

## Seasonality of the N<sub>2</sub>O cycle of the Biobío River during the megadrought

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### ABSTRACT

The mechanisms involved in N<sub>2</sub>O production, consumption and air-sea interactions in fluvial systems are modulated and affected by several factors, including hydrological, chemical and anthropogenic impacts. In recent decades, the basin of the Biobío River (central Chile) has been impacted by an enhanced precipitation deficit and anthropogenic pressure, so the aim of the present work is to understand the spatial and seasonal dynamics of N<sub>2</sub>O during a megadrought that has been affecting the river since 2010. We also aim to determine the annual contribution of N<sub>2</sub>O to the atmosphere and the possible response of the Biobío River to projected climatic changes. Seasonal sampling of the water physical-chemical properties was carried out at 15 stations along 280 km of the river and its five tributaries; the stations were distributed between the pristine headwaters (700 m a.s.l.) and the outlet of the river at the Pacific Ocean. The longitudinal distribution of oxygen, nutrients, and nitrous oxide evidenced agricultural, urban and industrial impacts on the river water. Headwater areas presented the highest oxygen concentration and the lowest nutrient and N<sub>2</sub>O concentrations, all of which fluctuated with the variability in water discharge with the different seasons. In the middle river section, where agricultural, industrial and urban activities impacted the river, the nutrient and N<sub>2</sub>O concentrations increased up to 20 and 1.2 times, respectively, compared to those in the headwaters, and the outlet area showed the highest nutrient and N<sub>2</sub>O concentrations. Throughout the entire river, N<sub>2</sub>O oversaturation exhibited a pronounced seasonal cycle with maxima occurring during the dry season. Our results suggest that urban activities had the greatest impact on the Biobío River at its outlet. Furthermore, the inverse relationship between the N<sub>2</sub>O concentration and water discharge suggests that the predicted future decrease in water discharge may result in higher N<sub>2</sub>O values in the Biobío River that would expectedly enhance global warming further, through a positive feedback.

### INTRODUCTION

Nitrous oxide is a naturally present trace gas in the atmosphere that contributes to approximately 6% of the greenhouse effect, and it is involved in the stratospheric destruction of ozone (Crutzen, 1970). Aquatic systems represent a quarter of the natural sources of atmospheric N<sub>2</sub>O (Bange *et al.*, 1996), and freshwater systems contribute 40% of that output (Seitzinger and Kroeze, 1998). Anthropogenic sources (*e.g.*, agricultural and industrial activities) have been recognized responsible for the exponential increase in atmospheric N<sub>2</sub>O, which has been observed over the last 70 years with a current annual rate of 0.25-0.30% (IPCC, 2013).

Nitrous oxide is naturally generated by two microbial processes, nitrification and denitrification (Beaulieu *et al.*, 2011; Rosamond *et al.*, 2011), which processes occur in both the water and the sediment, and require reactive nitrogen sources (nitrate, nitrite, and ammonium) and organic carbon. The anthropogenic activities along these natural N<sub>2</sub>O source zones (*e.g.*, coastal zones, rivers and lakes) may enhance N<sub>2</sub>O production through the input of pollutants (increasing nutrient loads and/or decreasing oxygen concentrations due to the delivery of high levels of organic compounds or blooms of primary producers; Hinshaw and Dahlgren, 2012). Thirty percent of the N<sub>2</sub>O contribution from rivers comes from anthropogenic sources (Seitzinger and Kroeze, 1998), although this value could be as much as three times higher (IPCC, 2007) in response to increasing nutrient inputs. This association has been observed in slightly to highly polluted rivers, but there is high seasonal and spatial heterogeneity in their N<sub>2</sub>O output. Thus, our understanding about N<sub>2</sub>O budgets at a global scale remains still elusive.

Central Chile is characterized by a Mediterranean climate, and several rivers flow from the Andes Mountains to the Pacific Ocean. Throughout their trajectories, the rivers are subject to variable anthropogenic impacts that modify the biogeochemistry of their waters and the associated processes. Additionally, the region has been subject to an increasing hydric deficit occurring since 2010, termed as megadrought (Boisier *et al.*, 2016). Furthermore, a 20-30% precipitation deficit has already been projected

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by 2050 (Collins *et al.*, 2013). The Biobío River is the most important river in central Chile and the second most important river in the country. It is 380 km long, and it discharges its waters into the eastern South Pacific Ocean (36.8° S/73.2° W) with a mean monthly discharge ranging from 300 to 900 m<sup>3</sup> s<sup>-1</sup>. The Biobío River is a hotspot of biodiversity (Habit *et al.*, 2006; Figueroa *et al.*, 2013), but it has experienced increasing anthropogenic impacts (*e.g.*, dams, industries, forestry, and urban areas; Grantham *et al.*, 2013) with forest occupying ~21% of the basin area (Parra *et al.*, 2013). Its watershed consists of a network of rivers and streams with more than 15,000 tributaries and a growing human population (from ~628,576 people in 2002, Census 2002, to currently more than 1 million people, Census 2017). According to chemical parameters, such as oxygen, nutrients and heavy metal contents, the headwater of the Biobío River is considered to be in “good quality” (CONAMA, 2004); while the river presents moderate to severe levels of pollution in the middle and downstream river sections (Parra *et al.*, 2013).

Spatial and seasonal variability, as well as alterations in climate (*i.e.*, in precipitation, air temperature and river discharges) have an important impact on the biogeochemical processes of river. These may result in a change of greenhouse gas accumulation and emission rate into the atmosphere. A better understanding of the biogeochemical variability and its main driving factors in this highly vulnerable region will allow us to predict the

biogeochemical behaviour of the Biobío River under the projected hydric stress a more reliable way. Therefore, here we evaluate the distribution of N<sub>2</sub>O and biogeochemical variables along the Biobío River, with a special focus of their temporal variability.

## METHODS

### Sampling and analysis

The sampling stations were distributed from the headwaters (Alto Biobío) to the outlet (San Pedro de la Paz, Concepción) of the Biobío River (Fig. 1). Nine stations were sampled along the main watercourse, while 6 additional stations were sampled in its main tributaries (the Laja River, Duqueco River, Bureo River, Vergara River and Guaqui River) (Tab. 1). The monitoring of the Biobío River and its tributaries in the present study was conducted by the Centre of Environmental Science, EULA-Chile at the University of Concepción.

Samples were collected from near the water surface (~30 cm depth) every four months between March 2013 and December 2015. Water temperature and electrical conductivity were measured *in situ* by a multiparameter probe (Hydrolab Quanta). Samples for determining dissolved oxygen content were taken in 300-mL DBO bottles and analysed by the Winkler method (Carpenter, 1969) with a detection limit of 0.1 mg L<sup>-1</sup>. The surface

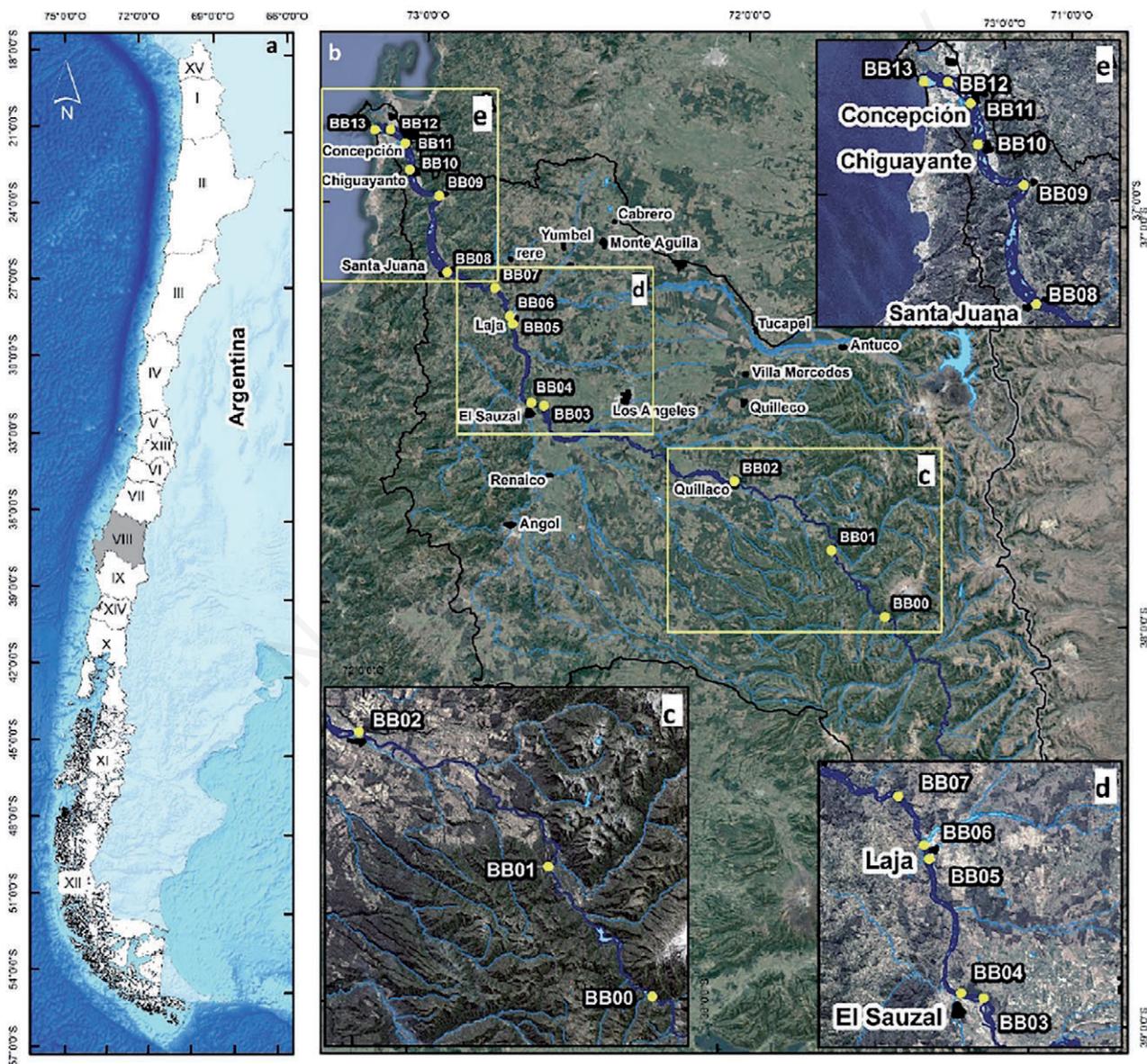
**Tab. 1.** Location of the stations sampled during the Programa de Monitoreo Biobío (PMBB) and the ranges of nitrate and nitrous oxide concentrations in the sampled sites of the Biobío River and its main tributaries.

	Latitude	Longitude	Distance from the mouth (km)	Nitrate (mg L <sup>-1</sup> )	N <sub>2</sub> O (nM) [saturation (%)]	Air-water N <sub>2</sub> O fluxes (μmol m <sup>-2</sup> d <sup>-1</sup> )
<b>HEADWATER</b>						
ABB0	-38.07	-71.37	276		10-16[88-167]	-3.89-12.02
BB0	-38.00	-71.51	256	0.03-0.44	10-19[80-140]	-4.00-16.72
BB1	-37.84	-71.68	228	0.01-0.21	10-15[71-128]	-10.39-5.36
<b>MIDDLE</b>						
BB3	-37.56	-72.59	119	0.15-1.10	12-21[91-211]	-1.50-25.67
BB4	-37.49	-72.65	106	0.12-1.19	10-14[87-145]	-2.45-7.51
BB7	-37.26	-72.73	75	0.41-1.19	10-15[91-184]	-1.44-16.38
Laja River	-37.27	-72.73	82	0.02-1.15	7-16	1.18-14.29
Duqueco River	-37.57	-72.49	133	0.72-2.72	12-17	2.17-12.32
Vergara River	-37.48	-72.68	107	0.37-1.74	11-13	3.02-7.70
Guaqui River	-37.37	-72.67	97	0.34-5.48	11-19	1.71-20.62
Bureo River	-37.58	-72.50	131	0.30-2.74	15-40	7.50-24.16
<b>OUTLET</b>						
BB8	-37.17	-72.92	56	0.16-1.19	10-27[86-370]	-1.76-45.80
BB11	-36.84	-73.06	10	0.14-1.24	11-28[83-370]	-2.18-46.27
BB13	-36.83	-73.15	0	0.32-2.02	11-35[82-411]	-2.32-55.34

water was also sampled for nutrients and stored at 4°C until the laboratory analyses. Nitrate and total phosphorus were analysed with the colorimetric method (4500 P and 4500 NO<sub>3</sub>, B-Standard Methods 22nd Edition, APHA 2012) by spectrophotometry (Lambda 25 Perkin Elmer). The detection limits were 0.001 mg L<sup>-1</sup> for nitrate and 0.01 mg L<sup>-1</sup> for total phosphorus.

Additional water samples for analysing N<sub>2</sub>O (in triplicate) were taken directly from the surface in 20-mL glass vials, avoiding bubble formation. N<sub>2</sub>O samples were

fixed with 50 µL of saturated HgCl<sub>2</sub> to inhibit biological activity and stored in the dark, and the concentrations were determined in the laboratory using a gas chromatograph (Greenhouse 2014, Shimadzu) equipped with an electron capture detector (ECD). Following the headspace technique (McAulliffe, 1971), 250 µL of the headspace was manually injected with a gastight syringe (Hamilton®), and a three-point calibration curve was determined with helium, air and N<sub>2</sub>O standards (0, 0.32 and 1 ppm, respectively; Scotty gas mixture, Air Liquid Co.).



**Fig. 1.** a) The regional context of the study area in South America. b) Detail of the Biobío River area with the distribution of the sampling stations (yellow circles) along the river in the c) headwaters, d) middle section and e) outlet of the river. Sampling was conducted by the Programa de Monitoreo del Biobío (PMBB).

## Data analysis

To obtain the differences in N<sub>2</sub>O contents along the Biobío River, the river was divided into three sections: i) the headwaters, which is considered to be pristine, although there are three hydropower dams in the area (one of the stations was located upstream from the dams while the others were between the dams); ii) the middle river, where there are high levels of anthropogenic impacts (agriculture, forest plantation and urban activity); and iii) the outlet (the last 30 km of the river before it discharges into the Gulf of Arauco in the Pacific Ocean), which experiences strong urban pressure and is the location of more than 80% of the population of the watershed.

To investigate the existence of linear relationships between N<sub>2</sub>O and other physical (*e.g.*, water discharge and water temperature) and biogeochemical (*e.g.*, conductivity, oxygen and nutrients) variables showing such a wide range of values, the Spearman correlation was chosen. The corresponding probability values (P) at a significance level of 0.05 was obtained and is reposted in the text.

The differences in physical and biochemical parameters between the three main river stations (headwaters, middle and outlet) were determined by one-way analysis of variance (ANOVA).

## N<sub>2</sub>O fluxes

The calculation of gas exchange between the atmosphere and the river water (transfer velocity, *k*) requires the consideration of the turbulence. However, direct measurements of N<sub>2</sub>O exchanges were not possible in the present study. Therefore, to determine the variability of the source of atmospheric N<sub>2</sub>O, we estimated this exchange ( $F_{N_2O}$ ) using the N<sub>2</sub>O concentration of the water, water temperature and mean wind speed according to Wanninkhof (1992) as follows:

$$F_{N_2O} = k_w(C_w - C_a) \quad (1)$$

where  $k_w$  (m d<sup>-1</sup>) is the gas transfer coefficient, which depends on the wind speed,  $C_w$  is the N<sub>2</sub>O surface water concentration, and  $C_a$  is the equilibrium concentration of the N<sub>2</sub>O in the freshwater, which is calculated according to Weiss and Price (1980).  $C_a$  considers the measured water temperature, a mean atmospheric value for N<sub>2</sub>O of 323 ppb for the year 2013, and an assumed 0.25% annual increase in N<sub>2</sub>O (IPCC, 2013).

The daily wind speeds used to determine the N<sub>2</sub>O fluxes for the headwaters and middle river sites were obtained from the “María Dolores” meteorological station, while data from the Carriel Sur station were used for the fluxes at the river outlet. Data from both stations were available online (Chilean Meteorological Service).

## River discharge

The water discharge and water temperature data were obtained from the Dirección General de Aguas ([www.dga.cl](http://www.dga.cl)) from three fluvial stations: Llanquén (headwaters, 726 m asl), Coihue (middle river, 76 m asl) and Desembocadura (outlet, 13 m asl). The strong correlations between the water discharges among these hydrological stations ( $R^2=0.84$  between Coihue and Desembocadura,  $n=4661$ ;  $R^2=0.74$  between Coihue and Llanquén,  $n=4691$ ) allowed us to correct the time series by predicting missing values based on the correlations.

## RESULTS

### Meteorology and hydrography of the Biobío River

The monthly mean water discharge of the Biobío River ranged from 19-398 m<sup>3</sup>s<sup>-1</sup> at the headwaters (March 2015) to 146-2053 m<sup>3</sup>s<sup>-1</sup> at the river outlet (August 2014) (Fig. 2a), and the discharges varied spatially, seasonally and inter-annually. Along the river, the discharge increased between 4 and 40 times from the headwaters to the outlet depending on the season, and two maxima were observed every year: one in winter (July and August), when the highest precipitation occurred (Fig. 2b), and another one in spring (September), due to enhanced melting of ice at the headwaters. The lowest water discharge was observed at the end of the summer (March; Fig. 2a).

Precipitation presented a spatial pattern that differed from water discharge. The highest precipitation was observed at the headwaters, while the lowest was observed at the middle of the river (Fig. 2b). During the entire study period, the highest values of rainfall and water discharge values occurred in 2014 in all the three river sections.

The seasonal water temperature ranged between 5.3°C and 26°C (Fig. 2c). Temporally, the highest water temperatures were observed every summer (January), while the lowest were observed in winter. Additionally, the values generally increased from the headwaters towards the outlet of the river, without significant differences among years.

The wind speed at the middle and outlet of the Biobío River ranged from 2.8 to 6.3 m s<sup>-1</sup> (Fig. 2d). The wind speed maxima were observed in summer and winter, while the lowest values were observed in spring and autumn.

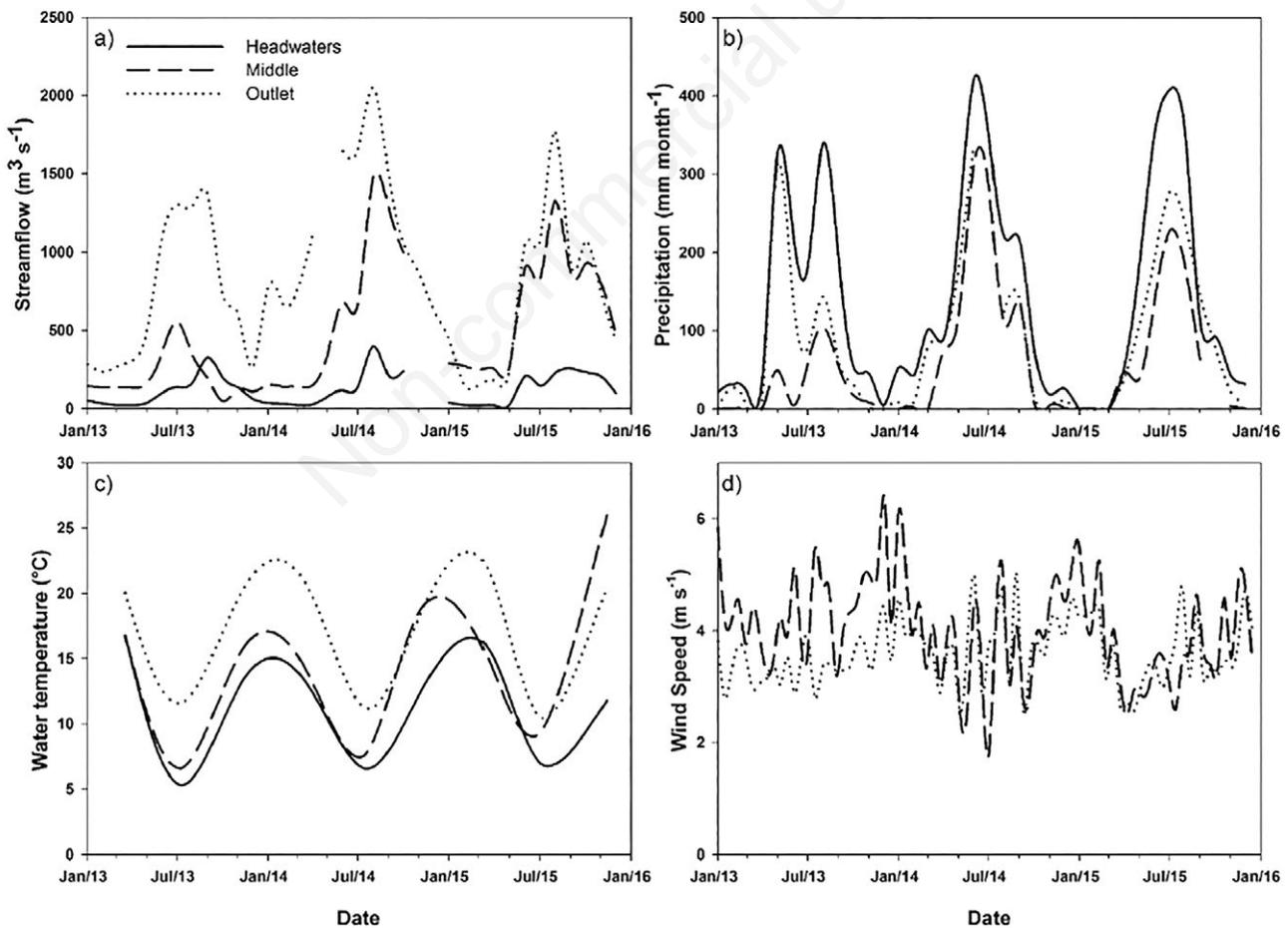
### Chemistry of the water in the Biobío River

The dissolved oxygen values varied seasonally in the Biobío River, with concentrations ranging between 8.2 and 12.5 mgL<sup>-1</sup> (Fig. 3a), and oxygen oversaturation occurred for a total of 75% of the study period (Fig. 3b). At the end of the summer (the dry season), there was

oversaturation along the river (100-107%), with the highest oxygen concentrations occurring at the headwater river stations with decreasing concentrations towards the middle and outlet stations. During this period, the oxygen concentrations did not correlate with the water discharge significantly. In winter, when the maximum water discharge was observed, there was no significant spatial variability in the oxygen saturation along the river, even with changes in water temperature, and the river mostly exhibited oxygen subsaturation. Finally, at the beginning of each summer (a period with mean water discharge), the maximum oxygen saturation was observed in the middle river section. During the study period, the oxygen concentrations differed significantly among the river sections (ANOVA,  $P < 0.05$ ) and were negatively correlated with water temperature (Spearman corr.,  $r = -0.76$ ;  $P < 0.05$ ;  $n = 27$ ; Tab.2).

The nitrate concentrations ranged from 0.01 to 2.02

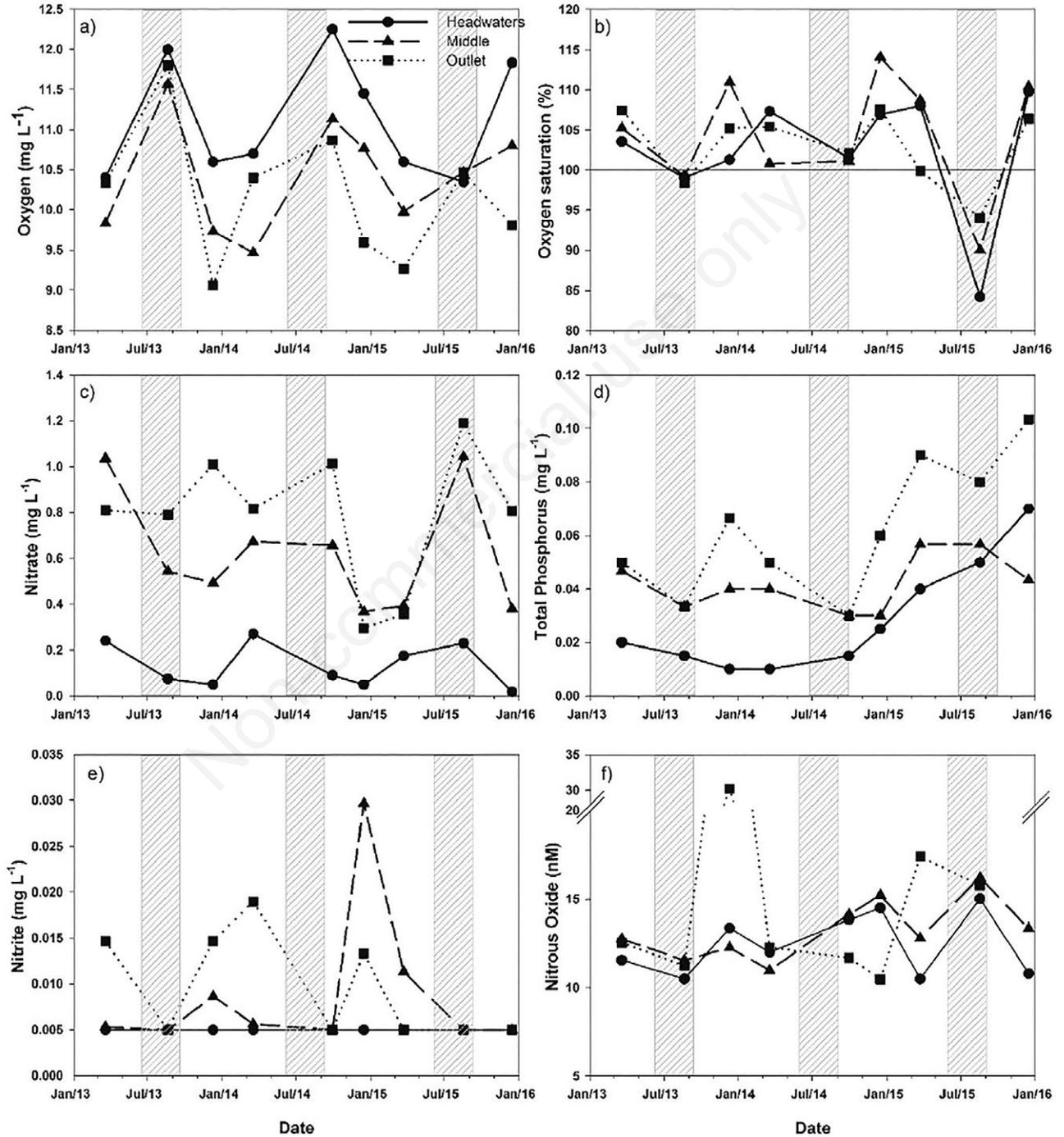
$\text{mgL}^{-1}$ , while the total phosphorus concentrations varied from 0.01 to 0.13  $\text{mgL}^{-1}$ , and both exhibited high spatial and seasonal variability (Fig. 3 c,d). Spatially, the concentrations of both nutrients generally increased from the headwaters towards the outlet of the river during the entire study period, with significant differences between river sections (ANOVA,  $P < 0.05$ ). A sharp increase in nitrate and total phosphorus concentrations was observed between the stations at the headwaters and those at the middle of the river, with increases of up to 20 times for nitrate and 4 times for total phosphorus, respectively. Compared to the middle river section, there was a less pronounced increase in the concentration of nutrients at the outlet area of the river, with increases of up to 110% and 148% for nitrate and total phosphorus, respectively. These sections (middle and outlet) only presented significant differences in total phosphorus concentrations (ANOVA,  $P < 0.05$ ).



**Fig. 2.** Temporal distribution of the hydrologic and meteorological variables in the Biobío River from 2013 to 2015: a) water discharge ( $\text{m}^3 \text{s}^{-1}$ ), b) precipitation ( $\text{mm month}^{-1}$ ), c) water temperature ( $^{\circ}\text{C}$ ), and d) wind speed ( $\text{m s}^{-1}$ ). The lines correspond to the headwaters (black line), middle section (dashed line) and outlet (dotted line) of the Biobío River.

Additionally, the nutrient concentrations showed seasonal variability. The nitrate concentration was generally higher in the wet season (*i.e.*, winter), showing a significant correlation with water discharge (Spearman

corr.,  $r=0.65$ ;  $P<0.001$ ;  $n=27$ ), while the total phosphorus concentration was higher in the dry season. For the entire study period, the highest concentrations of nitrate and total phosphorus, as well as the greatest difference in the



**Fig. 3.** Temporal distribution of the a) oxygen (mg L<sup>-1</sup>), b) oxygen saturation (%), c) nitrate (mg L<sup>-1</sup>), d) total phosphorus (mg L<sup>-1</sup>), e) nitrite (mg L<sup>-1</sup>) and f) nitrous oxide (nM) at the headwaters (circles and continuous line), middle (triangles and dashed line) and outlet (squares and dotted line) of the Biobío River.

concentrations between the headwaters and the outlet of the river were observed during 2015 in the wet season and the dry season, respectively (Fig. 3).

In contrast, the nitrite concentrations varied from  $<0.005$  to  $0.051 \text{ mgL}^{-1}$ . At the headwaters, the nitrite contents were always under the detection limit ( $<0.005 \text{ mgL}^{-1}$ ) and were also low at the middle of the river in winter when the maximum water discharge occurred. However, the nitrite concentration increased each December and March. The highest nitrite concentrations were observed in the outlet of the river, with concentrations up to  $\sim 4$  times higher than those occurring in the upstream stations in the dry season (Fig. 3e). The nitrous oxide concentrations ranged from  $9.7$  to  $35.4 \text{ nM}$ , with saturation levels ranging between  $71$  and  $411\%$  (Fig. 3 e,f). In  $\sim 85\%$  of the study period, the water of the Biobío River presented nitrous oxide supersaturation. Along the river, the lowest  $\text{N}_2\text{O}$  saturation was observed in winter, the season in which the highest water discharge occurred, and the highest  $\text{N}_2\text{O}$  saturation corresponded to the lowest water discharge (Fig. 3f). In these dry seasons, significant correlations were observed between  $\text{N}_2\text{O}$  and the water discharge in the middle (Spearman corr.,  $r=-0.80$ ;  $P=0.01$ ;  $n=4$ ) and outlet (Spearman corr.,  $r=-0.83$ ;  $P=0.01$ ;  $n=4$ ) river sections, but considering the entire study period,  $\text{N}_2\text{O}$  saturation was only correlated with water discharge at the outlet (Spearman corr.,  $r=-0.71$ ;  $P=0.06$ ;  $n=9$ ; Tab.2).

Spatially, the highest  $\text{N}_2\text{O}$  concentrations and saturations ( $9.7$ - $35.4 \text{ nM}$  and  $82$ - $411\%$ , respectively) were typically observed at the river outlet, and this section of the river presented the highest seasonal variability in  $\text{N}_2\text{O}$  concentrations. In contrast, the lowest concentrations and saturations of  $\text{N}_2\text{O}$  were observed at the headwaters ( $10.2$ - $19.0 \text{ nM}$  and  $71$ - $167\%$ , respectively) with low variability among seasons. The  $\text{N}_2\text{O}$  concentration was only significantly correlated with water discharge in the headwaters ( $r=0.96$ ;  $P<0.001$ ;  $n=9$ ).

In the middle of the river, the  $\text{N}_2\text{O}$  saturation was negatively correlated with river discharge ( $r=-0.86$ ;  $P=0.01$ ;  $n=9$ ) and positively correlated with water temperature ( $r=0.88$ ,  $P<0.001$ ;  $n=9$ ) and oxygen saturation ( $r=0.78$ ;  $P=0.01$ ;  $n=9$ ). At the river outlet, there was a positive correlation between the  $\text{N}_2\text{O}$  saturation values and water temperature ( $r=-0.85$ ;  $P=0.01$ ;  $n=9$ ) as well as between  $\text{N}_2\text{O}$  and nitrate and total phosphorus ( $r=0.85$  and  $0.65$ ;  $n=9$ , respectively; Tab. 2).

The  $\text{N}_2\text{O}$  fluxes ranged from  $-10.4$  to  $55.3 \text{ } \mu\text{molm}^{-2}\text{d}^{-1}$  (Tab. 1), indicating that the river provided  $\text{N}_2\text{O}$  to the atmosphere most of the time. The headwaters presented the lowest air-water  $\text{N}_2\text{O}$  fluxes, while the highest fluxes were observed at the river outlet. Seasonally, the highest fluxes were observed during the dry season. These flux values were raw approximations for an environment subject to turbulence induced not only by winds but also by

**Tab. 2.** Significant Spearman correlations between biogeochemical variables and the water discharge and water temperature at each section ( $n=9$ ) and for the entire ( $n=27$ ) Biobío River during the studied period. The probability values of each correlation are shown in parentheses.

	Oxygen				Oxygen saturation											
	Hw	Md	Otl	Whole	Hw	Md	Otl	Whole								
Water discharge	ns	0.72 (0.03)	0.69 (0.04)	ns	-0.76 (0.02)	ns	-0.77 (0.02)	ns								
Temperature	ns	-0.65 (0.06)	-0.77 (0.00)	-0.76 (0.00)	0.68 (0.04)	0.78 (0.01)	0.72 (0.03)	0.66 (0.00)								

	Nitrate				Total phosphorus				$\text{N}_2\text{O}$				$\text{N}_2\text{O}$ saturation			
	Hw	Md	Otl	Whole	Hw	Md	Otl	Whole	Hw	Md	Otl	Whole	Hw	Md	Otl	Whole
Water discharge	ns	ns	ns	0.65 (0.00)	0.63 (0.07)	ns	-0.65 (0.06)	0.45 (0.02)	0.96 (0.00)	0.87 (0.00)	ns	ns	-0.66 (0.07)	-0.86 (0.01)	-0.71 (0.06)	ns
Temperature	ns	ns	ns	ns	ns	ns	ns	ns	-0.73 (0.04)	ns	ns	ns	ns	0.88 (0.00)	0.74 (0.05)	0.78 (0.00)
Oxygen	-0.67 (0.05)	ns	0.89 (0.01)	-0.43 (0.03)	ns	ns	-0.59 (0.10)	-0.49 (0.01)	ns	ns	-0.89 (0.01)	ns	ns	ns	-0.88 (0.01)	-0.70 (0.00)
Oxygen saturation	ns	-0.80 (0.01)	0.69 (0.09)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.78 (0.01)	ns	0.41 (0.04)
Nitrate	—	—	—	—	ns	ns	ns	0.35 (0.07)	ns	ns	0.85 (0.01)	ns	ns	ns	ns	0.34 (0.09)
Total phosphorus	—	—	—	—	—	—	—	—	ns	ns	0.65 (0.09)	ns	ns	ns	0.85 (0.01)	0.36 (0.07)

ns, not significant Spearman correlation ( $P>0.1$ ).

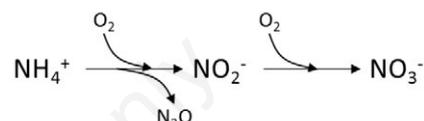
topography (*i.e.*, the slope of the land). However, the N<sub>2</sub>O supersaturation that was generally observed in the water implied that the river had the capacity to be a net N<sub>2</sub>O contributor to the atmosphere.

## DISCUSSION

Rivers are ecosystems with ecological and socio-economic importance (Palmer *et al.*, 2009). Many of them are subject to constant anthropogenic pressure, and multiple studies have focused on determining their biogeochemical behaviour and how human activities impact microbiological processes, thus increasing greenhouse gas production (Mosier *et al.*, 1998; Kumar *et al.*, 2011). These studies show high heterogeneity in river behaviour and the main factors driving greenhouse gas production that include organic carbon and nutrient availability, water temperature, river discharge, sediment contributions and eutrophication. In Chile, there is a wide range of river types (Figueroa *et al.*, 2013) with different discharges and sensitivities to climate change and/or anthropogenic impacts. In the last few decades, the Biobío River has been increasingly impacted by anthropogenic activities and strongly affected by a hydric deficit, and it has been predicted that the precipitation contributing water to the watershed would decrease over the next few decades (Boisier *et al.*, 2016; Yevenes *et al.*, 2018). Under such a scenario, alterations in the dynamics of a river (*e.g.*, hydrological, chemical, biological and microbiological) can affect positively or negatively its functioning (*e.g.*, the coupling of the air-water gas exchange), with variable feedback on the environment.

The biogeochemistry of the Biobío River during the analysed time period showed a clear differentiation according to the season and the level of anthropogenic activity. The thermal pattern associated with the glacial source of the pristine headwaters (Pedreros *et al.*, 2013),

drives the solubility of oxygen in the water and then the equilibrium concentration. Thus, although high oxygen concentrations are observed in winter, the low water temperatures result in high equilibrium concentrations and the lowest oxygen saturation of the river. These oxygen saturation values contrast with the generally observed N<sub>2</sub>O oversaturation (up to 167%), suggesting the relevance of the microbial processes involved in the N<sub>2</sub>O cycle and the influence of the biogeochemical variables on the cycle. Nitrification, an aerobic autotrophic microbial process, produces N<sub>2</sub>O as a by-product during the oxidation of ammonium to nitrite as follows:

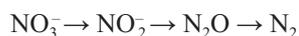


The production of N<sub>2</sub>O by nitrification increases with a decrease in the oxygen concentration from 0.1 to 10% of the NO<sub>3</sub><sup>-</sup> produced (Goreau *et al.*, 1980). In the headwaters, where pristine conditions prevail and oxygen levels are high, nitrate should mainly come from nitrification. Considering the concentration of nitrate from nitrification, the lower limit of N<sub>2</sub>O production reported by Goreau *et al.* (1980) (*i.e.*, 0.1% N<sub>2</sub>O/NO<sub>3</sub><sup>-</sup>) is sufficient to explain the observed N<sub>2</sub>O supersaturations. However, in summer, when the N<sub>2</sub>O saturation maxima were observed, the N<sub>2</sub>O production is not only explained by nitrification, suggesting that other processes may act as a source of N<sub>2</sub>O, or consumption of the produced nitrate was active. In this period, the increase in the chlorophyll concentrations (up to 9 times that observed in winter) along the entire river (R. Figueroa, *personal communication*; ATE 06-2018, Centro EULA-Chile, Universidad de Concepción) suggests enhanced primary productivity. This increase in primary production, combined with low river discharges and higher water

**Tab. 3.** List of some previously reported N<sub>2</sub>O and nitrate concentrations in rivers.

	N <sub>2</sub> O (nM) (saturation %)	NO <sub>3</sub> (mg L <sup>-1</sup> )	Author
Tuolumne River	10.2-41.8	<0.3	Hinshaw and Dahlgren, 2012
Merced River	9.5-44.5	<0.3	
San Juan River	7.0-36.4 (186-729)	0.01-5.38	
Sacramento River	9.1-15.2	0.07-0.40	
Alsea River	8.2-15.6 (92-166)	0.11-0.36	de Angelis and Gordon, 1985
Shanghai River	n.r. (521-1073)	0.09-37.8	Yu <i>et al.</i> , 2013
Changjiang River	8.8-17.7 (154-235)	0.79-1.08	Yan <i>et al.</i> , 2012
Iroquois River	13.65-27.67 (134-209)	10.2-10.6	Laursen and Seitzinger, 2004
Milestone River	11.5-13.3 (104-123)	1.3-2.4	
Biobío River	10-35 (71-411)	0.08-2.02	This study

temperatures, may trigger higher nitrate consumption (by uptake and denitrification) and lower oxygen concentrations in both the water and the sediment. The decrease in oxygen enhances the anaerobic microbial processes involved in  $\text{N}_2\text{O}$  production, such as denitrification. Denitrification, an anaerobic heterotrophic process that uses  $\text{NO}_3^-$  as an electron acceptor during the respiration of organic matter that produces  $\text{N}_2$  gas, generates  $\text{N}_2\text{O}$  as an intermediate product in the following sequential reduction of nitrate:

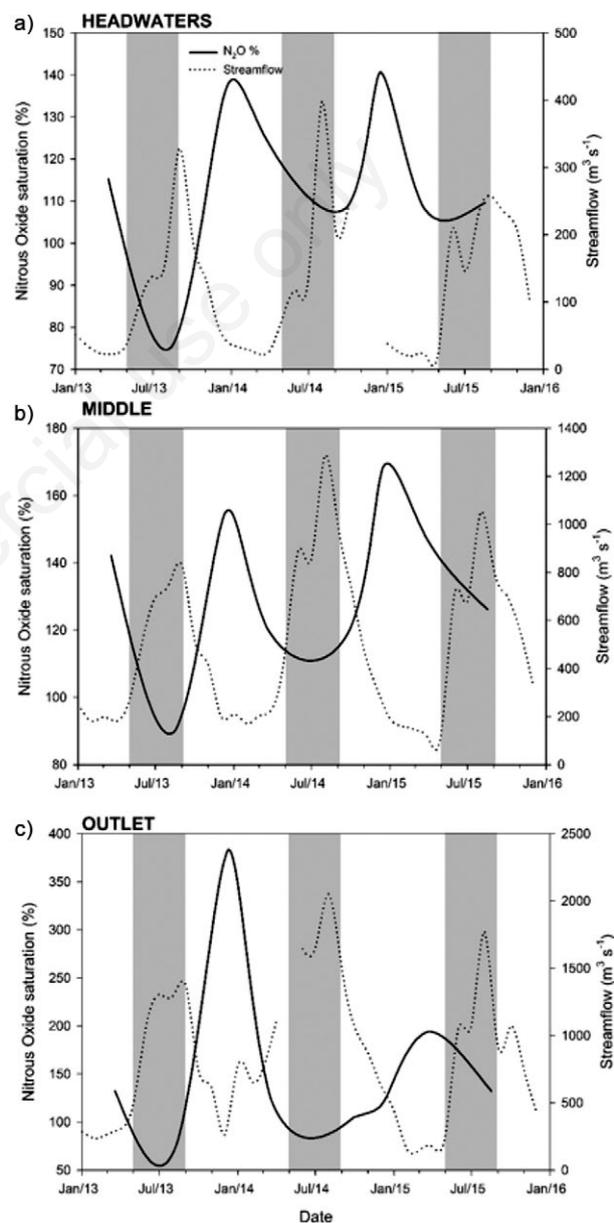


This pattern may explain the significant correlation between the  $\text{N}_2\text{O}$  concentration and the water discharge (Fig. 4), and the lack of correlations between the  $\text{N}_2\text{O}$  concentration and biogeochemical variables such as nutrients and oxygen. Considering these processes, there are two characteristics of the headwaters suggesting that the observed  $\text{N}_2\text{O}$  concentration comes from the *in situ* microbial community without the intervention of outside sources; i) the continuous degasification due to the strong turbulence that the waters experience because of geomorphology leads to, and ii) the scarce human population, thus limiting the anthropogenic contribution of nutrients containing carbon compounds. Despite these levels of  $\text{N}_2\text{O}$  supersaturation, which result in a contribution to the atmosphere, the observed saturation values are the lowest in the river.

An important characteristic in the headwaters section is the presence of three large dams (*i.e.*, Central Pangué, Ralco and Angostura) that may influence the distribution and production of greenhouse gases (Guérin *et al.*, 2006). As the water flows through the turbines, degasification reduces the concentration of gas, while downstream from the dams, microbial processes that generate greenhouse gases might be favoured by the reduction in flow by the dams (Ohio River; Beaulieu *et al.*, 2010). However, no evident impacts of the dams on  $\text{N}_2\text{O}$  concentrations could be identified in the present study. In fact, the first headwater station, which is located upstream of dams, presented  $\text{N}_2\text{O}$  supersaturations (up to 167%), similar to those located downstream of the dams.

In the middle river section downstream, the concentration of  $\text{N}_2\text{O}$  and other physical and biogeochemical variables differed considerably from that upstream (Figs. 3 and 4). Additionally, the water discharge increased, as is expected due to precipitation accumulation and runoff towards the river. This region, which is located in the central valley of Chile, is characterized by a Mediterranean climate and has the highest mean daily air temperatures in the study region during the summer ( $>20^\circ\text{C}$ , [www.dga.cl](http://www.dga.cl)). These environmental conditions are highly favourable for agricultural crop production as well

for urbanization and, consequently, the development of several industries (metallurgic, pulp and paper, and oil refinery industries; Parra *et al.*, 2013). In fact, agriculture is a major activity in the Biobío River basin, and up to 75% of the water discharge in summer is diverted through irrigation channels ( $\sim 171 \text{ m}^3 \text{ s}^{-1}$  during seven months of irrigation; CONAMA 2013). Much of the fertilizers and



**Fig. 4.** Distribution of nitrous oxide saturation (black line) and water discharge (dotted line) during the study period at the headwaters (a), middle section (b) and outlet (c) of the Biobío River. The shaded boxes indicate the winter season. Note that the scales for  $\text{N}_2\text{O}$  and water discharge differ between the plots to represent temporal differences.

chemicals used in this activity end up in the river through runoff, implying, among other impacts, an input of nutrients. The effects of these anthropogenic activities associated with the middle section of the river were observed as strong increases in the dissolved nitrogen and total phosphorus concentrations in the water (up to 20 and 4 times, respectively, compared to the concentrations upstream). This spatial increase was repeatedly observed during the whole - highest during the dry season -, independently of season, and therefore the dilution rate of inputs from the urban and industrial sources.

In this middle river section, the Biobío River receives inflow from several tributaries (*e.g.*, the Laja River, Vergara River, Bureo River, *etc.*), which all together form a complex network of streams. In the tributaries, nitrate and total phosphorus concentrations are up to 2.5 and 3 times higher, compared to main river, respectively (Parra *et al.*, 2013). The contribution of the tributaries to the water discharge of the Biobío River ranges between 5 and 20% ([www.dga.cl](http://www.dga.cl)), and acts as an important source of the main flow, including nitrate concentration that further trigger favourable conditions for N<sub>2</sub>O production. The tributaries also had N<sub>2</sub>O concentrations up to 5 times higher than those in the Biobío River, thus contributing to the concentrations in the main flow.

In the studied rivers and streams, increasing nitrate results in higher N<sub>2</sub>O production by denitrification. Considering that N<sub>2</sub>O production by denitrification is less than 1% of the denitrified nitrate (Beaulieu *et al.*, 2011), the observed N<sub>2</sub>O supersaturation in the Biobío River requires the denitrification of less than 0.18% of the observed nitrate concentration, so it is plausible that denitrification contributes to the production of N<sub>2</sub>O. However, no significant relationship was observed between the N<sub>2</sub>O and nitrate concentrations, while the oxygen concentration in the water was high throughout the study period. The lack of a direct relationship might be explained by different mechanisms, such as the availability of NO<sub>3</sub><sup>-</sup> increasing N<sub>2</sub>O production, but not yield by denitrification (Beaulieu *et al.*, 2011), the partial contribution of N<sub>2</sub>O by denitrification from the sediment, the physical dynamic of turbulence in this shallow system, or the influence of other processes involved in N<sub>2</sub>O production and/or accumulation (*e.g.*, nitrification). The latter is reinforced by the relationship between N<sub>2</sub>O saturation and oxygen saturation. In addition, the inverse correlation between N<sub>2</sub>O saturation and water discharge (Fig. 4), strengthens the conclusion that the environmental conditions during the dry season favour N<sub>2</sub>O production. In a region with strong hydric stress such as central Chile (Boisier *et al.*, 2016), these results might be highly relevant, considering the projections of future water deficits.

Once N<sub>2</sub>O is produced and accumulated in the riverine

waters, it is quickly exchanged with the atmosphere, where the mixing induced by wind or turbulence should be the factor driving gas accumulation/degasification. Thus, the relatively quick transfer of N<sub>2</sub>O to the atmosphere might prevent us to find a strong correlation with salts, such as nitrate, which remain dissolved in the water. The high N<sub>2</sub>O fluxes (up to 25.67 μmol m<sup>-2</sup>d<sup>-1</sup>) in this region account for this transfer, even considering that these estimates are conservative for an environment, where the turbulence is probably the main factor driving the transfer of gases to the atmosphere, as it is in other rivers (Clough *et al.*, 2007).

The river outlet presented the highest water discharge and the highest nutrient and N<sub>2</sub>O concentrations in the Biobío River. In this section, there are no tributaries, but there is a large urban population inhabiting both sides of the river (~680,000 people; Census 2017) that produces an increase in nutrients and a slight decrease in oxygen concentrations compared to the middle and upstream river sections. The nitrate and total phosphate concentrations were more than 2 times higher than those observed in the middle river, while the oxygen decreased by up to 10%. The N<sub>2</sub>O concentrations presented high levels of supersaturation (up to 411%), but they were generally lower than those in the middle part of the river. Although the rates of the microbial processes responsible for N<sub>2</sub>O production were not evaluated in this study, the correlations between N<sub>2</sub>O saturation and nitrate concentration and between N<sub>2</sub>O and oxygen concentration suggest that the N<sub>2</sub>O was related to nitrification, which is consistent with the reported evidence of a linear dependence between increasing nitrate and decreasing oxygen (Goreau *et al.*, 1980). However, the positive correlation between the nitrate concentration and the oxygen concentration is contradictory considering that nitrate should increase with decreasing oxygen if nitrification is a dominant process. The water in the outlet of the Biobío River has been characterized by the presence of high concentrations of organic carbon (Pérez *et al.*, 2015), and in this shallow aquatic system (1-2 m deep), the amount of organic matter that reaches the bottom changes from <1% in winter to up to 7% in summer (Bertrán *et al.*, 2001). The degradation of organic matter not only decreases the oxygen concentrations in the sediment, thereby triggering other redox processes such as denitrification, but also increases the ammonium available for nitrification; both of these processes increase the N<sub>2</sub>O concentration. The increase in ammonium along the river has been recently reported to be more than 10 times the amount in the outlet than in the headwaters (Yevenes *et al.*, 2018). The increase in the nitrite concentration observed in this section, the highest in the Biobío River, suggests that denitrification is indeed occurring at rates that allow for its accumulation. In this

shallow water column, the products from biological processes occurring in the sediment provide direct inputs to the water. If these products are gases, then they are quickly exchanged with the atmosphere, making this section of the river the highest source of  $N_2O$ .

The registered  $N_2O$  saturations along the river were in the range of unpolluted rivers and considerably lower than those in some other river systems (*e.g.*, 25 times lower than the South Platte River, USA; Rosamond *et al.* 2012) (Tab. 3). Although the Biobío River is impacted by anthropogenic activity, the lower concentrations of nutrients and  $N_2O$  observed may indicate that anthropogenic pressure has little impact on its biogeochemistry, at least for the variables studied in the present work. In fact, its water quality has been classified as “exceptional” according to concentrations in specific chemical variables (Parra *et al.*, 2013).

The present research was conducted during the so-called megadrought (Boisier *et al.* 2015), a five-year long drought period corresponding to extremely low water discharges. Although the water discharge decreased considerably during the megadrought, it has also been decreasing continuously since the 1950s (Fig. 5). The decadal average of the cumulative discharge at the outlet has decreased by up to 42% since the 1930s (Fig. 5), and the year of 2010s represent the strongest known hydric deficit in the history of the river. Thus, the results obtained in the present study may reflect the biogeochemical response of fluvial systems similar to the Biobío River during an extreme drought event. Under such conditions, remarkable spatial and seasonal differentiation may occur in the production and emission of  $N_2O$  to the atmosphere. Drought conditions, however, may enhance  $N_2O$  production, suggesting that future water stresses - as expected for the Biobío River over the next 75

years (Landman *et al.*, 2014) - may lead to increased  $N_2O$  production as a further positive feedback to global warming.

## CONCLUSIONS

Rivers have a major influence on the production of different greenhouse gases. The powerful greenhouse gas  $N_2O$  is distributed heterogeneously in rivers both spatially and temporally. In environments subjected to increasing anthropogenic stress, as well as significant hydric stress like in central Chile, it is necessary to evaluate the current status and the factors driving  $N_2O$  both in space and time.

Between 2010 and 2016, central Chile experienced a megadrought by an extreme level of hydric stress. Here, the evaluation of  $N_2O$  from one of the most important hydrologic system, the Biobío River during the megadrought will allow us to predict and better understand the effect of hydric stress events and anthropogenic pressure in a more reliable way in similar systems. Our results show that the Biobío River was an important source of atmospheric  $N_2O$  in response to the megadrought between 2013 and 2015.  $N_2O$  oversaturation in the water was higher during the dry seasons, with an inverse relationship to water discharge. The impact of anthropogenic activities was evidenced by a remarkable increase in  $N_2O$  oversaturation at the outlet of the river, the most urbanized and industrialized river section. These results highlight the importance of climatic conditions in affecting the contribution of  $N_2O$  to the atmosphere in the region, and may suggest a positive feedback of increasing  $N_2O$  production with hydric stress; the latter being predicted to further increase in the near future.

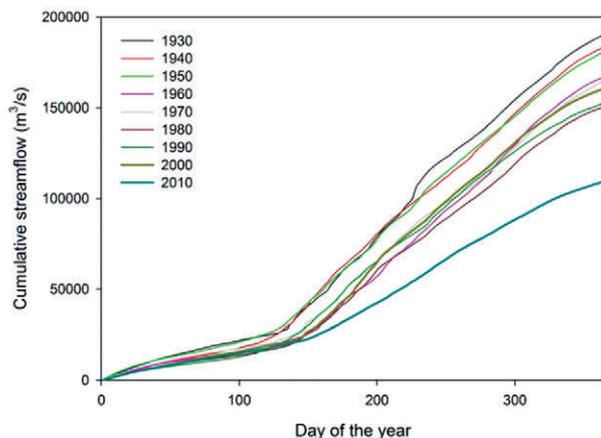
Future works may focus on determining the biogeochemical response to increased  $N_2O$  and to other greenhouse gases; and may further assess the impact of  $N_2O$  production in both the sediment and the rivers under a low discharge and higher temperature climate scenario.

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**Fig. 5.** Decadal cumulative average water discharge at the outlet of the Biobío River since 1930.

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