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A BIOLOGICAL AND NITRATE ISOTOPIC ASSESSMENT FRAMEWORK TO UNDERSTAND EUTROPHICATION IN AQUATIC ECOSYSTEMS

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ABSTRACT

Eutrophication is a globally significant challenge facing aquatic ecosystems, mostly associated with human induced enrichment of these ecosystems with nitrogen and phosphorus. Given the complexity of assigning eutrophication issues to local primary N sources in field-based studies, this paper proposes a multi-stable isotope and biological framework to track nitrogen biogeochemical transformations, inputs and fate of nitrate in groundwater-dependent shallow lakes. Three representative freshwater ecosystems from the Pampa Plain (Argentina), with different land uses and topographic features were selected. Groundwater (N=24), lake (N=29) and stream (N=20) samples were collected for isotope ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-, $\delta^{18}O$-H_2O) and hydrogeochemical (major ions and nutrients) determinations, and in the case of surface water, also for biological determinations (chlorophyll-*a,* fecal coliforms and nitrifying bacteria abundance). Both chemical and isotopic characteristics clearly indicated that denitrification was limited in lakes and streams, while evidence of assimilation in shallow lakes was confirmed. The results suggested that groundwater denitrification plays a role in the nitrate concentration pattern observed in the Pampeano Aquifer. The proportional contribution of nitrate sources to the inflow streams for all years were estimated by using Bayesian isotope mixing models, being ammonium nitrified in the system from soil and fertilizers ~ 50 - 75%, sewage/manure ~20 - 40% and atmospheric deposition ~5 - 15%. In this sense, agricultural practices seem to have a relevant role in the eutrophication and water quality deterioration for these watersheds. However, limnological, bacterial and algal variables, assessed simultaneously with isotopic tracers, indicated spatio-temporal differences within and between these aquatic ecosystems. In the case of Nahuel Rucá Lake, animal manure was a significant source of nitrogen pollution, in contrast to La Brava Lake. In Los Padres Lake, agricultural practices were considered the main sources of nitrate input to the ecosystem.

Keywords: eutrophication - nitrate isotopes - geochemical and biological proxies - Bayesian isotope mixing models - temperate shallow lakes - Argentina

1. INTRODUCTION

Nitrate contamination of water is a worldwide environmental problem that, in most cases, is the consequence of human activities. Excess nitrogen from agricultural and urban activities promotes NO_3^- (and other N species) leaching to groundwater and surface runoff, which affects the aquatic environment (Pauwels and Talbo 2004). Ecologically, the excess nitrate (NO_3^-) in surface waters can cause cultural eutrophication, the process of ecosystem deterioration due to nutrient enrichment in water, mainly with phosphorus (P) and nitrogen (N), which can ultimately lead to excessive algal and/or aquatic plant growth and severe degradation of the aquatic ecosystem and water resources (Seitzinger, 2008; Schindler, 2012; Xu et al., 2016).Given the adverse ecological, social and economic impacts associated with eutrophication (Kopprio et al., 2014; Goody et al., 2016; Yao et al., 2017), research efforts have been directed towards understanding the causes of this process and targeting mitigation strategies.

The protection of surface water and groundwater from NO_3^- pollution requires the identification and quantification of the contributions of primary nitrogen sources within the catchment. However, given the wide variety of potential NO_3^-sources that may exist within a catchment, the non-conservative behavior of nitrogen (N) as it is dispersed from a source, and the co-existence of several biogeochemical processes that alter nitrate and other nitrogen-related concentrations, it is often difficult to determine the predominant NO_3^- sources using conventional water quality monitoring techniques (Kendal et al., 2007; Xu et al., 2016).

The dual isotope approach ($\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) has been applied to assess nitrate pollution since it can be used to trace nitrogen dynamics including identifying nitrate sources (Aravena et al., 1993; Kendall, 1998; Mayer et al. 2002; Matiatos, 2016) and tracing nitrogen transformation processes such as nitrification, assimilation, N_2 fixation and denitrification (Mariotti et al., 1988; Böttcher et al., 1990; Xu et al., 2016; Zhang et al., 2018).This approach is based on the realization that NO_3^- originating from different sources exhibits distinct isotopic compositions ("fingerprint"). Moreover, transformations between nitrogen chemical species are often induced by microbial activities, during which N and O isotopic fractionation may occur, and consequently, the original isotopic fingerprint of the source materials can be altered (Xu et al., 2016).

Even though biological cycling of nitrogen often changes isotopic ratios in predictable and recognizable directions that can be reconstructed from the isotopic compositions (Kendall, 1998; Kendall et al., 2007), interpretation of surface water $\delta^{15}N$ and $\delta^{18}O$-NO_3^- requires a more complex framework. This means that without additional independent information, there are several possible explanations for the data that are more complex than simply assigning a source of NO_3^- based on the $\delta^{15}N$ values or assigning a single process based on a simplistic pattern in the $\delta^{18}O$- vs $\delta^{15}N$-NO_3^- values (Venkiteswaran et al., 2019).

Biological transformations in these aquatic systems alter the original $\delta^{15}N$ and $\delta^{18}O$-NO_3^- valuein different ways than the traditional groundwater-denitrification model; hence, multiple N-cycling processes and sources interact to produce the values measured in surface waters (Thibodeau et al., 2013; Xu et al., 2016; Botrel et al., 2017; Venkiteswaran et al., 2019; Soto et al., 2019).The combination of a suite of environmental isotopes ($\delta^{15}N$- and $\delta^{18}O$-NO_3^-, $\delta^{18}O$-H_2O) (Venkiteswaran et al., 2019) in conjunction with other physical-chemical (*e.g.* Dissolved Oxygen-DO, Cl^-, NO_3^-) (Li et al., 2013; Yu et al., 2018; Zhang et al., 2018; Hu et al., 2019) and biological indicators such as chlorophyll-*a* and fecal coliforms (Vrzel et al., 2016; Briand et al., 2017; Archana et al., 2018) offers a powerful means of nitrogen source identification, which can be used to investigate human influences on inland waters, providing a good indication of nutrient inputs to the surface water bodies (Sugimoto et al., 2009).

Due to its characteristics, the Pampa Plain in Argentina is one of the most threatened areas in the world as a consequence of cultural eutrophication. It is an extended plain region with fertile soils and numerous wetland systems and very shallow lakes (Iriondo and Kröhling, 2007), which undergo environmental stress mostly due to excess nitrogen from human-related activities (mainly agriculture, urbanization and recreation). Moreover, considering the effluent-influent condition in most of these shallow lakes, groundwater pollution can be considered as another potential source of nitrate input to these aquatic systems, and, on the other hand, lake water quality status is expected to have an impact on groundwater quality.

Pampean shallow lakes play an important role in the equilibrium of physical and biological systems among several ecosystem services *i.e*. nutrient cycling, water and climate regulation, recreation, provision of habitat for resident and transient species, etc. Most of them contribute in the conservation of biodiversity by providing habitat for resident and transient species, allowing for their survival, feeding and reproduction. The lakes are considered highly vulnerable ecosystems, which can be deteriorated when the nutrient balance is disrupted due to human-induced activities.

The aim of this study is to investigate the eutrophication processes occurring in freshwater ecosystems by combining nitrate isotopes with conventional chemical and biological assessment approaches. Better identification of the nitrogen pollution sources and thorough understanding of the biogeochemical processes affecting local nitrogen concentrations can lead to the development of effective management practices to preserve water quality and remediation plans for rivers and lakes under stress (Mayer and Wassenaar, 2012; Yue et al., 2014).

2. STUDY AREA

The watersheds of three groundwater-dependent shallow lakes were studied: La Brava, Los Padres and Nahuel Rucá (Fig. 1). These ecosystems represent freshwater with different land use, soil and topographic characteristics, hydrological functioning and management regulations for natural resource protection (Table 1).They are temperate shallow, polymictic and permanent lakes and belong to Mar Chiquita Coastal Lagoon Watershed (10,000 km^2); the latter aquatic system belongs to a MAB Reserve (Man and Biosphere Program, UNESCO). The watersheds of La Brava and Los Padres lakes are surrounded by the Tandilia mountain ranges, *i.e.* a block-mountain system with a maximum elevation of 250 meters above sea level (m.a.s.l.), and the land use in the watersheds is mainly agricultural. The watershed of Nahuel Rucá Lake is located in a plain geomorphological environment, and the land use is mostly related to cattle farming. Each lake is recharged by one inflow stream, which forms in the mountains and flows through agricultural lands before discharging to the lake, and is discharged through an outflow stream.

From a hydrogeological point of view, the loess-like sediment Quaternary deposits presented in the region, called Pampean sediments, are the most important hydrogeological sequence. These sediments, ranging in thickness from 70-100 m, form an unconfined multilayer aquifer called Pampeano Aquifer with variable hydraulic conductivity values as a result of the heterogeneity in the grain size and the variable proportion of clay content (Martínez and Bocanegra, 2002). Post-Pampean sediments overlay the Pampean sediments or lie directly on basement rocks. They are made up of sandy, silty, clayey and calcareous sediments of fluvial-lacustrine, Aeolian and marine origin. These sediments, originating from Aeolian processes, are mainly located in the highest topographical areas. Conversely, those originating from other agents are restricted to the valleys of the area (Kruse et al. 1998). While the Pampeano sediments are considered the most relevant unit in hydrogeological terms due to its greater continuity and thickness, the Post-Pampean sediments are restricted to small thin patches, usually less than 5 m thick, found in high areas, which makes them poor groundwater reservoirs.

The lake watersheds host urban-rural land use areas with potential contamination sources for the water resources originating from the complete lack of sewerage network ("wastewaters") and the use of agricultural fertilizers, such as urea and diammonium phosphate ("Synthetic fertilizers leachate") (Baccaro et al., 2006; De Gerónimo et al., 2014; Bedmar et al., 2015; Lima et al., 2019). In addition, most of the population in the area does not have access to piped water supply and households often use other types of home drillings to extract water, which increases the risk of consuming non-potable groundwater due to high levels of nitrate (Lima et al., 2019).

The three shallow lake ecosystems under consideration have dissimilar land cover that could be associated to specific potential pollutant loads. In a 2.5 km-radius buffer from each lake, Romanelli et al., 2013 identified the dominance of agricultural activities in La Brava Lake (63.65 %), horticulture in Los Padres Lake (63.88 %; Horticultural Belt of Mar del Plata City with greenhouse and open-field cropping systems) and pastures with livestock in Nahuel Rucá Lake (44.15 %). Moreover, close to Los Padres and La Brava lakes there are two permanent villages (Los Padres and La Brava) with 1,672 and 115 permanent residents, respectively (INDEC, 2012), and many tourism-related facilities along their shores (Romanelli et al., 2014b). Nahuel Rucá Lake is located within two private farmlands with cattle-breeding as the dominant economic activity. Moreover, farm housing facilities are located close to the lake with complete lack of sewerage network and home drillings to extract groundwater. Overall, human-induced stresses to these lakes are mainly triggered by agricultural (non-point and continuous source), residential and recreational activities (point and discontinuous source).

3. MATERIALS AND METHODS

3.1 Sampling and analytical procedures

Water samples (groundwater N=24, streams N=9 and lakes N=11) from La Brava, Los Padres and Nahuel Rucá lake watersheds were collected in 2016-2017 for hydrochemical analysis (major ions, nitrate, Total Phosphorus-TP and phosphate) and environmental stable isotope analysis ($^{18}O/^{2}H-H_2O$ and $^{15}N/^{18}O-NO_3^-$). An additional sampling campaign was conducted in autumn and spring of 2018 (dry and wet periods particularly for this year, respectively) comprising 5 sampling sites in each lake ecosystem (1 inflow stream, 3 lake and 1 outflow stream samples) (Fig.2). Lake (N=29) and stream (N=20) subsurface water samples were obtained, and groundwater samples (N=24) were collected from mills, domiciliary and irrigation wells. The samples for nitrate isotope determinations were field filtered through 0.45 μm glass microfiber filters and stored in acid-rinsed 100 mL polyethylene bottles. Once the samples were collected, they were stored at 4 °C until further analysis.

Water temperature, pH and electrical conductivity (EC) were measured *in situ,* and additionally water clarity (secchi disk) and water depth at lake water sampling sites. Chemical analysis included major ions, nutrients and dissolved oxygen determination following standard methods (APHA, 1998).

Stable isotopes ($\delta^{18}O$ and $\delta^{2}H$) in water were analyzed by using a laser spectroscopy Liquid-Water Isotope Analyzer Los Gatos Research 45-EP at the Hydrochemical and Isotopic Hydrology Laboratory (Universidad Nacional de Mar del Plata). The analytical uncertainties were ±0.15 and ±1.0‰ for $\delta^{18}O$ and $\delta^{2}H$ respectively. The isotopic analysis for $^{15}N-NO_3^-$ and $^{18}O-NO_3^-$ were performed at the Isotope Hydrology Laboratory (IAEA) by using a newly developed Ti (III) reduction method (Altabet et al., 2019), a one-step chemical conversion method that employs Ti (III) chloride to reduce nitrate to N_2O gas in septum sample vials. The N_2O headspace was measured for ^{15}N and ^{18}O by coupling with an IRMS (Isoprime ID 100).The analytical uncertainties were ±0.2 and ±0.4‰ for $\delta^{15}N/\delta^{18}O\text{-}NO_3$, respectively. The stable isotope ratios were expressed in delta (δ) units and a per mil (‰) notation relative to an international standard. Values of $\delta^{15}N$ were reported relative to N_2 in atmospheric air (AIR) and $\delta^{18}O$, $\delta^{2}H$ values were reported relative to Vienna Standard Mean Ocean Water (VSMOW).

To characterize the N isotopic compositions of main potential sources involved, direct sampling and measurement of local soil and most commonly used chemical fertilizers were conducted. Three composite soil samples (1 - 20 cm depth) obtained by physically mixing three individual soil cores taken within each lake watershed, were collected from farmlands close to the lake and the inflow stream sampling sites. These sites were considered as representative of the dominant upstream soil type, which is likely to be the main source of soil organic N entering into the lake. Chemical fertilizers samples, including urea and Diammonium Phosphate, were obtained from markets and farmers. The soil samples were dried in an oven at 60 ºC for 48 h, homogenized to powder and quartered to determine the $\delta^{15}N$ values of the soil organic matter. Fertilizer samples were treated in the same manner as soil samples. The samples were analyzed at MaiMA Laboratory (University of Barcelona, Spain) using an Elementar Analyzer Flash EA 1112 coupled in a continuous flow to an IRMS Delta C Finnigan MAT with an interphase Conflo III Finnigan MAT (EA-CF-IRMS), with an analytical uncertainty of ±0.5 ‰ for bulk $\delta^{15}N$.

Additionally, surface water samples were analysed in the laboratory for: 1. Dissolved Oxygen (DO); 2. Phytoplankton *chlorophyll a, b* and *c* (Chl*a, b* and *c*) only in lakes; 3. Bacterial indicators of sanitary quality of water (Total-TC and Thermotolerant Coliforms-TtC *and Escherichia coli*-EC); and 4. Nitrifying bacteria (ammonium-oxidizing and nitrite-oxidizing bacteria, AOB and NOB respectively). The DO content was determined by the Winkler Method. The phytoplankton analysis included the collection of 2.5 l water sample in each lake sampling site to estimate chlorophyll *a, b* and *c* concentration. Samples were filtered and extracted in 90% acetone. The extract was analyzed in a spectrophotometer and the total Chl*a*, *b* and *c* concentrations were calculated following the trichromatic equations given by Jeffrey and Humphrey (1975) (in Jeffrey et al., 1997). Moreover, dominant phytoplankton species were identified by optical microscopy using appropriate bibliography. For microbiological determinations, water samples were collected in 250 ml sterile glass bottles, stored in a cool box and transported to the Laboratory of Microbiology (Universidad Nacional de Mar del Plata). Microbial enumeration was performed using the Multiple-Tube Fermentation Method, expressed as MPN/100 ml. For TC, TtC and EC enumeration, the five-tube fermentation methodology was implemented (APHA, 1998; Folabella et al., 2015) and for AOB and NOB, the three-tube fermentation methodology was used (Frioni, 1987; APHA, 1998).

3.2 Bayesian isotope mixing model

Expected values from the main nitrate sources to these three watersheds were combined from measured data and literature (Kendall, 1998). A Bayesian isotope mixing model (MixSIAR, Stock and Semmens 2013) has been implemented as an open-source R package and applied to estimate the relative contributions of natural and anthropogenic sources to the dissolved nitrate in the watersheds (Shi et al., 2019; Soto et al., 2019). An error structure of "process error" was used, which considers the variation in the isotope values of the mixture due to the sampling process. The sources included in the models were: (1) chemical fertilizers and soil organic NH_4^+ that is nitrified in the system from measured values, (2) wastewater from manure and urban systems (+20.0 ± 5.0 for $\delta^{15}N$ and +5.0 ± 5.0 for $\delta^{18}O$), and (3) atmospheric deposition (+5.0 ± 7.5 for $\delta^{15}N$ and +40.0 ± 5.0 for $\delta^{18}O$). The $\delta^{15}N$ and $\delta^{18}O$ values of dissolved nitrate for (2) and (3) were taken from literature (Kendall et al., 2007), as it was not possible to collect representative samples. From an agricultural point of view, it was not possible to isotopically distinguish ammonium from soil N and ammonium from fertilizers used in the area, and thus they were considered as a single source. There are two main chemical fertilizers used in the area (urea and DAP) even though their isotopic composition differentiates, in combination, they overlap with the isotope values of soils, hence, the mixing model will not be able to discriminate between these two possible sources. Since the proportional use of each fertilizer in the watershed is not known, it was not possible to discard one of these. The NO_3^- based fertilizers are of limited use in the study area, so they were excluded from the list of potential sources of nitrogen pollution. No isotope discrimination (*i.e.* isotopic differences between the initial and the remaining dissolved nitrate after biological processing) was assumed in the models for the inflow river samples since no biological reactions of denitrification and assimilation were detected in these rivers (see Results and Discussion). However, discrimination related to assimilation processes in the lake samples was incorporated in the model. The three Markov chain Monte Carlo (MCMC) lengths in each model were run with 100,000 iterations, removing 50,000 for burn-in and thinning by a factor of 25.

The models were conducted in the inflow river samples since potential isotope effects on the source isotopic compositions such as denitrification and assimilation as well as evaporation are not expected in a high degree (see Results and Discussion for further explanations in mixture sample selection). Proportional contributions of nitrate sources in the lake for the period 2016/2017 and 2018 were also estimated. In the mixing models, a factor of 33% (± 5%) was attributed to assimilation; this level of assimilation was arbitrarily selected to match the primary productivity (estimated by Chl *a*) and the percent of nitrate concentration reduction between inflow river water and lakes. Therefore theoretical fractionation factors for assimilation were incorporated into the models using Rayleigh isotope fractionation model below:

$$\delta_t - \delta_o \sim \varepsilon_{assimilation} * \ln(f) \qquad \text{(Eq. 1)}$$

Where δ_t is the isotopic composition of dissolved nitrate at a time t; δ_o is the initial isotopic composition of a N source; $\varepsilon_{assimilation}$ is the average fractionation factor associated to assimilation (from Table 1 of Granger et al. (2004); and f is the remaining nitrate fraction. Assimilation processes typically trigger enrichment of ^{15}N and ^{18}O in a ratio 1:1 (Granger et al., 2004), and so we used the same fractionation factor value for ^{18}O than the one estimated for ^{15}N.

4. RESULTS AND DISCUSSION

4.1 Hydrochemical data

Table 2 shows the ionic composition of groundwater, stream and lake samples within Los Padres, La Brava and Nahuel Rucá lake watersheds. The groundwater ionic composition in Los Padres and La Brava Lake watersheds was characterized by Mg-HCO_3 water type close to the Tandilia Range System, whereas downstream in the hilly area, Na-Mg-HCO_3 to Na-HCO_3 waters types dominated. In the case of Nahuel Rucá Lake Watershed the groundwater samples showed mainly a Na-Cl and less a Na-HCO_3-Cl water type. A common hydrogeochemical evolutionary trend along the regional flow path (Chevotarev, 1955) was noticeable, water types evolved from Mg-HCO_3 type in the recharge area (Tandilia Range System) to Na-Cl type towards the discharge area (ocean).

The water samples from Los Padres Lake showed a Na-Mg-HCO_3 water type, while in La Brava Lake it was mainly Na-HCO_3 type. Nahuel Rucá Lake composition ranged from a Na-HCO_3-Cl to a Na-Cl-HCO_3 water type. Groundwater is a significant water source to these three lakes, directly discharging into the lake bed and indirectly via the gaining inflow streams. In addition, in the case of La Brava Lake, it also receives a water contribution from the fissure system present in La Brava Range (western margin of the lake) (Romanelli et al., 2014b).

Related to nitrate content in water samples (*i.e.* groundwater, lakes and streams), Nahuel Rucá Lake Watershed had the higher values (+45.9 ± 37.09 mg/l) followed by Los Padres (+31.4

± 28.5 mg/l) and then by La Brava (+24.7 ± 12.5 mg/l) lake watersheds. A greater area of contribution due to Nahuel Rucá Lake Watershed hydrogeological position (discharge area) together with the existence of several N sources associated to agricultural and farming activities partly explained the highest nitrate concentrations in the watershed.

In 2018, the mean nitrate concentration in lake water were +7.0 ±4.56, +17.7 ±11.6 and +4.7 ± 2.6 mg/l in spring and +5.7 ± 2.01, +15.0 ± 9.0 and +6.4 ± 3.26 mg/l in autumn, for Nahuel Rucá, La Brava and Los Padres lakes, respectively. The nitrate concentrations in Nahuel Rucá and La Brava lakes were slightly higher in spring than in autumn which could be attributed to increased input from nitrogenous fertilizers through surface runoff. Particularly, Los Padres Lake is surrounded by the Horticultural Belt of Mar del Plata City which is characterized by greenhouse and open-field cropping systems. Several authors suggest that greenhouse production can have a smaller environmental impact compared to open-field crops due to limited transfer of agrochemical fertilizers outside the greenhouses (Muñoz et al., 2008; Boulard et al., 2011).These facts could explain the lesser nitrate input to the lake during the wet season.

The nitrate concentrations in the lakes were lower than in their water inputs (inflow streams and groundwater) (Fig.3), indicating that nitrate attenuation processes such as assimilation by autotrophic organisms or denitrification might be taking place.

The ratio of NO_3^-/Cl^- was used to identify mixing and nitrate retention processes, such as denitrification and assimilation (Liu et al., 2006; Li et al., 2013; Yu et al., 2018). Animal manure, sewage, and urban wastewater discharge show high Cl^- concentrations and low NO_3^-/Cl^- values, whereas agricultural effluents typically have low Cl^- concentrations and high NO_3^-/Cl^- values (Liu et al., 2006).The values of NO_3^-/Cl^- can also decrease through denitrification (Li et al., 2013). Figure 4 shows the variations in the NO_3^-/Cl^- ratios of surface water and groundwater samples. The relative low NO_3^-/Cl^- values observed in the lakes and their outflow stream samples (with the exception of the outflow stream samples from La Brava Lake) were attributed to nitrate reduction processes such as denitrification and assimilation that decreased the nitrate concentrations. The high NO_3^-/Cl^- values and low Cl^- content in the groundwater from Los Padres and La Brava lake watersheds showed that nitrate pollution comes from agricultural activities, whereas in the case of groundwater from Nahuel Rucá Lake Watershed, the high Cl^- content and low NO_3^-/Cl^- ratios indicated that animal manure and/or domestic effluents are probably the dominant sources of nitrogen pollution.

4.2 Biological data in surface water

4.2.1 Fecal coliforms

All surface water samples showed detectable bacterial presence (total and fecal coliforms and *E. coli*) (Fig. 5), which evidenced water pollution from animal or human. Both inflow and outflow streams exhibited the highest bacterial values compared to lake water samples. This is probably because, the inflow streams flow through agroecosystems previous to their discharge into the lakes and the outflows streams (except for El Sotelo Stream-SSTC) flow close to human settlements.

The highest bacterial values for the lake water samples were detected close to the waterbird breeding colonies (gulls, herons and egrets), which are located in the inflow stream delta in the case of Los Padres (WLP002) and La Brava (WLB038) lakes, and in the NE sector of Nahuel Rucá Lake (WNR001 and WNR002). Excessive nitrogen loads can increase the survival time and recovery rate of the indicator *E. coli* in water (Telesford-Checkley et al., 2017). In Nahuel Rucá Lake, the very high *E. coli* bacteria detected at WNR003 site were attributed to feces from livestock activities, whereas the smaller values were linked to sewage effluents from fishing clubs.

4.2.2 Nitrifying bacteria

Nitrification consists of the two-step process of NH_4^+ oxidation to NO_3^- mediated by several different autotrophic bacteria or archaea. The first step is mediated by ammonium-oxidizing bacteria (AOB) and produces NO_2^-, which in turn is oxidized to NO_3^- by nitrite-oxidizing bacteria (NOB) (Sugimoto et al., 2009). Nitrite and ammonia provide good indicators of recent fecal contamination (Debela et al., 2018). Nitrifying bacteria were detected in all lakes and streams (Figure 6). The highest concentrations were recorded close to the waterbird colony which is probably related to N- avian input or cattle breeding farms (livestock manure N input) which trigger increased microbial activity. Nahuel Rucá Lake showed the highest nitrifying bacteria values during all sampling periods compared to the other shallow lakes, which was attributed to intensive livestock activities around the lake, together with N-synthetic fertilizers leachate, leading to greater availability of substrates for the nitrifying bacteria (Vallejo et al., 2011).

All lake samples showed the highest NO_2^- values during spring, probably due to the temperature effect on bacteria growth. Nahuel Rucá Lake showed the highest NO_2^- values during all sampling periods compared to the other lakes. However, the nitrifying bacteria concentrations did not show any seasonal pattern.

4.2.3 Phytoplankton community

Based on phytoplankton community results and according to TP, water clarity and phytoplankton Chl-a values (Table 3) (Carlson, 1977) the three shallow lakes were classified as turbid, high productive and eutrophic systems (being LP > LB > NR). The phytoplankton composition evidenced the presence of cyanobacteria bloom (*Anabaena aphanizomenoides)* in Los Padres Lake during most sampling periods (with the exception of autumn 2018) and in La Brava Lake during samples collected in 2017. *Anabaena aphanizomenoides*, a filamentous N^2-

fixing cyanobacteria species, was associated to the algae blooms in both waterbodies. The rest of the lake water samples showed the dominance of centric diatoms such as *Cyclotella meneghiniana* and *Navicula spp.,* and chlorophytes such as *Staurastrum sebaldi* and *Cosmarium formosulum*, among others.

The absence of cyanobacterial blooms in Nahuel Rucá Lake was evident. High electrical conductivity and sulfate values in some eutrophic lakes, like the latter aquatic system (Table 2), attributed to saline groundwater inflow, may inhibit algae and cyanobacterial growth, thereby promoting healthier conditions (Gibson et al., 2016). Phytoplankton from Nahuel Rucá Lake was dominated during all the study period by centric diatoms such as *Aulacoseira granulata* and *A. granulata var. angustissima*. The presence of these species in shallow lakes is linked to well mixed and moderately eutrophic waters (O' Farrell et al., 2001). A more diverse phytoplanktonic community was also observed, with the presence of benthic diatoms such as *Surirella robusta* and *S. ovalis*, among others. This waterbody, in comparison to Los Padres and La Brava lakes, had the lowest depth and water clarity, the latter mainly caused by sediment resuspension. The low light penetration and the sediment resuspension might have promoted the presence of benthic taxa in phytoplankton, mainly represented by pennate diatoms (Pacheco et al., 2010).

In this work, the estimated phytoplanktonic chlorophyll *a*, *b* and *c* concentrations also confirmed the community composition in each shallow lake. When cyanbacteria dominate the water column (as in the case of Los Padres Lake) chlorophyll *a* values are higher than chlorophylls *b* and *c*. On the other hand, when phytoplankton is diverse with taxa of different algal divisions (*e.g.* chlorophytes and euglenophytes mainly with Chl *b*, diatoms among others, mainly with Chl *c*) or dominated by diatoms (as in Nahuel Rucá Lake or in some occasions in La Brava Lake) no notorious differences among chlorophyll *a*, *b* and *c* are evidenced.

4.3 Sources and fate of nitrate in aquatic ecosystems

The dual-isotopic composition of NO_3^- (Fig. 7A) in Los Padres and La Brava lake watersheds suggested that soil organic N, manure and urban wastewater were the dominant nitrate sources in most of the water samples. All water samples from Nahuel Rucá Lake Watershed showed the highest $\delta^{15}N$ and $\delta^{18}O$ values in comparison to the other two lake watersheds, indicating that manure and septic wastes should be the dominant sources of nitrogen pollution for the watershed. Besides the predominant land-use (pastures with cattle-breeding), the geomorphology (plain area) and the hydrogeological position of the lake watershed (discharge area) could also influence the isotopic enrichment of groundwater samples; low hydraulic gradients and groundwater velocities can potentially decrease DO levels favoring denitrification.

Very few samples, WLP001, WNR003 and SSTC (Fig. 7 A) fell into the overlapping isotopic values of nitrate-based and ammonium-based synthetic fertilizers. This preliminary interpretation is misleading, because synthetic fertilizers have been used in the area. We believe that the isotopic signal from the fertilizers in the lake has been partly masked by biogeochemical processes (Saccon et al., 2013) except for sampling sites with direct N input from point sources within the lake such as waterbird colonies and cattle-breeding.

The presence of fecal coliforms in surface water samples, evidencing water pollution from animal or human, was consistent with $\delta^{15}N$ NO_3 values which overlap the range of expected known values reported for nitrate for septic systems (+8 - +10‰) (Aravena et al. 1993; Martínez et al, 2014) and from avian inputs. Since waterbirds use these three lakes to feed, rest or nidify in so they could provide an important amount of nitrates through their depositions, which could increase the $\delta^{15}N$ values in these aquatic ecosystems (Sebastián-González et al., 2012). Ornithogenic nitrogen is ^{15}N-enriched due to the isotopic fractionation occurring during volatilization of the ammonia from bird feces. Consequently, the stable nitrogen isotope ratio ($\delta^{15}N$) is a proxy for ornithogenic influence. Gull guano isotopic signature varied seasonally, ranging from +8.2 to +14.7‰ for $\delta^{15}N$ (Vizzini et al., 2016). This isotopic signature was similar to the values detected in those lake sites located close to the waterbird breeding colonies location according to Josens et al., 2009 (+8.0, +8.6 and +7.9‰ for $\delta^{15}N$ in WLP002, WLP038 and WNR001, respectively), indicating that avian input could be another nitrate source of certain importance in some localized areas of the lake. Particularly in Nahuel Rucá Lake, the highest $\delta^{15}N$ value detected in WNR003 (+11.5 ‰) together with the high *E. coli* levels, suggested a livestock manure- N input from the cattle breeding farm located next to the lake.

Regarding the isotopic composition of dissolved nitrate during 2018 sampling (Fig. 7A, see supplementary information), all samples collected during spring (wet period) evidenced the highest $\delta^{18}O$ values (> 10 ‰) in comparison to samples from autumn. Since the main synthetic fertilizers applied in the region are urea and diammonium phosphate (DAP), nitrate-based fertilizers were not expected to be widely used in the study area. Therefore, the high $\delta^{18}O$ values were attributed to atmospheric deposition inputs (see Supplementary information). The pattern of decreasing NO_3^- concentrations in lakes and streams was also consistent with proportionally increasing in precipitation.

Seasonal and spatial variation between the sites can be difficult to compare because of the variability in $\delta^{18}O\text{-}H_2O$ since the $\delta^{18}O\text{-}NO_3^-$ axis is reported relative to the typical standard VSMOW. So, nitrate isotope biplot of the same data where the $\delta^{18}O\text{-}NO_3^-$ axis is reported relative to the ambient $\delta^{18}O\text{-}H_2O$ values (Venkiteswaran et al., 2019) in the water samples at the same time of sampling was done (Fig. 7B), Here, data are more clearly expressed relative to the appropriate environmental conditions. In this sense, appropriate site-specific ranges of $\delta^{18}O\text{-}NO_3^-$ produced *in situ* were obtained for the three watersheds. Most of the samples were distributed below the value where the $\delta^{18}O\text{-}H_2O$ is entirely retained in the $\delta^{18}O\text{-}NO_3^-$. Hence, 100% contribution of $\delta^{18}O\text{-}H_2O$ is not the case in our study and gives support to use 2: 1 and 3: 1 scenarios (see below).

Spatial $\delta^{15}N$ variation in lake watersheds are shown in Fig 8. In Los Padres Lake watershed $\delta^{15}N$ values increased along the inflow stream flow direction. Moreover, the $\delta^{15}N$ content in the southern coastline of Los Padres Lake (+8.0 ‰,) was similar to the $\delta^{15}N$ signature of the inflow stream (+8.6 ‰). Even though groundwater is also a recharge water source to the aquatic system, it exhibited elevated $\delta^{15}N$ values (+12.51 ‰) upstream. Mixing of variable "external" and "internal" sources could explain lake composition. In the case of La Brava Lake Watershed, an increase in $\delta^{15}N$ along the groundwater flow system direction is evident from the recharge to the discharge area (lake). La Brava Lake in its southern coastline had a similar $\delta^{15}N$ fingerprint to those upstream groundwater samples, indicating nitrate delivered by groundwater input due to its gaining condition. For Nahuel Rucá Lake, the $\delta^{15}N$ values in the southwestern margin were similar to the mean $\delta^{15}N$ value of the upstream groundwater (+13.9 ‰), which indicated the nitrate input from groundwater to the lake.

While groundwater can be identified as a potential non-point nutrient source (Barton et al., 2013), its role remains poorly understood. This is partly due to the difficulty in quantifying groundwater nutrient loading in surface waters (Burnett et al., 2003) *(i.e.* groundwater discharges to surface waters either by indirect discharge into tributaries or direct discharge into the lakes) and also because groundwater is not a single source (*i.e.* multiple N-cycling processes and sources interact to produce the values measured in groundwater) (Grannemann and Van Stempvoort, 2016).

4.4 Biogeochemical processes

4.4.1 Nitrate production processes: nitrification

For a long time, it has been argued that up to 1/3 of the O atoms in regenerated nitrate derives from DO (Andersson and Hooper 1983; Hollocher 1984). However, more recent work has shown that the relative contribution of ambient O from surrounding water and O_2 could deviate from the rule of 2:1 (Mayer et al., 2002; Casciotti et al., 2010; Snider et al., 2010; Mongelli et al., 2013). Without the role of other reactions/processes, the contribution of water:oxygen to the final $\delta^{18}O\text{-}NO_3^-$ value could vary from 2:1 (0.66 H_2O - 0.33 O_2) to 3:0 (1.0 H_2O – 0.0 O_2) (Snider et al., 2010; Venkiteswaran et al., 2019). This range of values includes values such as 3:1 (0.75 H_2O – 0.25 O_2), which is a middle value case between the two extreme cases. Regardless of the exact mechanisms that determine the $\delta^{18}O$ of newly-nitrified nitrate, the $\delta^{18}O$ of water leaves its imprint in the $\delta^{18}O$ of nitrate that has been re-generated. Oxygen exchange with H_2O during nitrification reduces or eliminates the isotopic signal from the O_2 incorporated during the initial NH_4^+ oxidation step, thereby producing NO_3^- with $\delta^{18}O$ values closer to those of the available H_2O.

The estimation of the theoretical values for nitrification based on a contribution of 2:1 for water:oxygen and using local water $\delta^{18}O\text{-}H_2O$ data (Supplementary information) was performed for each inflow stream and groundwater sample from the three watersheds. The ^{18}O isotope signature was relatively constant in time for the nitrate processed through nitrification ($\delta^{18}O\text{-}NO_3$ = 4.4 ± 0.6 in Los Padres, 4.4 ± 0.5 in La, and 5.0 ± 0.6 in Nahuel Rucá lake watersheds, respectively) and these results were relatively similar than the expected values. On the contrary, when measured $\delta^{18}O\text{-}H_2O$ data from lake samples were used, the theoretical values for the lakes were slightly higher than those estimated by using inflow river and groundwater sample data ($\delta^{18}O\text{-}NO_3$ = 5.8 ± 0.1 in Los Padres, 5.7± 0.3 in La Brava, and 7.5 ± 0.6 in Nahuel Rucá lakes, respectively). This seems to be related to evaporation processes taking place in these lakes (*i.e.* ^2H and ^{18}O enrichment in lake samples compared to other samples).

A cross plot of $\delta^{18}O\text{-}NO_3^-$ for nitrification versus measured $\delta^{18}O\text{-}NO_3^-$ considering both scenarios of contribution 2:1 and 3:1 for water:oxygen was done; since there is uncertainty associated to the $\delta^{18}O\text{-}O_2$ values and to the level of water contribution, the 3:1 contribution was arbitrarily chosen for the comparison exercise. No significant differences were detected when changing the amount of atoms that come from water or O_2 (Fig. 9); however, this highlights the importance of analysing $\delta^{18}O$-O_2 values in future studies for a better interpretation of NO_3 isotope data(Wassenaar and Koehler 1999).

Most of the samples showed a significant deviation from the 1:1 line. This finding was explained by considering additional processes responsible for the enrichment (*e.g*. through denitrification, assimilation and/or mixing with deposition) or depletion (*e.g.* wide range of $\delta^{18}O$-O_2 values in high productive lakes) of isotopic values in each direction. The $\delta^{18}O$-NO_3 values, which were lower than the theoretical values of nitrification can be explained by considering that in surface water the $\delta^{18}O$-NO_3^- is a function of the isotopic composition of dissolved oxygen, which can vary according to productivity patterns (P:R ratios) (Venkiteswaran et al., 2007).Therefore, this variation associated to $\delta^{18}O$ values of dissolved O_2 and its relationship with the isotopic composition of the water ($\delta^{18}O$–H_2O) can drive the decreasing pattern (Snider et al., 2010; Venkiteswaran et al., 2019). Regarding lake samples from 2018, all of them were located above the 1:1 line evidencing a mixing with atmospheric deposition, with the highest $\delta^{18}O$-NO_3^- values during spring probably due to the increase of rainfall values.

4.4.2 Nitrate removal processes

4.4.2.1 Groundwater denitrification

All measured isotope ratios for groundwater samples are plotted on diagrams of $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- in Figure 10. Denitrification processes in surface water was excluded as a possible source of transformation as all samples had DO > 2 mg/L (4.5 to 8.8mg/l) (Table 3), a scenario that does not favor microbial removal of NO_3^- by denitrification (Pina-Ochoa and Alvarez-Cobelas, 2006; Kendall et al., 2007; Zhang et al., 2018).

Keeling plots ($\delta^{15}N$-NO_3^- versus $1/[NO_3^-]$) are generally very useful to distinguish between mixing and fractionation processes (Kendall et al., 1998; Wong et al., 2018). The latter typically results in progressively increasing $\delta^{15}N$-NO_3^-values as NO_3^- concentrations decrease and yields a positive linear relationship. Meanwhile, mixing of NO_3^- from two or more sources can result in concomitant increase of both $\delta^{15}N$-NO_3^- and NO_3^- concentrations and results in (1) a linear negative trend (*e.g.* mixing of one source with low NO_3^- concentration and $\delta^{15}N$ values and another one with high nitrate levels and $\delta^{15}N$ values), but also in (2) a straight line with a slope close to zero (*i.e.* other scenarios of mixing). These plots for groundwater revealed that $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- were positively correlated with $1/[NO_3^-]$ suggesting denitrification processes in the aquifer (Fig. 10 A, B and D). However, we assumed that these relationships were linear due to the lack of larger sample sizes for accurate non-linear relationship modeling.

Positive relationships between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- were detected at different extents in groundwater samples from the three lake watersheds and the obtained slope values were between 0.4 and 0.9, which overlap the range of expected known values reported for denitrification (0.5-0.8) (Böttcher et al., 1990; Aravena and Robertson, 1998; Granger and Wankel, 2016).The linear relationship between $\delta^{15}N$ and $\delta^{18}O$ values provides additional unambiguous evidence that denitrification is responsible for nitrate concentration decline and enrichment in nitrogen isotope values in the Pampeano Aquifer. Deviations in $\delta^{18}O$-NO_3^- vs. $\delta^{15}N$-NO_3^- from expected 1.3 to 2.1 for denitrification (Böttcher et al., 1990) are explained by isotopic over-printing from coincident NO_3^- production by nitrification (*i.e.* relatively lower $\delta^{18}O$-H_2O values, characteristic of freshwater systems, which contribute to lower $\delta^{18}O$ values for nitrified NO_3^-) (Granger and Wankel, 2016).

4.4.2.2 Algal growth in the lakes: assimilation occurrence

Both the chemical and isotopic characteristics in addition to the presence of enough DO levels (> 4.53 mg/l) clearly indicated that the signs of denitrification were absent in pelagic waters. However, it is important to highlight that denitrification it is likely to occur in the lake bed but the data show that the proportion of nitrate removed by denitrification in the whole aquatic system is of low consideration (*i. e*. the amount of denitrified nitrate in the lake bed is minimal with respect to the total amount of nitrate dissolved and, therefore, this process is not reflected in the isotopic composition).

While it is unlikely that denitrification is playing a major role in lake samples, there are certain indicators of assimilation. Combined with information on NO_3^-/Cl^- (low ratio), the pattern of decreasing NO_3^- concentrations was consistent with proportionally increasing Chl*a* concentrations (Fig. 11) during 2016-2017 and 2018 samplings, which could be attributed to a nitrate retention process (*i.e.* assimilation). Even though the existence of elevated Chl*a* suggests the presence of phytoplankton that would be capable of assimilating N, preferentially ^{14}N, a negative relationship between ^{15}N and Chl*a* was evident in most of the cases, so isotopic compositions are not solely influenced by assimilation and includes the isotopic 'fingerprint' of other processes. Therefore, lake samples were included in the evaluation of nitrate sources of contamination in mixing models assuming the fractionation due to assimilation.

4.5 Quantification of the relative contributions of main potential NO_3^- sources to the watersheds

The relative contribution of N sources to the dissolved nitrate fraction was estimated using Bayesian isotope mixing models and, for this purpose, the sources of ammonium nitrified in the system from soil and fertilizers were investigated as a whole. These sources related with nitrification processes were the predominant contributor to the dissolved nitrate pool in the three watersheds, indicating that agricultural practices have a relevant role in the eutrophication and water quality deterioration for these watersheds (Table 4). Nonetheless, there is a large spatial and temporal variability in these watersheds. For inflow water samples from La Brava and Nahuel Rucá lake watersheds, the influence of wastewater (from sources such as domestic sewage and cattle farming) and of atmospheric deposition is higher on average (~40% and 15%, respectively) relative to Los Padres inflow waters (~20% and 5%, respectively). But, these differences are not significant considering all samples together from distinct periods of time. In general, for all models, the variation associated to the contribution estimations are relatively high making significant spatial and temporal differences for one source limited likely due to the low sample size for each grouping. In the temporal point of view, we highlight the increase (Δ ~20%) of average proportional contribution of atmospheric deposition over time and, in a lesser extent, of wastewater for La Brava inflow lake samples; and with opposite trends on the extent for Los Padres inflow lake samples.

Lake watersheds receive water inputs form the inflow waters and groundwater with a certain level of nitrate contamination, and this nitrate will be ultimately transformed to algal particulate material (Chl *a* as a proxy) via assimilation processes. Taking into consideration the effects on the isotopic composition of the remaining nitrate after being assimilated by the algae in our models, we estimated that again the agricultural and soil ammonium nitrified in the watershed seems to be the predominant contributor for all sites and years (~ 60-90%). Some patterns were also determined. A slight tendency of higher wastewater contribution in Nahuel Rucá lake was found in the period 2016/2017, but an opposite trend was determined in 2018 for the mean values

(NR < LB < LP). This is in good agreement with the results in a temporal scale from inflow water samples.

It is challenging to understand and quantify the N inputs using only isotopic information into Bayesian mixing models since it could be unclear whether the source term has been altered, due to the variety of processes involved in N transformations, or not, by simple source apportionment assumptions commonly made in dual nitrate isotope biplots. In that sense it is important to recognize the usefulness of having other proxies to constrain the phenomena such as geochemical and biological data. Moreover, measuring locally appropriate sources of nitrogen as potential initial $\delta^{15}N$ values is the appropriate way to constrain the ^{15}N axis in the biplot instead of relying on the broad assumption that a single set of boxes, derived from a limited number of measurements, are globally appropriate (Bateman and Kelly, 2007; Venkiteswaran et al., 2019). In addition, it is important to combine $\delta^{18}O\text{-}H_2O$ values (and $\delta^{18}O\text{-}O_2$) with $\delta^{18}O\text{-}NO_3$ of the aquatic system (at the same time of sampling) since data are more clearly expressed relative to the appropriate environmental conditions that recognize that nitrogen is biologically cycled and will be largely imprinted with the ambient $\delta^{18}O\text{-}H_2O$ (Venkiteswaran et al., 2019).

Under this approach, considering the other proxies (NO_3^-, Cl and DO content together with *E. coli* levels and Chl *a* in phytoplankton), the locally relevant $\delta^{15}N$ source values (Table 5) and with site-specific ranges of $\delta^{18}O\text{-}NO_3$ produced *in situ*, it has been possible to select and define the appropriate inputs to the Bayesian mixing models considering in this study. The use of Bayesian models is a promising tool because uncertainties associated to isotope measurements and expected processes occurring (*i.e*. discrimination) can be incorporated.

CONCLUSIONS

The nitrate values in lakes, streams and groundwater exhibited a high dispersion (8.0 to 166 mg/l) implying spatial variation and different intensity of water degradation due to nitrogen pollution. The dual isotope approach (^{15}N vs. ^{18}O) showed that surface water and groundwater samples undergo quality deterioration due to nitrogen contamination originated from agricultural and soil ammonium nitrified in the watershed, manure and or septic waste and in a lesser extent atmospheric deposition. Other N sources to the lakes are less well constrained, but could include N_2 fixation either in the lakes (cyanobacterial blooms dominated by N_2-fixing species *Anabaena* were detected during all sampling periods) or on field crops from the surrounding lands, and avian inputs.

Lakes receive water inputs from the inflow waters and groundwater with a certain level of nitrate contamination. Nitrate isotope variations observed in the three aquatic systems are mostly influenced by sources of ammonium nitrified in the system from soil and fertilizers, however, several differences among the different shallow lakes were supported through limnological, bacterial and algal studies carried on simultaneously. Nahuel Rucá Lake was characterized by the lowest water clarity, depth and DO values with a dominance of centric and benthic diatoms in the phytoplanktonic community (lowest productivity), and the highest nitrifying bacteria abundance. Besides, high *E. col* levels and high Cl^- in the lake, together with the Bayesian model results, indicated that animal manure is also an important source of nitrogen pollution to the system, with a lesser extent during dry periods. La Brava Lake presented the highest water clarity and depth, intermediate DO and nitrifying bacteria concentrations and, the presence of cyanobacteria bloom (2017) and centric diatoms (intermediate productivity). In addition, low *E. coli* levels and low Cl- concentrations within the lake along with mixing model estimations supported that domestic sewage was not a significant contributor to the present dissolved nitrate in the la*ke.* Finally, Los Padres Lake evidenced the presence of cyanobacteria bloom during most of the sampling periods (highest productivity) with the highest DO values and moderate nitrifying bacteria abundance; agricultural practices have a relevant role in the eutrophication and water quality deterioration of the system.

Dual nitrate isotope approach has provided some evidence of source tracking and processing in many natural case studies. Nonetheless, the use of dual isotope approach only is certainly limited for proper interpretation with the knowledge we have nowadays. Aquatic ecosystems are complex structures where different processes and contamination source mixing is very likely occurring at the same time. Thus, the combined use of different environmental parameters and chemical/isotopic tracers give a more realistic point of view for what is happening. The use of Bayesian models is a promising tool because uncertainties associated to isotope measurements and expected processes occurring (*i.e.* discrimination) can be incorporated. This is a novel technique used in this study in order to understand these systems easily.

Nitrogen biogeochemical processes were identified in surface water and groundwater based on different correlations between isotopic, hydrogeochemical and biological parameters, such as: $\delta^{15}N\text{-}NO_3^-$vs.$\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N/\delta^{18}O\text{-}NO_3^-$ vs. NO_3^- and $\delta^{15}N\text{-}NO_3^-$ vs. $1/[NO_3^-]$ for denitrification; phytoplankton Chl *a* vs. $\delta^{15}N\text{-}NO_3$, Chl*a* vs. NO_3^- for assimilation, and $\delta^{18}O\text{-}NO_3$ vs. $\delta^{18}O\text{-}H_2O$ for nitrification. This combined approach shows that denitrification plays a role in the nitrate concentration pattern observed along the groundwater flow system in the Pampeano Aquifer. However, other attenuation processes such as assimilation was present in these lakes.

Both nitrate isotopes and fecal contamination indicators confirm that there is serious health risks related to fecal pollution in these lake waters, which leads to exposure to pathogens via recreation and, considering the losing condition of lakes downstream, also via irrigation or human/animal water consumption from groundwater of the watershed.

Data resulting from this work has multiple impacts: firstly, it serves as an important baseline for nitrate isotope studies of eutrophication in surface water, being used for the first time in the country. Secondly, it will inform environmental managers, and policy makers of the most important sources of nitrogen pollution and N transformation processes, which may be considered targets for future mitigation strategies. Thirdly, it provides a new multidisciplinary approach to improve regional surface water quality management.

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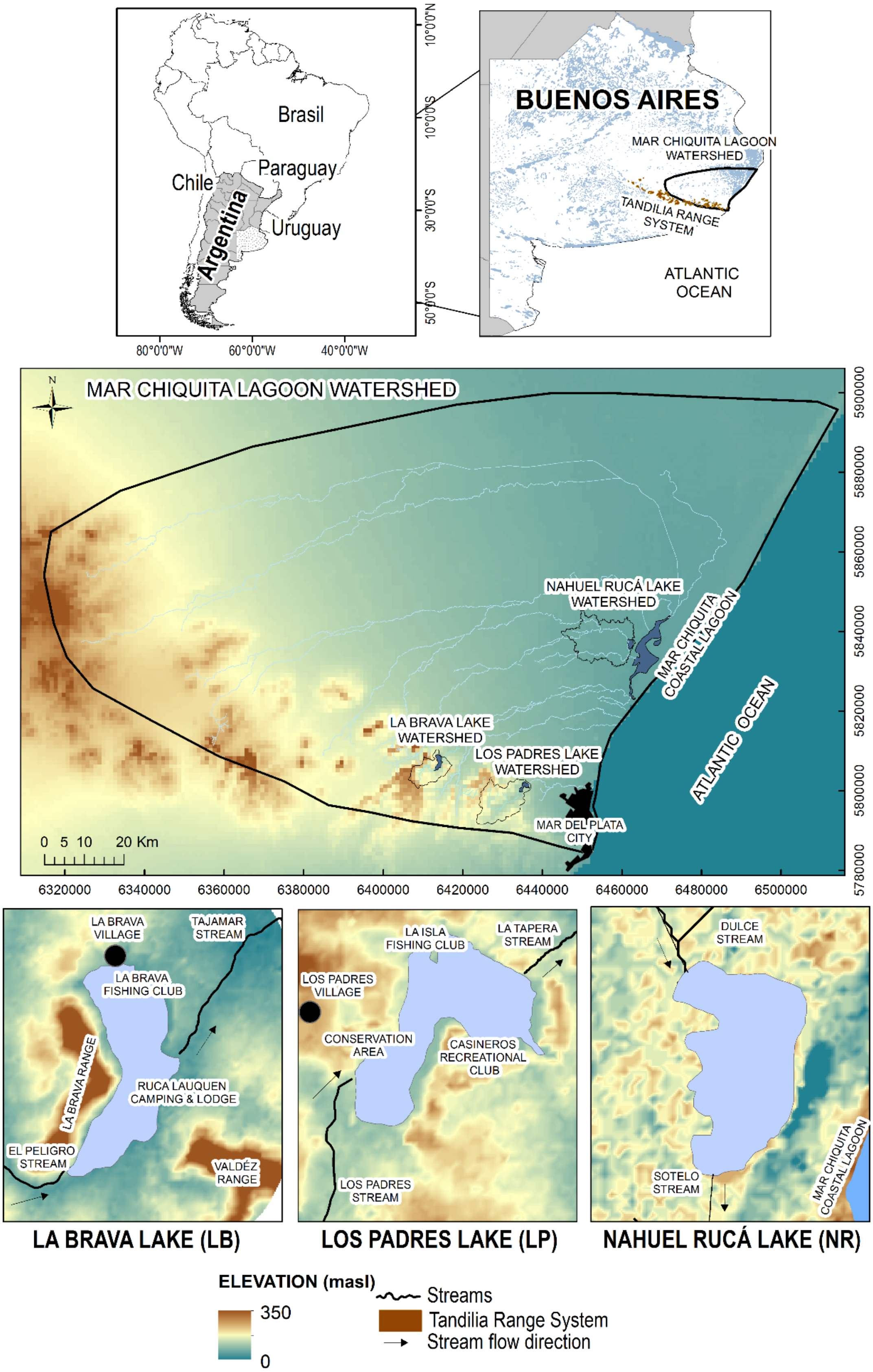
Figure 1



Brasil
Paraguay
Chile
Argentina
Uruguay
10°0'0"N
10°0'0"S
30°0'0"S
50°0'0"S
80°0'0"W
60°0'0"W
40°0'0"W
BUENOS AIRES
MAR CHIQUITA LAGOON WATERSHED
TANDILIA RANGE SYSTEM
ATLANTIC OCEAN
MAR CHIQUITA LAGOON WATERSHED
NAHUEL RUCÁ LAKE WATERSHED
MAR CHIQUITA COASTAL LAGOON
ATLANTIC OCEAN
LA BRAVA LAKE WATERSHED
LOS PADRES LAKE WATERSHED
MAR DEL PLATA CITY
0 5 10 20 Km
6320000
6340000
6360000
6380000
6400000
6420000
6440000
6460000
6480000
6500000
5900000
5880000
5860000
5840000
5820000
5800000
5780000
LA BRAVA VILLAGE
TAJAMAR STREAM
LA BRAVA FISHING CLUB
LA BRAVA RANGE
RUCA LAUQUEN CAMPING & LODGE
EL PELIGRO STREAM
VALDÉZ RANGE
LA ISLA FISHING CLUB
LA TAPERA STREAM
LOS PADRES VILLAGE
CONSERVATION AREA
CASINEROS RECREATIONAL CLUB
LOS PADRES STREAM
DULCE STREAM
SOTELO STREAM
MAR CHIQUITA COASTAL LAGOON
LA BRAVA LAKE (LB)
LOS PADRES LAKE (LP)
NAHUEL RUCÁ LAKE (NR)
ELEVATION (masl)
350
0
Streams
Tandilia Range System
Stream flow direction

Figure 2

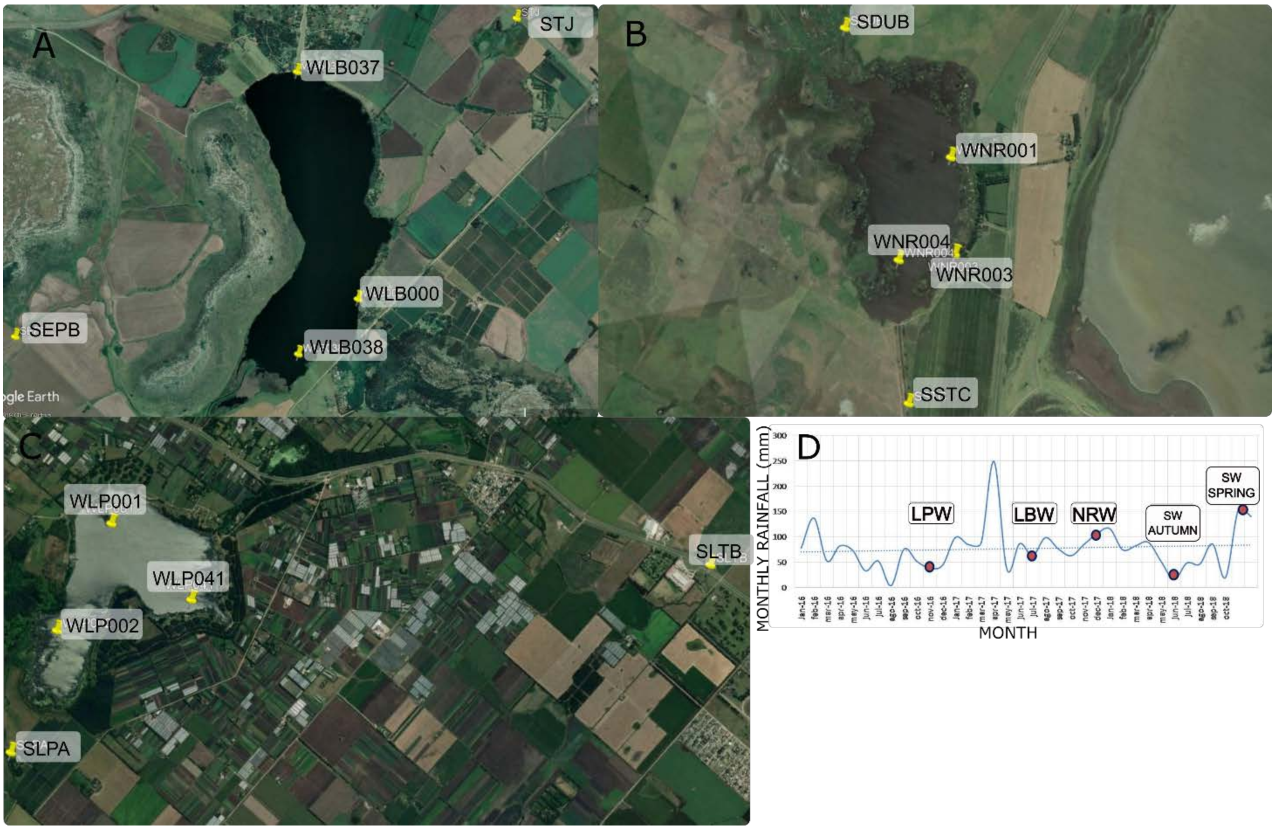
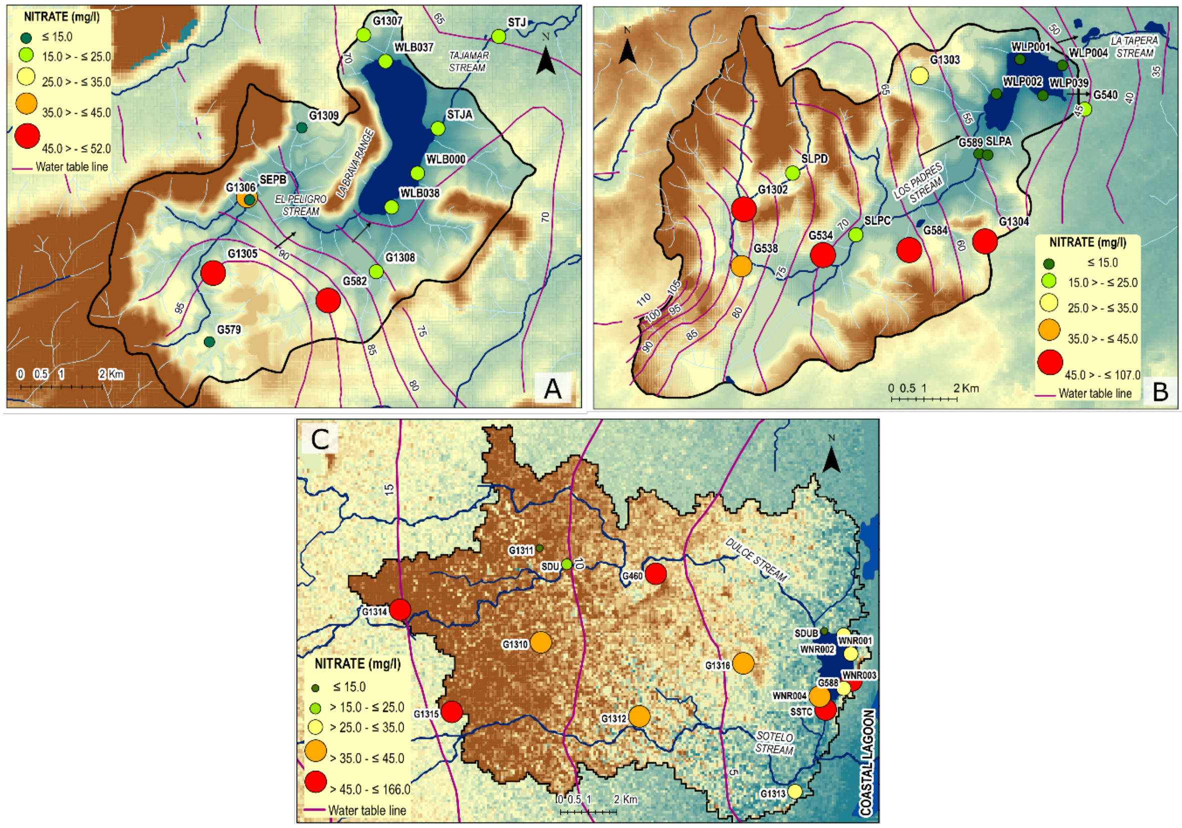

A
STJ
WLB037
WLB000
SEPB
WLB038
B
SDUB
WNR001
WNR004
WNR003
SSTC
C
WLP001
WLP041
WLP002
SLPA
SLTB
D
MONTHLY RAINFALL (mm)
MONTH
LPW
LBW
NRW
SW AUTUMN
SW SPRING

Figure 3



NITRATE (mg/l)
≤ 15.0
15.0 > - ≤ 25.0
25.0 > - ≤ 35.0
35.0 > - ≤ 45.0
45.0 > - ≤ 52.0
Water table line
G1307
STJ
WLB037
TAJAMAR STREAM
G1309
STJA
LA BRAVA RANGE
WLB000
G1306
SEPB
EL PELIGRO STREAM
WLB038
G1305
G1308
G582
G579
0 0.5 1 2 Km
A
G1303
WLP001
WLP004
LA TAPERA STREAM
WLP002
WLP039
G540
G589
SLPA
LOS PADRES STREAM
SLPD
G1302
SLPC
G534
G584
G1304
G538
NITRATE (mg/l)
≤ 15.0
15.0 > - ≤ 25.0
25.0 > - ≤ 35.0
35.0 > - ≤ 45.0
45.0 > - ≤ 107.0
Water table line
0 0.5 1 2 Km
B
C
G1311
SDU
G460
DULCE STREAM
G1314
G1310
SDUB
WNR001
WNR002
G1316
WNR003
NITRATE (mg/l)
≤ 15.0
> 15.0 - ≤ 25.0
> 25.0 - ≤ 35.0
> 35.0 - ≤ 45.0
> 45.0 - ≤ 166.0
Water table line
WNR004
G588
SSTC
G1315
G1312
SOTELO STREAM
G1313
0 0.5 1 2 Km
COASTAL LAGOON

Figure 4

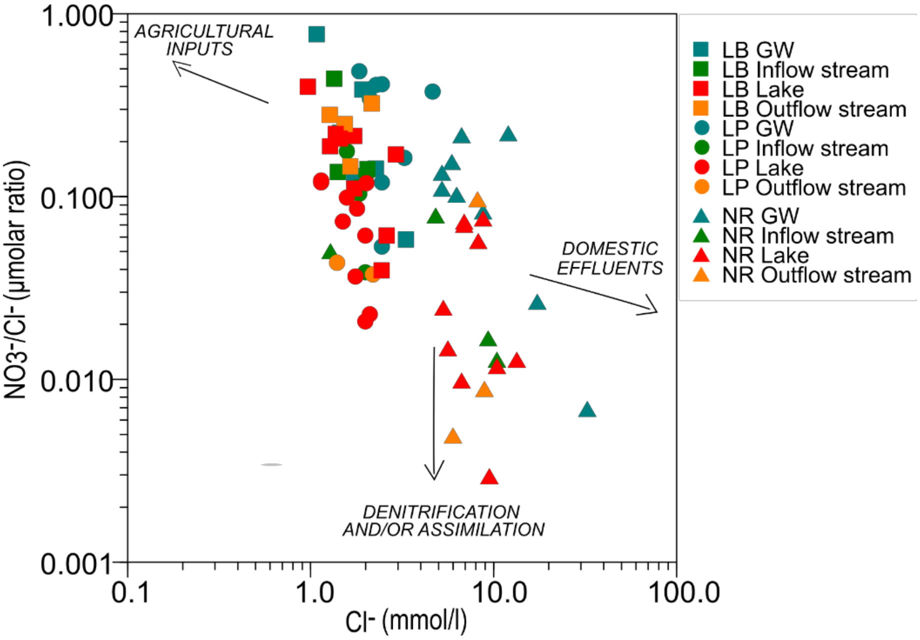
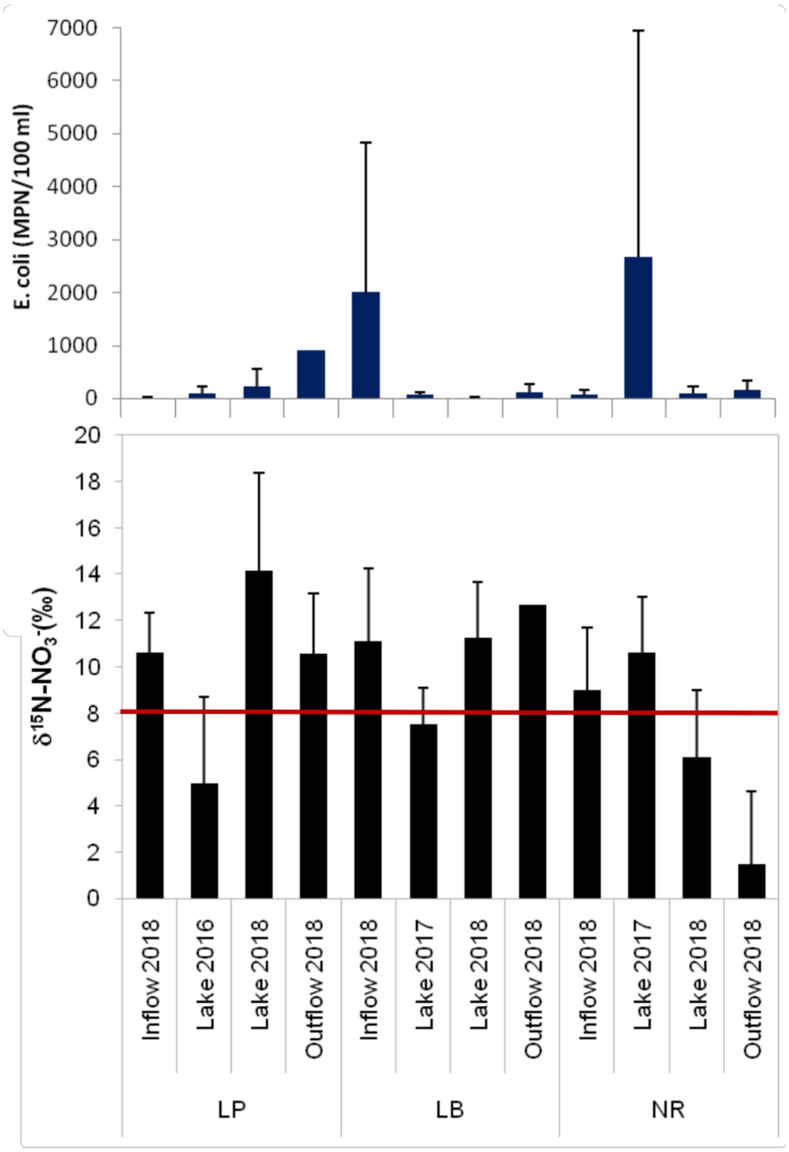

AGRICULTURAL INPUTS
DOMESTIC EFFLUENTS
DENITRIFICATION AND/OR ASSIMILATION
1.000
0.100
0.010
0.001
0.1
1.0
10.0
100.0
NO3-/Cl- (µmolar ratio)
Cl- (mmol/l)
LB GW
LB Inflow stream
LB Lake
LB Outflow stream
LP GW
LP Inflow stream
LP Lake
LP Outflow stream
NR GW
NR Inflow stream
NR Lake
NR Outflow stream

Figure 5



E. coli (MPN/100 ml)
7000
6000
5000
4000
3000
2000
1000
0
δ15N-NO3-(‰)
20
18
16
14
12
10
8
6
4
2
0
Inflow 2018
Lake 2016
Lake 2018
Outflow 2018
Inflow 2018
Lake 2017
Lake 2018
Outflow 2018
Inflow 2018
Lake 2017
Lake 2018
Outflow 2018
LP
LB
NR

Figure 6

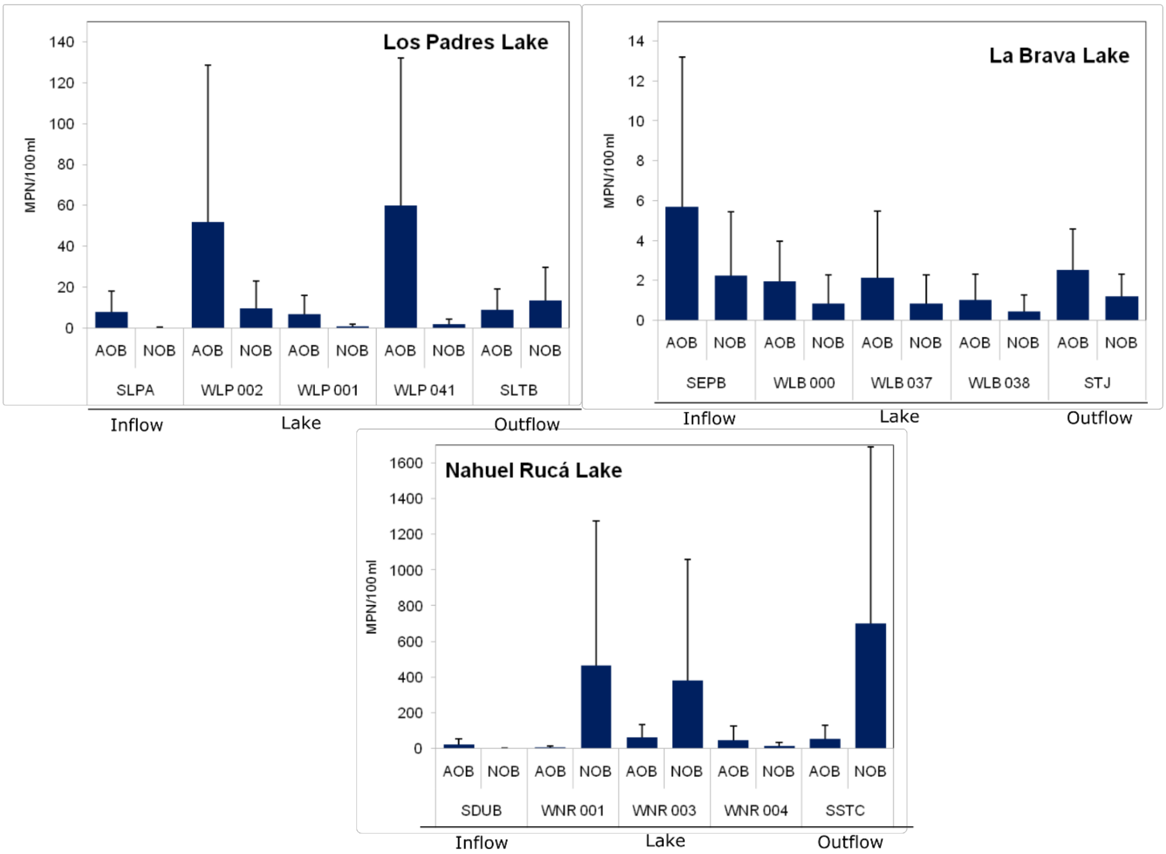

Los Padres Lake
MPN/100 ml
140
120
100
80
60
40
20
0
AOB NOB AOB NOB AOB NOB AOB NOB AOB NOB
SLPA WLP 002 WLP 001 WLP 041 SLTB
Inflow Lake Outflow
La Brava Lake
MPN/100 ml
14
12
10
8
6
4
2
0
AOB NOB AOB NOB AOB NOB AOB NOB AOB NOB
SEPB WLB 000 WLB 037 WLB 038 STJ
Inflow Lake Outflow
Nahuel Rucá Lake
MPN/100 ml
1600
1400
1200
1000
800
600
400
200
0
AOB NOB AOB NOB AOB NOB AOB NOB AOB NOB
SDUB WNR 001 WNR 003 WNR 004 SSTC
Inflow Lake Outflow

Figure 7

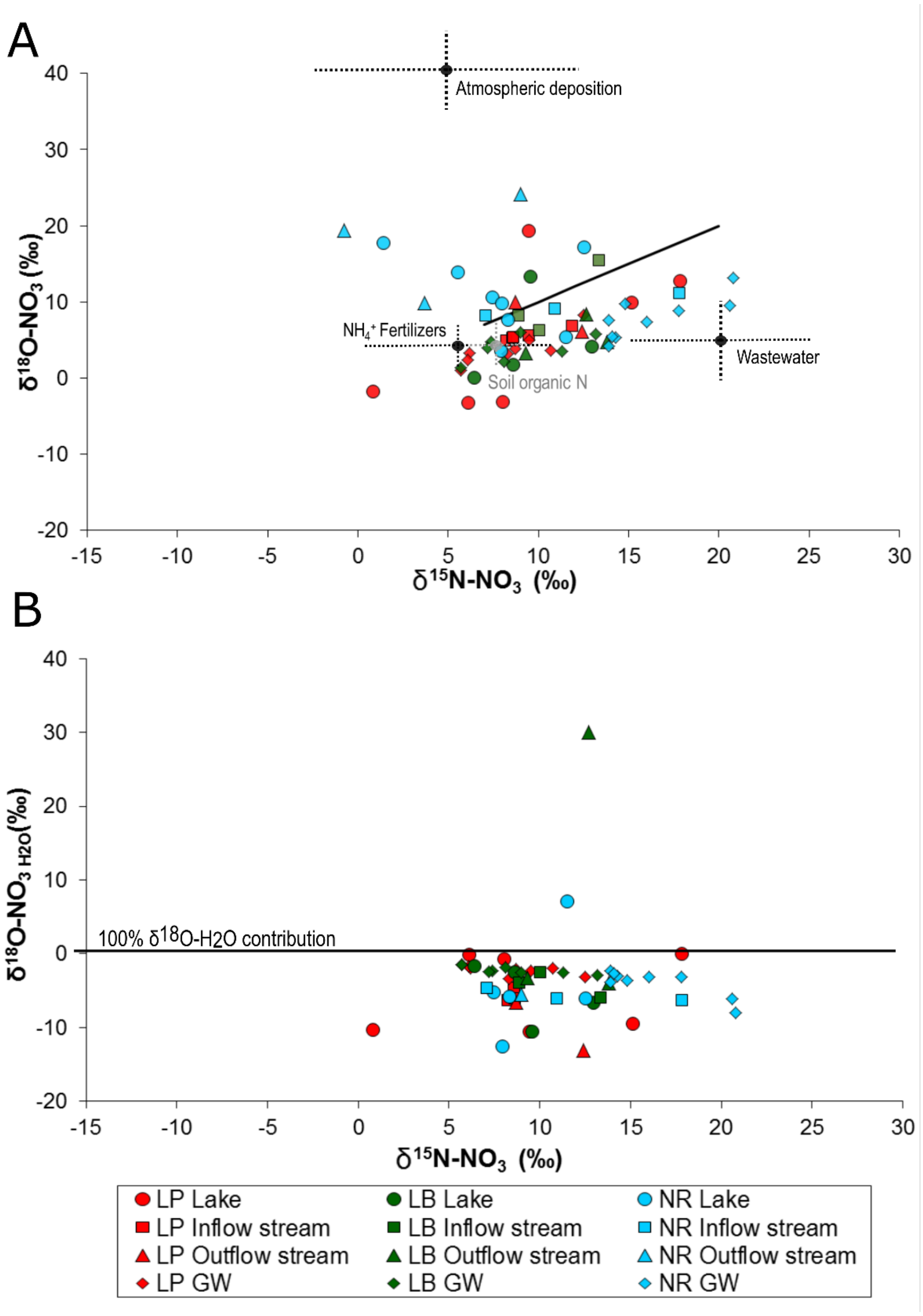

A
Atmospheric deposition
NH_4^+ Fertilizers
Soil organic N
Wastewater
$\delta^{18}O\text{-}NO_3$ (‰)
$\delta^{15}N\text{-}NO_3$ (‰)
B
100% $\delta^{18}O\text{-}H_2O$ contribution
$\delta^{18}O\text{-}NO_{3\ H2O}$(‰)
$\delta^{15}N\text{-}NO_3$ (‰)
LP Lake
LB Lake
NR Lake
LP Inflow stream
LB Inflow stream
NR Inflow stream
LP Outflow stream
LB Outflow stream
NR Outflow stream
LP GW
LB GW
NR GW

Figure 8

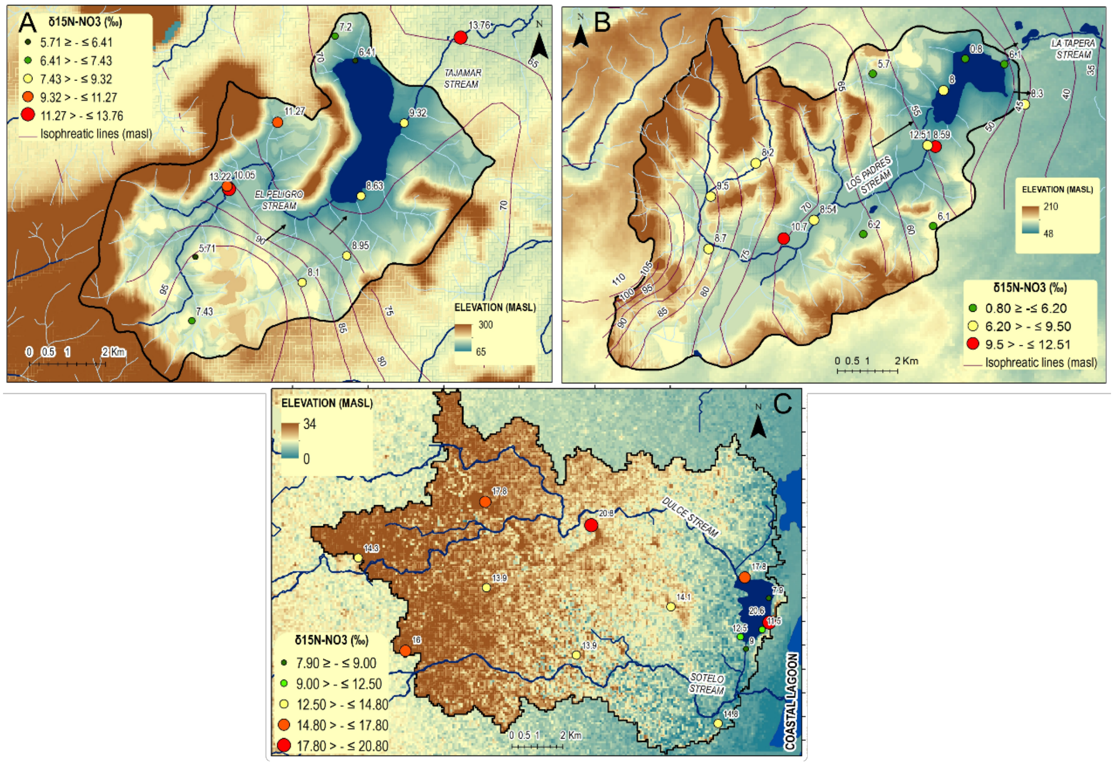

A
δ15N-NO3 (‰)
5.71 ≥ - ≤ 6.41
6.41 > - ≤ 7.43
7.43 > - ≤ 9.32
9.32 > - ≤ 11.27
11.27 > - ≤ 13.76
Isophreatic lines (masl)
TAJAMAR STREAM
EL PELIGRO STREAM
ELEVATION (MASL)
300
65
0 0.5 1 2 Km
B
LA TAPERA STREAM
LOS PADRES STREAM
ELEVATION (MASL)
210
48
δ15N-NO3 (‰)
0.80 ≥ -≤ 6.20
6.20 > - ≤ 9.50
9.5 > - ≤ 12.51
Isophreatic lines (masl)
C
ELEVATION (MASL)
34
0
DULCE STREAM
SOTELO STREAM
COASTAL LAGOON
δ15N-NO3 (‰)
7.90 ≥ - ≤ 9.00
9.00 > - ≤ 12.50
12.50 > - ≤ 14.80
14.80 > - ≤ 17.80
17.80 > - ≤ 20.80

Figure 9

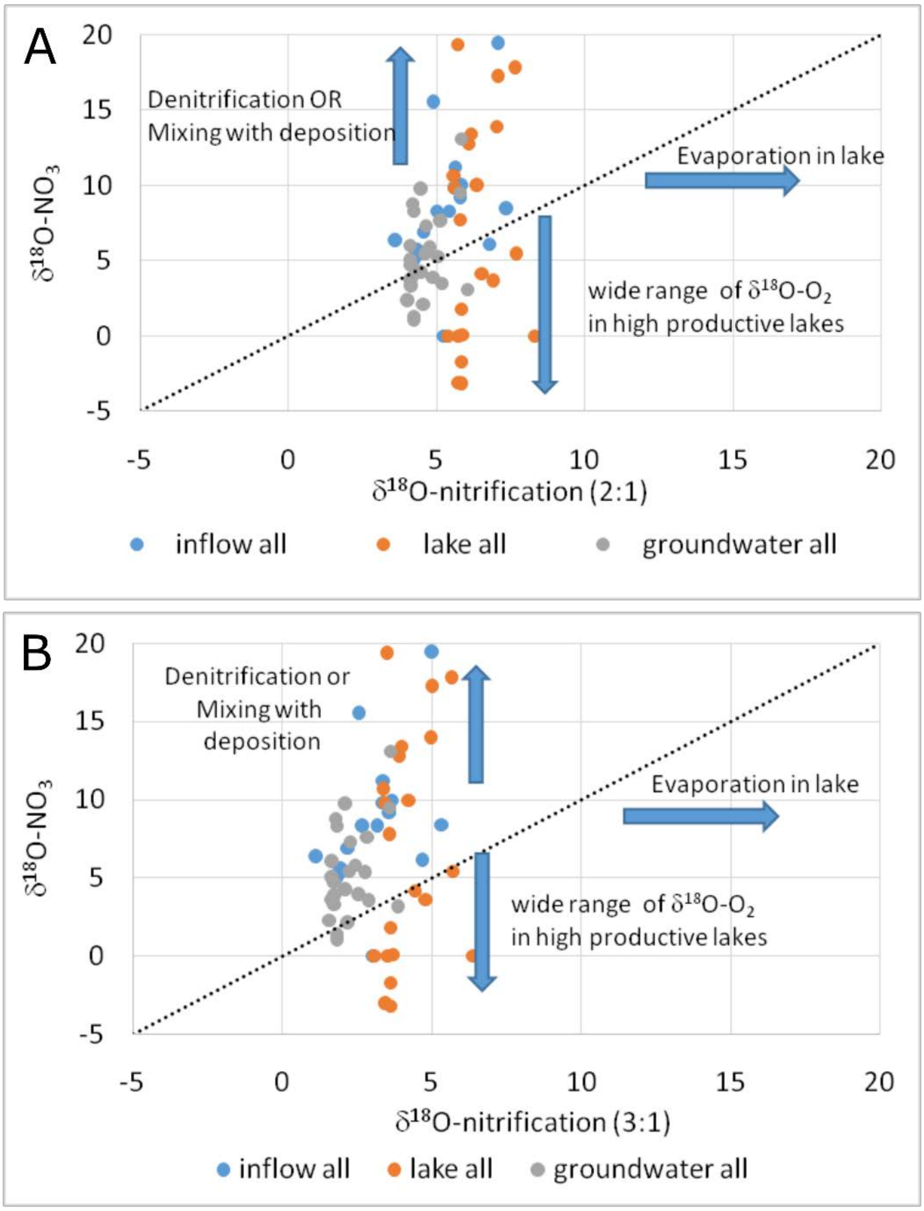
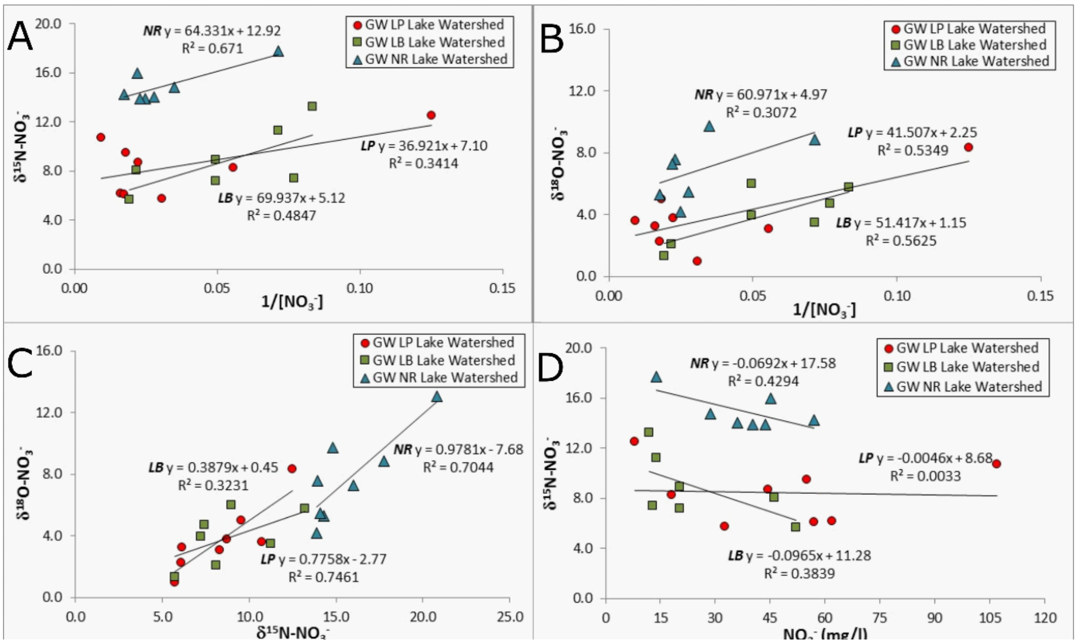
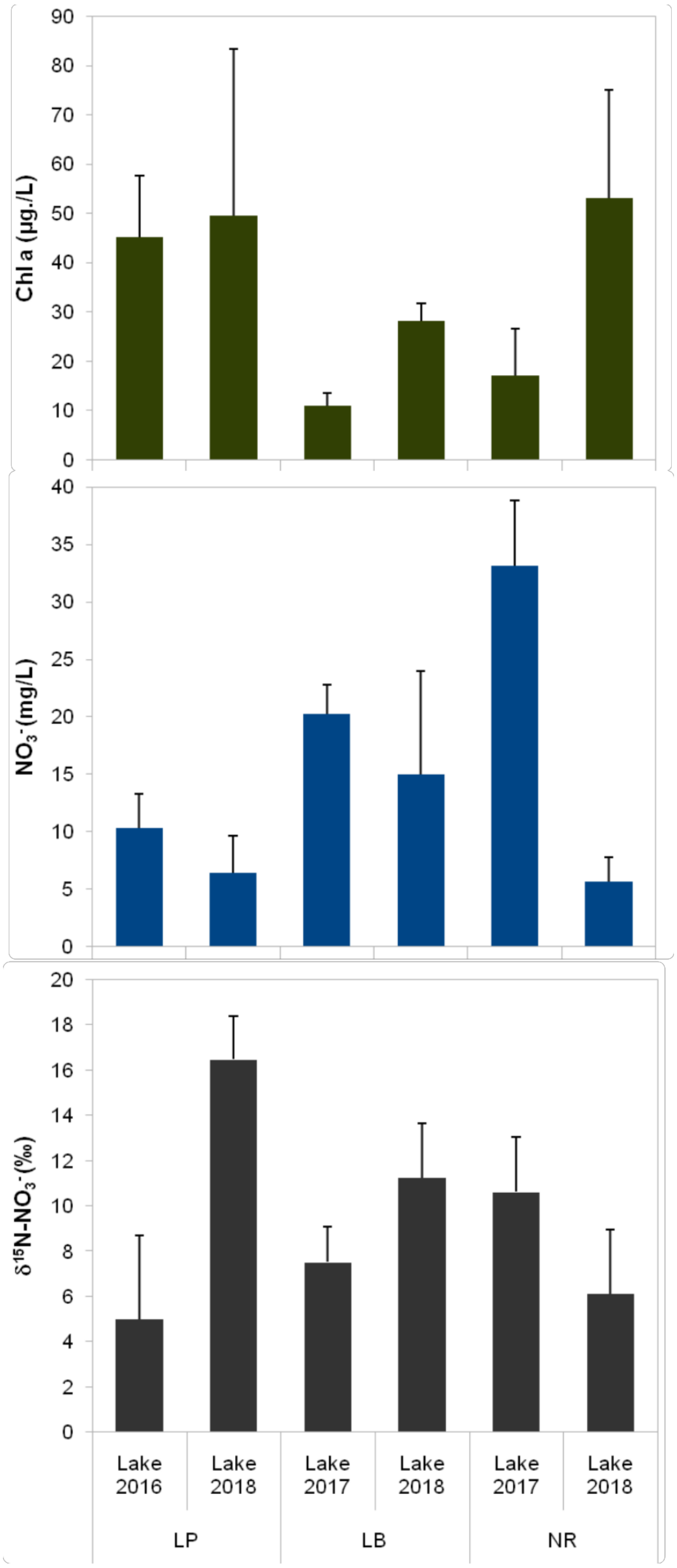

A
Denitrification OR
Mixing with deposition
Evaporation in lake
wide range of $\delta^{18}O-O_2$
in high productive lakes
$\delta^{18}O-NO_3$
$\delta^{18}O$-nitrification (2:1)
inflow all
lake all
groundwater all
B
Denitrification or
Mixing with
deposition
Evaporation in lake
wide range of $\delta^{18}O-O_2$
in high productive lakes
$\delta^{18}O-NO_3$
$\delta^{18}O$-nitrification (3:1)
inflow all
lake all
groundwater all

Figure 10



A
NR y = 64.331x + 12.92
R² = 0.671
LP y = 36.921x + 7.10
R² = 0.3414
LB y = 69.937x + 5.12
R² = 0.4847
GW LP Lake Watershed
GW LB Lake Watershed
GW NR Lake Watershed
δ15N-NO3-
1/[NO3-]
B
NR y = 60.971x + 4.97
R² = 0.3072
LP y = 41.507x + 2.25
R² = 0.5349
LB y = 51.417x + 1.15
R² = 0.5625
δ18O-NO3-
1/[NO3-]
C
LB y = 0.3879x + 0.45
R² = 0.3231
NR y = 0.9781x - 7.68
R² = 0.7044
LP y = 0.7758x - 2.77
R² = 0.7461
δ18O-NO3-
δ15N-NO3-
D
NR y = -0.0692x + 17.58
R² = 0.4294
LP y = -0.0046x + 8.68
R² = 0.0033
LB y = -0.0965x + 11.28
R² = 0.3839
δ15N-NO3-
NO3- (mg/l)

Figure 11



Chl a (µg./L)
90
80
70
60
50
40
30
20
10
0
NO_3^- (mg/L)
40
35
30
25
20
15
10
5
0
$\delta^{15}N$-NO_3^- (‰)
20
18
16
14
12
10
8
6
4
2
0
Lake 2016
Lake 2018
Lake 2017
Lake 2018
Lake 2017
Lake 2018
LP
LB
NR

Table 1. Main characteristics of the three shallow lake watersheds.

	Shallow lakes		
	La Brava	Los Padres	NahuelRucá
Altitude (masl)	71.62	53.18	3.19
Slope (%)	5 - 20	2 - 3	0.2 - 0.7
Geomorphological environment	Ranges	Hills	Plain
Watershed area(km^2)	53.56	102.6	153.9
Lake area (km^2)	4.00	2.16	2.71
Maximum depth (m)	4.00	2.10	0.60
Water type	Na-HCO_3	Na-HCO_3	Na-HCO_3-Cl
Trophic state	Eutrophic	Eutrophic	Eutrophic
Aquatic macrophytes	Marshy and free- floating species	Marshy and free- floating species	Marshy, submerged and free-floating species
Gate for hydrometric level regulation	No	Yes (outflow stream)	Yes (outflow stream)
Lake-aquifer relationship	Effluent-influent	Effluent-influent	Effluent-influent
Location according to regional GW flow system	Recharge area	Recharge area	Discharge area
Constructed area along the coastline (%)	2.20	6.57	0.00
Predominant land cover	Agricultural crops	Horticultural crops	Pastures with cattle breeding
Management regulations for natural resource protection	"Protected landscape of Provincial Interest" (2018)	Natural Reserve. Intangible and multiple-purpose zones (1982)	No. Included in private farmlands

Shallow lake	Site	Description	pH	EC (µS/cm)	PO_4^{-2} (mg/L)	NO_3^- (mg/L)	Na^+ (mg/L)	K^+ (mg/L)	Ca^{+2} (mg/L)	Mg^{+2} (mg/L)	Cl^- (mg/L)	HCO_3^- (mg/L)	CO_3^{-2} (mg/L)	SO_4^{-2} (mg/L)
LOS PADRES	GW	Groundwater	7.4 ± 0.2	919 ± 239.5	11.2 ± 8.5	48.01 ± 30.6	152.7 ± 81.3	13.7 ± 4.6	11.7 ± 5.3	62.5 ± 15.7	93.1 ± 30.8	667 ± 156.6	0	11.4 ± 5.6
	SLPA	Inflow stream	7.9 ± 0.08	864 ± 39.4	22.1 ± 22.5	9.6 ± 4.3	150 ± 60.8	8.7 ± 3.2	17 ± 9	48.2 ± 8.7	65.1 ± 3.5	597.7 ± 237.9	7.8 ± 13.5	17.3 ± 2.9
	WLP002	Conservation area. Waterbird colonies	8.5 ± 0.1	627.7 ± 59.5	6.16 ± 1.4	7.37 ± 2.9	100 ± 26.5	10 ± 8.2	18 ± 7	36.6 ± 12.05	54.3 ± 12.3	372.3 ± 64.3	17.7 ± 30.6	22.3 ± 0.6
	WLP001	La Isla Fishing Club	8.6 ± 0.06	644.7 ± 13.3	7.08 ± 0.6	9.64 ± 4.4	110 ± 34.6	9.7 ± 1.5	24.7 ± 5.7	30.5 ± 11.1	63.7 ± 10.1	359.3 ± 136.7	43.2 ± 21.9	38 ± 30.6
	WLP041	Casineros Recreational Club	8.6 ± 0.1	624.3 ± 20.4	13.5 ± 11.02	4.63 ± 3.3	110 ± 52.9	9.7 ± 4.04	22.3 ± 10.9	32.3 ± 12.1	60.5 ± 18.2	375 ± 94.9	37.2 ± 32.3	22.7 ± 3.2
	SLTB	Outflow stream	7.9 ± 0.2	635 ± 5.6	9.73*	4.35 ± 0.9	115 ± 49.5	7 ± 1.4	27.5 ± 2.1	24.5 ± 18.03	62.2 ± 19.5	453 ± 236.2	0	17.5 ± 0.7
LA BRAVA	GW	Groundwater	7.8 ± 0.4	921.4 ± 171.2	12.5 ± 6.5	25.4 ± 16.6	165.7± 68.3	6.6 ± 3.4	10.3 ± 6.9	34.4 ± 20.2	67.9 ± 25.3	553.9 ± 140.8	0	16.7 ± 11.1
	SEPB	Inflow stream	8.15 ± 0.2	416.8 ± 252.3	5.9 ± 0.6	22.3 ± 13.05	126.7 ± 20.5	6.7 ± 3.9	13 ± 7.3	38.9 ± 18.6	56.3 ± 11.1	390 ± 25.8	0	31.3 ± 13.3
	WLB000	Ruca Lauquen Camping andLodge	8.8 ± 0.3	600.3 ± 36.8	21.16 ± 21.4	13.7 ± 8.7	133.3 ± 15.3	8.3 ± 4.2	35 ± 24.9	16.3 ± 10.1	69.3 ± 14.4	393.3 ± 38.7	46.3 ± 15.1	19 ± 6.9
	WLB037	Balcarce Fishing Club	8.9 ± 0.2	606 ± 34.2	17.28 ± 19.1	14.57 ± 4,4	136.7 ± 32.1	7.3 ± 1.1	25.7 ± 8.4	17.8 ± 7.03	62.5 ± 25.3	384 ± 89.8	40.7 ± 35.4	29.7 ± 24.2
	WLB038	SE coastline. Waterbird colonies	8.7 ± 0.3	609.7 ± 34.6	13.44 ± 1.8	24.6 ± 6.1	173.3 ± 28.9	4.3 ± 3.1	20.7 ± 3.1	21.4 ± 0.5	61.8 ± 36.4	517.7 ± 29.7	29 ± 28.5	17.7 ± 2.1
	STJ	Outflow stream	8.8 ± 0.2	622.7 ± 47.5	25.65 ± 15.5	26.87 ± 14.8	146.7 ± 40.4	11 ± 1	36.7 ± 20.5	21.8 ± 8.2	59.6 ± 15.7	461 ± 14.4	48.9 ± 7.4	27.3 ± 11.1

NAHUEL RUCA	GW	Groundwater	7.5 ± 0.2	3052.6 ± 1586.04	8.2 ± 3.1	57.9 ± 45.6	397.8 ± 258.3	11.4 ± 5.1	34.8 ± 13.4	24.8 ± 12.6	388.9 ± 313.6	499.8 ± 137.2	0	217 ± 145.2
	SUDB	Inflow stream	8.5 ± 0.4	2142.7 ± 408.9	6.71 ± 1.9	7.33 ± 3.06	346.7 ± 61.1	16 ± 12	61.3 ± 36.3	31.9 ± 9.9	245.7 ±174.8	617.3 ± 172.2	27 ± 46.8	114 ± 12.5
	WNR001	E coastline. Forest area	8.4 ± 0.2	1720 ± 322.1	6.1 ± 2.5	14.5 ± 14.9	290 ± 70	9.3 ± 6.4	67.9 ± 40.1	22.2 ± 13.4	280.7 ± 74	579 ± 139.5	28.5 ± 38.3	110.7 ± 9.01
	WNR003	SE coastline. Livestock	8.2 ± 0.3	1484.7 ± 290.1	8.96 ± 2.5	12.3 ± 15.4	253.3 ± 70.2	12 ± 2	56.2 ± 32.5	20.5 ± 3.8	257 ± 68.7	499 ± 163.3	21 ± 36.4	94 ± 6.2
	WNR004	SW coastline. Waterbird colonies	8.5 ± 0.3	1658 ± 348.1	6.96 ± 0.1	20.2 ± 18.6	293.3 ± 102.6	11.8 ± 7.3	58.3 ± 34.5	30.3 ± 15.01	320.7 ± 142	500.3 ± 142.6	32.7 ± 28.6	103.7 ± 6.5
	SSTC	Outflow stream	8.56 ± 0.3	1660 ± 370.5	5.99 ± 2.3	18.67 ± 26.3	316.7 ± 111.5	10.7 ± 8.1	50.9 ± 40.7	31.4 ± 23.4	269.3 ± 53	475.3 ± 98.2	54.7 ± 48.01	114 ± 14.4

Table 2. Mean (± SD) of water ionic composition, pH, electric conductivity (EC) and nutrients (PO_4^{-2} and NO_3^-) in Los Padres, La Brava and Nahuel Ruca Lake Watershed.

Table 3. Mean (± SD) of the principal limnological parameters in Los Padres, La Brava and Nahuel Rucá shallow lakes. DO: dissolved oxygen.

Shallow lake	Site	Location	Depth (m)	Water clarity (m)	Water temperature (ºC)	DO (mg/L)
LOS PADRES	SLPA	Inflow stream	0.88*	Nd	18.5 ± 3.9	8,8*
	WLP 002	Conservation area. Waterbird colonies	0.82 ± 0.29	0.23 ± 0.09	18.2 ± 3	7.0 ± 1.72
	WLP 001	La Isla Fishing Club	0.7 ± 0.28	0.3 ± 0.16	19.1 ± 2.2	8.3 ± 2.1
	WLP 041	Casineros Recreational Club	0.8 ± 0.6	0.3 ± 0.13	20.0 ± 1.9	7.9 ± 2.21
	SLTB	Outflow stream	0.22*	nd	17.6 ± 2.7	7.95*
LA BRAVA	SEPB	Inflow stream	0.14*	0.14*	14.2 ± 6.9	6.36*
	WLB 000	Ruca Lauquen Camping and Lodge	1.1 ± 0,24	0.5 ± 0.17	14.5 ± 5.03	7.2 ± 1.23
	WLB 0037	Balcarce Fishing Club	1.23 ± 0.22	0.57 ± 0.2	13.5 ± 5.9	7.34 ± 1,9
	WLB 0038	SE coastline. Waterbird colonies	0.58 ± 0.18	0.37 ± 0.08	14.17 ± 5.08	5.9 ± 1.15
	STJ	Outflow stream	0.2*	0.2*	16.5 ± 6.7	5.93*
NAHUEL RUCA	SUDB	Inflow stream	0.48*	0.25*	16.3 ± 10.9	6.7*
	WNR 001	E coastline. Forest area	0.6 ± 0.07	0.2 ± 0.05	16.3 ± 9.2	6.14 ± 1.8
	WNR 003	SE coastline. Livestock	0.43 ± 0.01	0.17 ± 0.01	19.0 ± 10.6	6.6 ± 2.06
	WNR 004	SW coastline. Waterbird colonies	0.5 ± 0.07	0.16 ± 0.03	18.5 ± 8.5	5.7 ± 0.35
	SSTC	Outflow stream	0.36*	0.19*	18.7 ± 10.34	5.51*

*value of one sample; nd: no data available.

Table 4. Proportional estimated contributions of N sources (atmospheric deposition, nitrification, and wastewater-manure/septic waste) to nitrate in the inflow streams and shallow lakes sampled in 2016-2017 and 2018. Mean (± SD) values estimated using Bayesian mixing models. The number of samples (N) analyzed for each collection site is shown. LP: Los Padres Lake; LB: La Brava Lake; NR: Nahuel Rucá Lake.

Sample	Period	N	Atm deposition	Nitrification	Wastewater
INFLOW STREAM[a]					
LP	nov-16	3	0.04 ± 0.03	0.75 ± 0.13	0.21 ± 0.12
	apr-18 (dry)	1	0.08 ± 0.06	0.59 ± 0.23	0.34 ± 0.21
	oct-18 (wet)	1	0.09 ± 0.07	0.48 ± 0.22	0.43 ± 0.21
LB	jul-17	1	0.08 ± 0.06	0.57 ± 0.22	0.35 ± 0.21
	jun-18 (dry)	1	0.12 ± 0.07	0.58 ± 0.21	0.3 ± 0.19
	dec-18 (wet)	1	0.28 ± 0.10	0.30 ± 0.19	0.42 ± 0.19
NR	nov-17	1	0.16 ± 0.08	0.25 ± 0.19	0.59 ± 0.19
	jun-18 (dry)	1	0.11 ± 0.07	0.63 ± 0.20	0.25 ± 0.18
	oct-18 (wet)	1	0.13 ± 0.08	0.49 ± 0.21	0.38 ± 0.20
LP_all	-	5	0.04 ± 0.03	0.74 ± 0.12	0.21 ± 0.11
LB_all	-	3	0.14 ± 0.06	0.49 ± 0.20	0.37 ± 0.18
NR_all	-	3	0.13 ± 0.06	0.48 ± 0.20	0.39 ± 0.19
LAKE[b]					
LP_all	nov-16	3	0.05 ± 0.06	0.88 ± 0.09	0.06 ± 0.06
LB_all	jul-17	2	0.06 ± 0.06	0.85 ± 0.11	0.09 ± 0.10
NR_all	nov-17	3	0.06 ± 0.55	0.82 ± 0.11	0.12 ± 0.10
LAKE[b]					
LP_all	apr-oct-18	3	0.12 ± 0.06	0.59 ± 0.20	0.29 ± 0.19
LB_all	jun-dec-18	2	0.06 ± 0.05	0.76 ± 0.17	0.18 ± 0.17
NR_all	jun-oct-18	5	0.06 ± 0.04	0.87 ± 0.07	0.06 ± 0.06

a Assumed no denitrification and no assimilation; *b* Certain assimilation is assumed.

Table 5. Locally relevant $\delta^{15}N$ source values.

Sampling date	Analyses	Sample type	$\delta^{15}N$ (‰)	STD
05/06/2018	^{15}N of Total N	soil Los Padres Lake	6.85	0.2
26/06/2018	^{15}N of Total N	soil La Brava Lake	8.94	0.2
29/06/2018	^{15}N of Total N	soil Nahuel Rucá Lake	8.04	0.2
11/07/2018	^{15}N- NH_4^+	NH_4^+ fertilizer (DAP)	11.11(*)	0.1
11/07/2018	^{15}N- NH_4^+	NH_4^+ fertilizer (Urea)	-0.60	0.3

(*) Atypical value; it was considered in the model since the main reason for using a Bayesian approach is that it facilitates representing and taking fully account of the uncertainties related to parameter values.