to NO_3^- . While inorganic oxidation would not significantly change the initial $\delta^{15}\text{N}$, microbial nitrification (e.g., oxidation of NH_4^+ to NO_3^-) might increase the $\delta^{15}\text{N}$ of NO_3^- by a few ‰ within irrigated soils (Feigin et al., 1974). In contrast, $\delta^{18}\text{O}$ of NO_3^- is more controlled by $\delta^{18}\text{O}$ of water and atmospheric O_2 (Kendall et al., 2007). Generally, two molecules of water oxygen and one molecule of atmospheric oxygen are incorporated to NO_3^- according to the following equation (e.g., Aleem et al., 1965; DiSpirito and Hooper, 1986; Kendall et al., 2007):

$$\delta^{18}\text{O}_{\text{nitrate}} = 2/3 \times \delta^{18}\text{O}_{\text{water}} + 1/3 \times \delta^{18}\text{O}_{\text{atmospheric oxygen}} \quad (2)$$

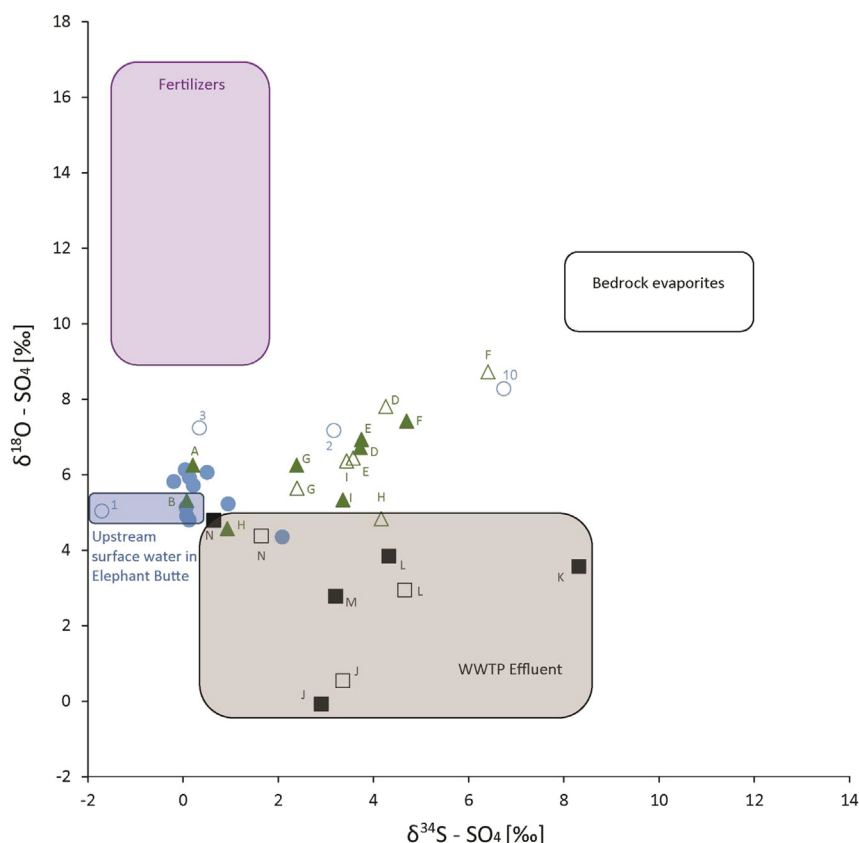


Fig. 7. Variations of $\delta^{18}\text{O}$ and $\delta^{34}\text{S}$ of dissolved SO_4^{2-} in the Rio Grande watershed. Boxes show the isotope compositions of major SO_4^{2-} endmembers determined with new data (this study – WWTP effluents) and by *Szynkiewicz et al. (2015)* for the fertilizer and bedrock evaporite endmembers. Numbers and letters indicate site locations presented on *Fig. 1*. Symbols are the same as on *Fig. 3*.

Equation (2) is a rough estimation of $\delta^{18}\text{O}$ of NO_3^- assuming two constant sources of oxygen from water and air with limited alteration by subsequent processes (e.g., microbial denitrification, evapotranspiration). We used this equation and the $\delta^{18}\text{O}$ of atmospheric O_2 (+23.5‰; *Kropnick and Craig, 1972*) and the $\delta^{18}\text{O}$ of water samples (−10.5 to −3.6‰; *Suppl. Tab. 3*) for determining the theoretical $\delta^{18}\text{O}$ of NO_3^- from oxidation of ammonium-rich fertilizers in the studied area. Accordingly, it was determined that the $\delta^{18}\text{O}$ of NO_3^- from oxidation of local solid fertilizers would range from +0.9 to +6.6‰ (average +2.6‰, $n = 23$; *Fig. 4*). Note that this is by ~15–20‰ lower compared to the $\delta^{18}\text{O}$ of liquid fertilizers (+15.8 to +19.9‰). As seen on *Fig. 4*, the ammonium-rich fertilizer endmember plots slightly to the left from the Rio Grande and drain water samples that were distributed along the mixing line determined for the liquid fertilizer and waste effluent endmembers. This implies rather minor contributions of NO_3^- from oxidation of ammonium fertilizers into the Rio Grande and agricultural drains.

According to *Fig. 4*, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- originated from soil processes (+2 to +8‰ and −10 to +10‰, respectively; after *Kendall et al., 2007*) overlapped with the measured ones in some drain and river water samples. While isotope compositions of NO_3^- from soil nitrification plot in the middle of mixing line determined for the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of fertilizer and waste effluent endmembers, it is impossible to accurately evaluate soil NO_3^- contributions in the studied area. Nevertheless, the $\delta^{18}\text{O}$ of NO_3^- should show a strong correlation with $\delta^{18}\text{O}$ of water in aquatic environments dominated by NO_3^- fluxes from soil nitrification (e.g., *Wankel et al., 2006*; *McMahon and Bohlke, 2006*) because two out of three oxygens in NO_3^- come from ambient water (Equation (2)). However, there was no clear relationship between $\delta^{18}\text{O}$ of NO_3^- and water (*Fig. 8*), which

supports the conclusion that NO_3^- contributions from nitrification were rather minor/negligible in the investigated seasons.

4.4. Impacts of water salinity on NO_3^- cycling

Microbial denitrification is common in anoxic soils/groundwater and often leads to significant increases of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, followed by decreases of NO_3^- concentrations, in shallow groundwater (e.g., *Kendall et al., 2007*). This process has been important in bioattenuation of excess nutrient loads from agricultural activities (*Panno et al., 2001*). In the Rio Grande watershed during the non-irrigation season, two agricultural drains in west El Paso (Location E) and Tornillo (Location I) and one waste effluent in Las Cruces (Location K) showed noticeable increases of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, and general shift of isotope composition to the right side of the mixing line presented on *Figs. 3–4*. This implies that microbial denitrification might be locally important but it is not a dominant processes controlling NO_3^- cycling in the studied area.

Elevated water salinity is a potential physiological stressor for denitrifying microbes (*Rysgaard et al., 1999*). Some marine studies suggest that denitrification rates decrease under elevated Na and Cl concentrations (*Magalhaes et al., 2005*; *Seo et al., 2008*). In this study, two agricultural drains (Locations E and I) with the strongest isotope evidence of denitrification showed highest electric conductivity of 2.9 and 4.2 mS/cm, respectively, and high Cl^- (297–665 mg/L), Na^+ (269–562 mg/L), SO_4^{2-} (593–601 mg/L) concentrations (*Suppl. Tabs. 1–2*). This suggests that higher salinity is not a limiting factor for microbial denitrification in the studied portion of the semi-arid Rio Grande. In this area, local agricultural drains are designed to increase drainage of irrigation water in order

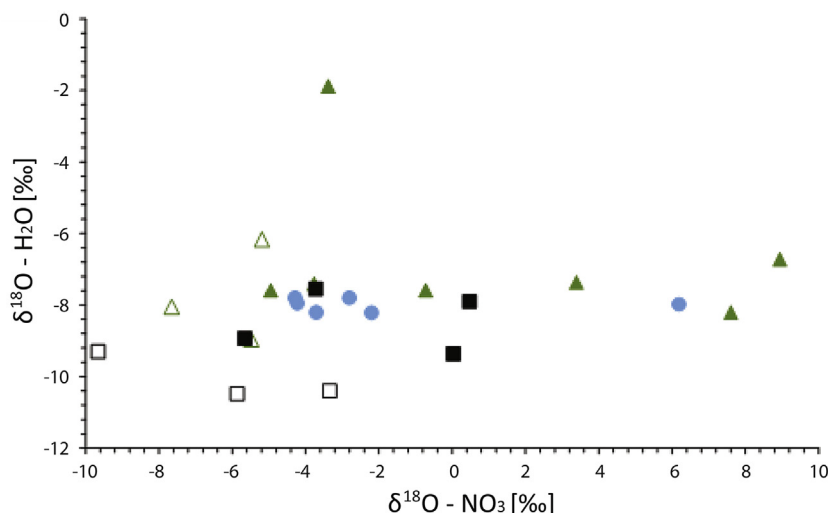


Fig. 8. Variations of $\delta^{18}\text{O}$ in dissolved NO_3^- compared to $\delta^{18}\text{O}$ of water in the Rio Grande, drains and effluents. Closed and open symbols represent irrigation and non-irrigation seasons, respectively. Symbols are same as on Fig. 4.

to avoid anoxic conditions in the shallow subsurface of the irrigated fields. As a result, microbial denitrification rates might be greatly slowed down by a quick drainage system. This observation is in a good agreement with previous studies showing that higher denitrification rates are usually more typical in soils with slow movement of water through soil profiles (e.g., Gormley and Spalding, 1979; Kendall et al., 2007).

5. Conclusions

In this study, two major NO_3^- sources were identified in the Rio Grande watershed during dry seasons using chemical and isotope tracers: fertilizers applied on agricultural fields and waste water effluents from large cities in Las Cruces, New Mexico and El Paso, Texas. Minor amounts of NO_3^- were likely added to the system by precipitation (urban runoff) and/or microbial processes in soil. According to the isotope mass balance constraint and spatial changes of NO_3^- concentrations, the contribution of waste effluent-derived NO_3^- gradually increased downstream due to increasing urbanization and large number of WWTPs. For example, in upstream locations near Las Cruces with smaller population, the waste effluents contributed ~0–30% of NO_3^- into the Rio Grande when compared to the fertilizer-derived NO_3^- contribution. Conversely, the waste effluent-derived NO_3^- accounted for up to ~70–100% in downstream locations south of Las Cruces and near El Paso. This suggests that large urban centers are important NO_3^- contributors into the aquatic system of the Rio Grande in southern New Mexico and western Texas. Microbial denitrification appears to be insignificant in natural bioattenuation of the excess nutrient loads from anthropogenic sources in the studied area. The observed small denitrification effect likely results from the quick drainage of irrigation water. Moreover, denitrification did not appear to be significantly affected by elevated water salinity.

The predicted transition to a more arid climate in the future will likely increase evaporation rates and decrease stream flows in American Southwest due to increasing temperatures and less snowpack in main recharge areas (e.g., Yuan and Miyamoto, 2004; Gutzler and Robbins, 2011). Additionally, the current population in the study area is expected to double during next 50 years. Therefore, it can be expected that NO_3^- loads will likely increase into the Rio Grande in the future, mainly from the increases of WWTPs servicing the growing population in the region. Additional

increases of NO_3^- concentrations might also result from increasing evaporation rates and smaller dilution due to less snow/rain fall.

Future monitoring studies in the Rio Grande watershed would benefit from more seasonal observations as well as sampling performed during key seasons. This is particularly important for understanding the NO_3^- contributions from precipitation during monsoon seasons. Further, plausible denitrification “hot-spots” could be better identified by studying N and O isotope compositions of NO_3^- on the flooded irrigated fields using monitoring piezometers and available groundwater wells.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.apgeochem.2017.09.012>.

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