Nutrient Export and Periphyton Biomass in a Stream-Lake Basin from the Patagonian Andean Region

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Abstract: We characterized land use, dissolved nutrients, and periphytic biomass in Patagonian Andean streams. Nutrient export, especially dissolved nitrogen increased with human population density. However, no correlation between nutrient concentration and algal biomass was found, which could instead be limited by riparian vegetation cover. Our results suggest that local N-limited ecosystems are liable to eutrophication by increased demographic pressure and that alternative wastewater treatment strategies are necessary for sustainable growth.

Keywords: Ecosystem Functioning; Water Quality; Eutrophication; Baseflow; Spatial studies

Rainfall water infiltrates or runs off drainage basins until it reaches a stream. Physicochemical characteristics of stream water thus reflect the results of the interactions between precipitated water and the basin it flows through. For example, a stream's N:P ratio depends on the flow paths of each individual watershed (Green *et al*., 2007). Eutrophication, the enrichment of surface waters with nutrients, is a key human-induced stressor in streams and often a consequence of intensified land use (Dodds & Smith, 2016). Stream eutrophication results in higher export and concentration of phosphorus (P) and nitrogen (N), usually followed by increased algal biomass and Cyanobacteria blooms (Álvarez *et al*., 2017). Since algal intracellular nutrient concentrations and stream nutrient concentration are directly linked, ambient N:P ratio can indicate if any of these elements acts as a limiting factor to primary productivity of the ecosystem. A review of field studies found that the optimal N:P molar ratio of freshwater benthic algae is 18:1, suggesting that higher ratios indicate P limitation, while lower ratios indicate N limitation (Kahlert, 1998). Thus, N:P ratios are a simple metric to evaluate the impact of land use changes in a basin on stream ecosystems.

Ecological characteristics of the Northwest Patagonian region are driven by the Andean range. This region has a temperate-cold climate with marked seasonality in precipitation (Paruelo *et al*., 1998). Its lakes and forests are generally N limited (Díaz *et al*., 2007; Diehl *et al*., 2008), while its Andisols-type soils are characterized by high

capacity to retain P (Satti *et al.*, 2003). The region harbours a wide network of stream ecosystems subject to a yearly cycle formed by three contrasting hydrological periods: stormflow, meltflow and baseflow (Sosnovsky *et al.*, 2020). Although large part of the region has been suffering increasing impact from human activities related to settlement since the early 20th century, studies linking this impact with eutrophication-driving nutrients like N and P are still scarce (Baffico, 2001; Miserendino *et al.*, 2011; Sosnovsky *et al.*, 2019). We expect that more intensive land use will translate into higher nutrient export and concentration of nutrients in fluvial ecosystems, as well as an increased periphyton biomass. Thus, we aimed to determine, for a stream-lake basin during the baseflow period, the existing relationships i) between human population density and dissolved nutrient export (i.e., N and P) and ii) between nutrient concentration and periphyton algal biomass.

Research was conducted in a suburban area within the city of San Carlos de Bariloche, Argentina. This city has approximately 110 000 inhabitants, distributed in several drainage basins. Stream Gutiérrez drainage basin *(41° 09' 36.18" S 71° 24' 37.19"W)* is large (162 km^2) and includes a large and deep lake (17 km^2, 111 m maximum depth) (Fig. 1). The lake has 7 inflow perennial streams; its seasonal average concentrations of soluble reactive phosphorus (Srp) and dissolved inorganic nitrogen (Din) are 3.4 mg m^{-3} and 4.6 mg m^{-3}, respectively (Díaz *et al.*, 2007). Gutiérrez stream, the outflow of the lake, flows 9 km through a wide gently sloped valley and has only a perennial affluent, the Cascada stream. Gutiérrez's riverbanks are extensively colonized by the exotic crack willow, *Salix fragilis*, and pass through a series of populated areas. Most settlements in the basin are not connected to the city's sewer system, using instead household sanitation systems to treat and dispose domestic wastewater. Newer or more affluent households use septic tanks and nitrifying gravel beds, while older ones usually employ cesspits. Thus, sewage input is directly related to local population density. In addition, a trout farm with a total production of ~10 tons year^{-1} pours its sewage into Gutiérrez stream.

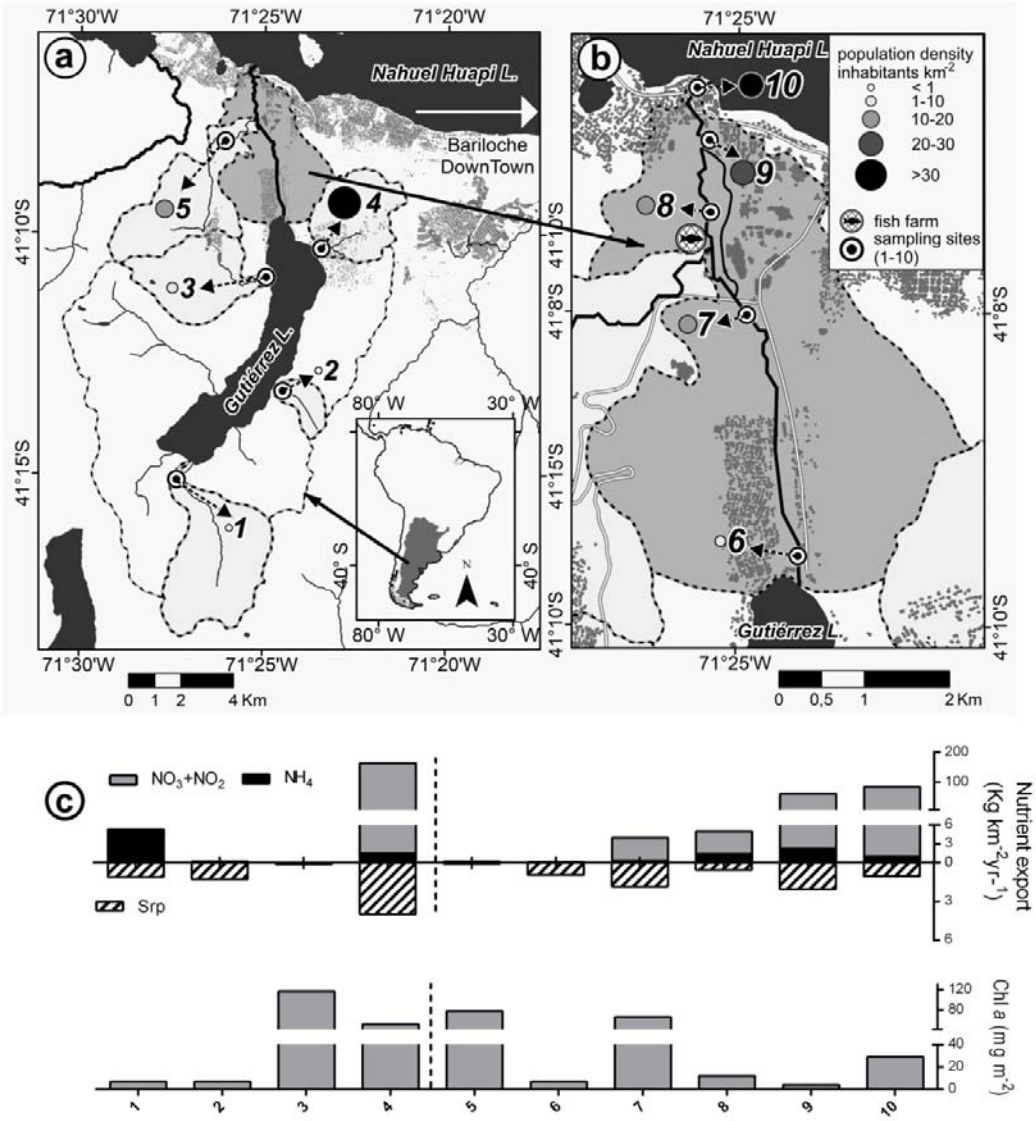

Fig. 1. a) Gutiérrez stream basin with the sampled sub-basins highlighted. Sampling sites at tributaries and mainstem of Gutiérrez stream are shown: 1) Torrontegui stream, 2) Melgarejo stream, 3) Pescadero stream, 4) Loncochinoco stream, 5) Cascada stream. b) 6-10) Gutiérrez stream. Population zones are shown in grey. c) Export of nutrients and concentration of Chlorophyll *a* (Chl *a*). The vertical dotted line represents the lake, to its left are its tributary streams, to its right are the Gutiérrez stream and its tributary. The Y axes corresponding to dissolved nitrogen (NO_3 + NO_2 + NH_4) and Chl *a* has two segments for a better visualization of the data.

We surveyed water quality along the Gutiérrez stream basin in April 2016. Six points along the stream, the Cascada tributary stream plus four other tributary streams flowing into the headwater lake were sampled (Table 1, Fig. 1). We determined the specific drainage area for each site and used demographic density data (inhabitants· km^{-2}) from 2010's National Census. We also estimated canopy cover from riparian vegetation using a concave gridded mirror; and measured discharge (Q), electrical conductivity (CE),

temperature (T) and turbidity. We filtered 0.5 liters of stream water through a MGF Munktell filter to determine the concentration of dissolved nutrients, Din and soluble inorganic phosphorus. We calculated Din as the sum of ammonium (NH_4), nitrates (NO_3) and nitrites (NO_2). We analysed NH_4 by the phenol method and NO_3 + NO_2 with a FUTURA Autoanalyzer (Alliance Instruments,Frepillon, France) through a reaction with sulfanilamide with a previous Cu-Cd-reduction for NO_3 (A.P.H.A., 2005). We considered soluble inorganic phosphorus as Srp (A.P.H.A., 2005). The export nutrients were calculated by the following formula:

$$Nutrient\ export = \frac{[N]\ Q\ t}{A}$$

Where $[N]$ is nutrient concentration, Q is discharge, t is time and A is drainage basin area, and export was standardized to kg of nutrient per km^2 and year.

We also determined chlorophyll *a* (Chl *a*) concentration, corrected for pheopigments (Lorenzen, 1967), as a proxy for periphyton algal biomass. We choose two representative boulders and sampled periphyton from each one of the boulders, using a piece of cylindrical plastic pipe measuring 16 cm^2 in cross-section and a brush to standardize the sampled area and remove the algae. We used linear regressions to explore the relationship between i) population density and nutrient export, and ii) nutrient concentration and Chl *a* concentration. Variables for which data failed tests for normal distribution were log-transformed.

Number/Site	Drainage basin (km^2)	Discharge ($m^3 s^{-1}$)	EC ($\mu S cm^{-1}$)	Temperature (°c)	Turbidity (NTU)	Nutrient concentration ($mg m^{-3}$)				N:P (molar)	Canopy (%)
						Nh_4	NO_2 + NO_3	Din	Srp		
1) Str. Torrontegui	17.0	0.158	92	6.7	0.32	17.1	0.9	18.0	3.7	8.1	7
2) Str. Melgarejo	2.6	0.016	198	8.1	1.47	nd	1.1	1.1	6.5	0.2	0
3) Str. Pescadero	11.3	0.006	74	9.6	0.41	nd	1.7	1.7	4.6	0.2	66
4) Str. Loncochinoco	9.4	0.099	146	9.3	1.14	4.4	488.2	492.6	12.0	21.0	0
5) Str. Cascada	13.0	0.003	87	7.6	0.07	nd	27.9	27.9	8.3	1.7	15
6) Gutiérrez rise	133.1	1.051	70	14.8	0.17	nd	0.5	0.5	3.7	0.1	3
7) Gutiérrez	144.2	1.132	76	12.6	0.24	1.0	14.8	15.8	7.4	1.3	0
8) Gutiérrez	158.3	0.231	88	11.5	0.83	30.2	78.3	108.5	12.0	7.6	100
9) Gutiérrez	160.9	0.938	80	11.7	0.32	12.0	326.0	338.1	11.0	16.6	44
10) Gutiérrez mouth	161.6	0.937	80	11.7	0.35	5.3	452.3	457.6	5.5	42.4	0

Table 1 Morphometric and limnological characteristics of the streams (Str.). Electrical Conductivity (EC); Ammonium (Nh_4); Nitrates + Nitrites (NO_3 + NO_2); Soluble reactive phosphorus (Srp); Dissolved inorganic nitrogen (Din), Phosphorus:Nitrogen ratio (N:P). Nd: not detectable

Tributary streams to Gutiérrez lake and stream show a highly heterogeneous distribution of variable values (Fig. 1a, Table 1, supplementary table ST1). These streams had considerably lower temperature and discharge values relative to those found along stream Gutiérrez. In contrast, their nutrient concentrations were higher than those measured at the stream's headwater (site 6) (Table 1). Torrontegui (site 1) and Melgarejo (site 2) streams, draining sparsely populated basins, showed the lowest Chl *a* concentrations (Fig. 1a). Despite the low nutrient export at Gutiérrez stream headwater, we detected an increasing trend in Din export and N:P ratio towards the stream mouth (Fig. 1b-c, Table 1). This trend was mainly driven by increased NO_3 + NO_2 and not NH_4; ammonium export was highest at the two sites immediately downstream of the trout farm. This increase in Din was not accompanied by increases in either Srp exports or Chl *a* concentration (Fig. 1c, Table 1). Lack of response to increased nitrogen load could be due to light limitations; for example, sites 8 and 9, which showed high Din values and low Chl *a* concentration were shaded by a very dense riparian canopy cover (Table 1).

Integrating our results across the whole study basin, we observe that the stream ecosystem seems to be responding to an increase in land use, given that Din and Srp exports were higher at sites draining more densely populated areas (Fig. 2a-b). This relationship was more noticeable for Din: the linear regression between Din and population density was significantly higher (P = 0.002. compared to P = 0.018 for Srp) and explained a larger amount of variance (R^2 = 0.72, compared to 0.52 for Srp). Similarly, the increase in population density was reflected in an increase in the N:P ratio, which surpasses 18: 1 once population reaches ~ 35 inhabitants per km^2 (Fig. 2c). This potential effect of land use on nutrient load was not followed by a response from periphyton (Din-Chl *a* R^2=0.02, n=10, F=0.17, P=0.69; Srp-Chl *a* R^2=0.01, n=10, F=0.05, P=0.82).

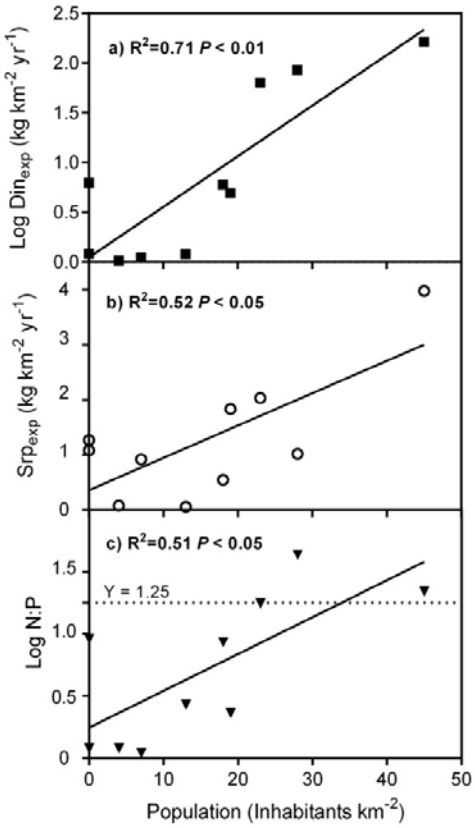

Fig. 2. Linear regressions Human density - Nutrients export. a) Dissolved inorganic nitrogen export (Din_{exp}) b) Soluble reactive phosphorus export (Srp_{exp}) c) Nitrogen:Phosphorus ratio in a molar basis. Dotted line indicates N:P = 18:1.

Our results reflect a relationship between population density and exports of dissolved nitrogen and phosphorus. On the one hand, the steepest response shown by Din values indicates that nitrogen was more readily incorporated into the stream ecosystem. This may be partially explained by leeching of nitrates from household-associated nitrifying beds (Beal *et al.*, 2005; De & Toor, 2017) and cesspits towards groundwater. In contrast to this pervasive and diffuse source of nitrates to the stream, ammonium increase is better explained by single point sources, likely excretion from fishes at the trout farm. On the other hand, septic chambers and Andisol soils seem to be better at retaining phosphorus derived from human activities. Thus, the specific interactions between human settlement, waste treatment strategies and natural environmental characteristics in this area skew nutrient export ratios towards nitrogen enrichment. In mountain areas as the study region, this enrichment becomes most relevant during the baseflow period,

when groundwater flow path makes the largest contribution to total stream discharge (Ahearn *et al.*, 2004).

The range of ambient N:P ratio has been used to define the transition between N and P limitation in benthic algae (Schanz & Juon, 1983). If the N:P ratio is greater than 20 then P can be assumed to be in limiting supply. If N:P is less than 10 then N can be assumed to be in limiting supply. The distinction of the limiting nutrient when ambient N:P ratios are between 10 and 20 is not precise. Since the range of N:P values recorded on our study site spans a wide range, lack of response from periphyton to changes in N:P ratio might be due to different sites being subject to different constraints to algal growth. For example, stream ecosystems found within a forest matrix can be limited by light availability (Hill *et al.*, 2001); indeed, we found that sites with low Chl *a* concentration despite high nutrient loads were usually shadier. Thus, we can postulate that in stream ecosystems as the one we studied, the response of primary producers to increased nutrient availability is modulated by the nature and structure of the riparian vegetation. Recently, a significant fraction of the invasive *S. fragilis* population has been removed presenting a great opportunity to experimentally test this hypothesis; and more generally, to measure the impact of riparian vegetation on stream productivity.

This work presents some points which deserve further study. NH_4 concentration in Torrontegui stream was unusually high and deserves further investigation. Moreover, since we have made our measurements only during the baseflow period, we still do not know if the same patterns relating population density and nutrient exports hold for stormflow and meltflow periods of the local hydrologic cycle. Furthermore, our understanding of the relationships between periphytic algae and nutrient availability would benefit from studying species and chemical composition of this primary producers (Carrillo *et al.*, 2018). Finally, the study of the environmental C:N:P relationships will give us a better comprehension of the auto-heterotrophic dynamics of Andean fluvial ecosystems of Patagonia.

Our research yields insights into stream eutrophication, as it links land uses with physicochemical characteristics of a N-limited drainage basin. The headwater lake acted as an efficient nutrient trap, while residential land use caused increased nutrient release. We can conclude that in the Northwest Patagonian region, household wastewater treatment strategies as those currently used within Gutiérrez stream basin are likely contributing to eutrophication of streams during the baseflow period. Thus, it would be necessary to consider and evaluate the use of alternative wastewater treatment systems

capable of reducing the release of nitrogen into groundwater. Since this basin is part of a touristic region that can seasonally increment its population density to almost twice its year-round density, devising mitigation and remediation measurements for human impact is imperative to achieve environmental sustainability.

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Number/Site	Population (inhabitants km^{-2})	Nutrient export ($kg\ km^{-2}\ y^{-1}$)				Chlorophyll *a* ($mg\ m^{-2}$)
		Nh_4	NO_2 + NO_3	Din	Srp	
1) Str. Torrontegui	0	5.0	0.3	5.3	1.08	7
2) Str. Melgarejo	0	nd	0.2	0.2	1.27	7
3) Str. Pescadero	4	nd	nd	nd	0.08	117
4) Str. Loncochinoco	45	1.5	162.2	163.7	3.97	52
5) Str. Cascada	13	nd	0.2	0.2	0.06	77
6) Gutiérrez rise	7	nd	0.1	0.1	0.92	7
7) Gutiérrez 7	19	0.3	3.7	4.0	1.83	66
8) Gutiérrez 8	18	1.4	3.6	5.0	0.55	12
9) Gutiérrez 9	23	2.2	59.9	62.1	2.03	4
10) Gutiérrez mouth	28	1.0	83.1	84.1	1.02	29

Table 1 annexed Data supporting Figures 1 and 2. Population density and limnological characteristics of the study sites (Str.) Streams. Ammonium (Nh_4); Nitrates + Nitrites (NO_3 + NO_2); Soluble reactive phosphorus (Srp). Nd: not detectable.