

1 **Rise of toxic cyanobacterial blooms is promoted by agricultural intensification in the basin of**  
2 **a large subtropical river of South America**

3

4 **Running head - 45 characters including spaces:** Agro-intensification promotes Cyanobacteria

5

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21

22 **Summary**

23 Toxic cyanobacterial blooms are globally increasing with negative effects on aquatic ecosystems,

24 water use and human health. Blooms' main driving forces are eutrophication, dams construction,

25 urban waste, replacement of natural vegetation with croplands, and climate change and variability.

26 The relative effects of each driver have not still been properly addressed, particularly in large river

27 basins. Here, we performed a historical analysis of cyanobacterial abundance in a large and

28 important ecosystem of South America (Uruguay river, c.a. 1,900 km long, 365,000 km<sup>2</sup> basin). We  
29 evaluated the interannual relationships between cyanobacterial abundance and land use change,  
30 river flow, urban sewage, temperature and precipitation from 1963 to the present. Our results  
31 indicated an exponential increase in cyanobacterial abundance during the last two decades,  
32 congruent with an increase in phosphorus concentration. A sharp shift in the increase rate of  
33 cyanobacterial abundance after the year 2000 was identified, resulting in abundance levels above  
34 public health alert since 2010. Path analyses showed a strong positive correlation between  
35 cyanobacteria and cropland area at the entire catchment level, while precipitation, temperature and  
36 water flow effects were negligible. Present results help to identify high nutrient input agricultural  
37 practices and nutrient enrichment as the main factors driving toxic bloom formation. These  
38 practices are already exerting severe effects on both aquatic ecosystems and human health and  
39 projections suggest these trends will be intensified in the future. To avoid further water degradation  
40 and health risk for future generations, a large scale (transboundary) change in agricultural  
41 management towards agroecological practices will be required.

42

43 **Key words.** Cyanobacterial blooms, land use, crops, temperature, precipitation, health risk.

## 44 **INTRODUCTION**

45

46 The occurrence of massive toxic blooms of cyanobacteria is an environmental and public health  
47 problem worldwide. Most blooms produce metabolites that are toxic to animals and humans, which  
48 are collectively referred to as cyanotoxins (Chorus & Welker, 2021; Preece et al., 2017; Svirčev et  
49 al., 2019). These cyanotoxins can lead to sickness and cause deaths of aquatic animals, including  
50 negative impacts on human health and other activities (Azevedo et al., 2002; Giannuzzi et al., 2011;  
51 Vidal et al., 2017; Zhang et al., 2015). Over the past few decades, an upsurge in the frequency,  
52 geographic distribution and severity of such blooms has recurred in many areas of the planet  
53 (Preece et al., 2017) and forecast models indicate that both bloom frequency and intensity will  
54 continue to increase (Fang et al., 2022). Therefore, disentangling the main driving causes to define  
55 management actions is of fundamental importance (Sukenik & Kaplan, 2021).

56

### 57 *Cyanobacterial bloom forcings*

58 Understanding cyanobacterial toxic bloom dynamics is a complex issue and there are several  
59 driving forces linked to their occurrence and toxicity. However, there is a 50-year consensus in the  
60 scientific community about the fundamental role that the input of nutrients to water bodies (due to  
61 cultural eutrophication) plays in driving algal biomass increment and cyanobacterial dominance  
62 (Huisman et al., 2018; Paerl et al., 2018). The identification of nutrient enrichment as a relevant  
63 driver can be traced back to early empirical studies (e.g. Vollenweider & Kerekes, 1982; Trimbee &  
64 Prepas, 1987), including manipulative experiments at the ecosystem-scale (Schindler, 1977) and has  
65 most recent support in cross-system field and laboratory studies of large data sets at different  
66 latitudes (Downing et al., 2001; Kosten et al., 2012). These studies demonstrate that bloom forming  
67 cyanobacteria benefit over other phytoplankton groups under high nutrients and low turbulence  
68 conditions (e.g. Kim et al., 2017; Scheffer et al., 1993).

69

70 Cultural eutrophication is originated by multiple human activities including agriculture, cattle rising

71 and poorly-treated sewage water from human settlements (Fang et al., 2022), which were traced  
72 back by paleolimnological studies (Bueno et al., 2021). Among the multiple human-related driving  
73 forces, agriculture is a prominent and persistent cause of diffuse nutrient loads and the world largest  
74 source of both phosphorus and nitrogen pollution to water bodies (Bennett et al., 2001; Chakraborty  
75 et al., 2017; MacDonald et al., 2011; Withers et al., 2014). A strong linkage has been identified  
76 between the industrialized cutting-edge production of crops and the emergence of toxic  
77 cyanobacteria outbreaks (Chakraborty et al., 2017; Massey et al., 2020).

78

79 There are other drivers that also promote cyanobacterial blooms and might exert synergistic effects  
80 with eutrophication (Meerhoff et al., 2022). Most cyanobacterial organisms have relatively low  
81 specific growth rates and thus toxic populations are not able to proliferate under low to moderate  
82 flushing (Bakker & Hilt, 2016; Kruk et al., 2021). Therefore, in eutrophic rivers, dam construction  
83 favors blooms by increasing water residence time (Massey et al., 2020; Paerl & Huisman, 2009).  
84 High temperatures also accelerate cyanobacterial metabolism, promoting their growth, the  
85 formation of blooms under stratified waters (Paerl & Huisman, 2008) and the stimulation of toxin  
86 production (Martínez de la Escalera et al., 2017; Massey et al., 2020). Changes in precipitation  
87 modify water residence time and geographical distribution of cyanobacterial blooms (Massey et al.,  
88 2020; Kruk et al., 2021). Moreover, the replacement of natural land cover with crops and the  
89 modification of trophic food webs accelerates the effects of eutrophication and climate change  
90 (Alcántara et al., 2022; Meerhoff et al., 2022). Thus, understanding the importance of individual  
91 drivers and potential synergies among them are both relevant to provide management actions to  
92 stakeholders at different levels (Fang et al., 2022; Alcántara et al., 2022; Kosten et al., 2012;).

93

#### 94 *Watersheds in Southern South America*

95 South America is one of the regions most affected by global agriculture, where large-scale cash  
96 crops have replaced natural vegetation (Doughty, 2010; Graesser et al., 2018; Perez et al., 2021;  
97 Tucci & Clarke, 1998). Large proportions of land are dedicated to agricultural production with

98 annual crops, such as soybean, maize, wheat and rice (Giller et al., 2021). These large-scale cash  
99 crop areas have transformed land use dynamics with important implications for food security,  
100 biodiversity conservation, and revenues (Bueno et al., 2021; Graesser et al., 2018; Tucci, 2001;  
101 Tucci & Clarke, 1998).

102

103 Within South America, the Uruguay river is a major and vital ecosystem (c.a. 1,900 km long) with a  
104 transboundary catchment area of 365,000 km<sup>2</sup> shared by three countries (Brazil, Argentina and  
105 Uruguay). This river supports key ecosystem services, drinking water for human consumption,  
106 recreation and tourism, navigation, as well as biological production at different trophic levels  
107 including fisheries (de Vasconcelos et al., 2014; Tucci & Clarke, 1998). It integrates the Río de la  
108 Plata basin, the second largest in South America (Guerrero et al., 1997; Milliman et al., 2008) and  
109 represents an important region for agriculture and livestock production in the world (de  
110 Vasconcelos et al., 2014; Tucci, 2001; Tucci & Clarke, 1998). During the last decades, natural  
111 grasslands supporting extensive livestock production have been replaced with large scale croplands,  
112 causing degradation of ecosystems services (de Vasconcelos et al., 2014; Modernel et al., 2016).  
113 High-resolution palaeoceanographic studies in the Río de la Plata demonstrated the effect of  
114 continental human activities on the aquatic ecosystems (Pérez et al., 2021a; Pérez et al., 2021b)  
115 attributed to land-use intensification and dam construction leading to significant soil erosion and  
116 transport/deposition into the inner shelf, enhanced by the El Niño Southern Oscillation (ENSO)  
117 events (Bonachea et al., 2010; Pérez et al., 2021a; Pérez et al., 2021b). The Uruguay river holds 10  
118 large dams (> 10 km<sup>2</sup>) and there are more than 90 further dams either planned or under construction  
119 (<http://globaldamwatch.org>) (Pérez et al., 2021a), which cause strong modifications to its  
120 hydrological cycle (Depetris & Pasquini, 2007a, b; Milliman et al., 2008; Tucci & Clarke, 1998).

121

122 Toxic cyanobacterial blooms are frequently registered in the Uruguay River (Bordet et al., 2017;  
123 Debastiani Júnior et al., 2016; O'Farrell & Izaguirre, 2014) and exported to the Río de la Plata  
124 Estuary (Nagy et al., 2002; Kruk et al., 2017; Martínez de la Escalera et al., 2017; Segura et al.,

125 2017). These blooms negatively impact health, including human intoxications (Giannuzzi et al.,  
126 2011; Vidal et al., 2017), local economies, tourism, and related recreational activities (Kruk,  
127 Martínez de la Escalera et al., 2021). Long term records of toxic cyanobacterial blooms and the  
128 analysis of their forcings are not common in large watersheds worldwide (Doubek et al., 2015; Salk  
129 et al., 2022), and particularly in the Southern Hemisphere, where these type of analysis are scarce  
130 (Silvarrey Barruffa et al., 2021). The objective of this study was to disentangle the contribution of  
131 different environmental drivers in determining cyanobacterial abundance. With this aim, we  
132 analyzed temporal trends which showed an increase in the frequency and severity of cyanobacterial  
133 blooms over a 60-year period in a large subtropical river basin (Uruguay river basin). Also, causal  
134 path models were used to evaluate the relative importance of each driving forcing (land use change,  
135 urban sewage, river flow and climate variability (temperature, precipitation) in explaining  
136 cyanobacterial abundance at the entire-basin level.

137

## 138 **METHODS**

139

### 140 *Study area*

141 The Uruguay river basin lies between 28°10'S and 37°08'S (Di Persia & Neiff, 1982) and it is  
142 shared by Brazil (percentage of surface area: 50.1%), Uruguay (32.5%) and Argentina (17.4%)  
143 (Figure 1). The river origin is located in Brazil at 200 meters above mean sea level and its main  
144 tributary is the Negro River (500 km long). Together with the Paraná River are the main tributaries  
145 of the Río de la Plata Estuary.

146

147 The Uruguay river hydrographic basin climate is located in a humid subtropical (*sensu* Köppen )  
148 and is set upon sedimentary and volcanic soils. It comprises mainly three dominant biomes from  
149 headwaters to the Río de la Plata: i) upper basin: mixed agro-eco-region, ii) middle basin: Campos  
150 biome and iii) lower basin: South American Pampas biome (Argentina, Uruguay) (Saurral et al.,  
151 2008; Viglizzo & Frank, 2006). The upper river is steep (43 cm km<sup>-1</sup>), comprises 750 km, has a

152 maximum average flow of  $9,387 \text{ m}^3\text{sec}^{-1}$  and exhibits large annual variations in water level. The Itá  
153 Hydroelectric Power Plant is the largest reservoir in this area (lake area:  $141 \text{ km}^2$ , basin area:  
154  $45,800 \text{ km}^2$ ) and it became operative in 2000. Additionally, eight dams are now built in the river  
155 and other 89 are planned for the next few years (<http://globaldamwatch.org>, Mulligan et al., 2020)  
156 (Figure 1). The middle zone of the river is 800 km long, has a  $9 \text{ cm km}^{-1}$  slope, and does not contain  
157 large reservoirs. The lower river is 350 km with a slope  $3 \text{ cm km}^{-1}$  and its hydrology is strongly  
158 influenced by the Salto Grande reservoir, constructed for hydroelectricity production (Concordia,  
159 Salto,  $31^\circ 16' 29'' \text{S } 57^\circ 56' 18'' \text{W}$ ). Salto Grande became first became operative in 1979 with a  
160 catchment area of  $224,000 \text{ km}^2$  and a lake surface area of  $783 \text{ km}^2$ . Finally, at Nueva Palmira, the  
161 Uruguay river flows into the Río de la Plata Estuary, and then into the Atlantic Ocean (Figure 1).

162

### 163 ***Cyanobacterial abundance and limnological variables***

164 Cyanobacterial information including abundance and blooms presence was compiled for the lower  
165 Uruguay river from different data sources, attaining a total number of 1,712 cases from 1963 to  
166 2019. From the total number of cases, 1,424 were retrieved from sampling sites located either  
167 upstream or in the Salto Grande (SG) dam and 288 from sites downstream this dam. The largest part  
168 of the data set was obtained from publicly accessible reports of the Uruguayan Government Water  
169 Institution (i.e., *Obras Sanitarias del Estado*, OSE, 2009), for the period between 1963 and 2008.  
170 This information included cyanobacterial cells abundance ( $\text{cells mL}^{-1}$ ) in surface water, dominant  
171 cyanobacterial species and the presence of blooms in six sites next to three water treatment plants:  
172 1) upstream SG reservoir: Bella Unión, 2) in the SG reservoir: Belén and Constitución, and 3)  
173 downstream the reservoir in Salto, Fray Bentos, Paysandú and Bassotti. From 2008 until 2019 we  
174 gathered information from other institutional sources, including the Argentina-Uruguay  
175 Administrative Commission (CARU, 2019, <https://www.caru.org.uy/web/>), SG Joint Technical  
176 Commission (CTM, Argentina-Uruguay, <https://www.saltogrande.org/>) and the National  
177 Environmental Observatory of Uruguay (MVOTMA-OAN, 2019;  
178 <https://www.ambiente.gub.uy/oan/>). These data sources included cyanobacterial cells abundance,

179 cyanobacterial species, and fecal coliforms abundance (as fecal indicator bacteria, FIB) in 16 sites  
180 from Bella Union (Artigas) to the SG reservoir main channel next to the dam. Total phosphorus  
181 concentration in water ( $\text{mg L}^{-1}$ ) was retrieved for the same sites for the period 2008 to 2019,  
182 including information from CTM (N = 245), CARU (N= 25) and our own monitoring program  
183 (Kruk et al., 2015; N = 72).

184

185 In all cases, surface water samples were taken with bottles and fixed with Lugol solution.  
186 Cyanobacterial and other phytoplankton species were counted with comparable methods with  
187 Sedgwick Rafter or Utermöhl counting chambers using a light microscope and estimating the cell  
188 abundance ( $\text{cell mL}^{-1}$ ) using the settling technique (Utermöhl, 1958). Utermöhl chambers were used  
189 for counting samples with low phytoplankton abundances, while Sedgwick chambers were used for  
190 high abundance samples including those dominated by cyanobacteria and with presence of blooms.  
191 The abundance of cyanobacteria was also used to determine alert levels following (Chorus &  
192 Welker, 2021; level 1: 2,000 - 100,000  $\text{cell mL}^{-1}$ , level 2: higher than 100,000  $\text{cell mL}^{-1}$ , vigilance  
193 stage: cell abundance higher than 200  $\text{cell mL}^{-1}$ ).

194

195 The effects of human settlements were estimated based on the abundance of fecal indicator bacteria  
196 (FIB). Samples for FIB detection and quantification were taken from surface water at the same area  
197 as cyanobacteria but with a higher frequency from 1987 to 2020, attaining a total number of 2,127  
198 cases. For the 1987-2005 period, the information was retrieved from the binational Procon-CARU  
199 project ([https://www.caru.org.uy/web/2020/05/programa-procon-caru-1987-2005-recopilacion-y-  
200 validacion-de-datos/](https://www.caru.org.uy/web/2020/05/programa-procon-caru-1987-2005-recopilacion-y-validacion-de-datos/)). For the 2014-2020 period, the information source was the Uruguay National  
201 Environmental Observatory (OAN) (<https://www.ambiente.gub.uy/oan/>).

202

### 203 *Climatological and hydrological variables*

204 Temperature and precipitation data in the Uruguay river watershed from 1963 to 2020 were  
205 obtained from the historical climate database produced by the Climatic Research Unit (CRU) of the

206 University of East Anglia and provided by the World bank climate knowledge portal, corresponding  
207 to number 252 watershed ([https://climateknowledgeportal.worldbank.org/watershed/252/climate-  
208 data-historical](https://climateknowledgeportal.worldbank.org/watershed/252/climate-<br/>208 data-historical)). The information included annual average values of maximum, minimum and  
209 average daily temperatures (°C) and the average annual precipitation (mm). Historical data of  
210 Uruguay river flow rates were retrieved from the Argentinian National System of Information  
211 (<https://snih.hidricosargentina.gob.ar>) and corresponded to station 3802 in Paso de los Libres.  
212 Monthly average water flow ( $\text{m}^3 \text{sec}^{-1}$ ) was selected from 1963 to 2020 and expressed as the  
213 maximum value and the accumulated flow per year.

214

### 215 *Land Use*

216 Annual land use and land cover maps from 2000 to 2019 for the entire Uruguay river basin were  
217 obtained from an assembly of classifications generated by MapBiomass initiatives (Table 1). The  
218 MapBiomass project is an international multi-institutional initiative whose goal is to generate annual  
219 land use and land cover maps based on the use of satellite images provided by Landsat 4, 5, 7 and 8  
220 and automatic classification processes of images. These included MapBiomass Pampa (Baeza et al.  
221 2022) and Atlantic Forest Tri-national initiatives (Souza et al., 2020).

222

223 Land use and land cover were grouped into six classes natural forest, forest plantation, grasslands  
224 and wetlands, farming, non-vegetated areas (that include bare soil and urban infrastructure) and  
225 water bodies and rivers (Souza et al., 2020). Farming includes annual crops (i.e. maize, soybean,  
226 wheat, etc.) and sown pastures. Information for a longer period (from 1989 and 2019) was only  
227 available for the Brazilian portion of the basin. Collection 6 of Map-Biomass Brazil was used to  
228 separate the composition of the class "annual crops and pasture" into different land use covers, such  
229 as: pastures, soybeans, other summer crops and a "mosaic of pastures and annual crops".

230

### 231 *Data analysis*

232 Cyanobacterial abundance was  $\log_{10}$  transformed for most analyses and graphical representations.  
233 Linear models were performed to evaluate the change in cyanobacterial abundance and total  
234 phosphorus yearly average with time and were compared using the differences in the Akaike  
235 information criteria ( $\Delta$ AIC). The fit of the segmented models followed a well developed technique  
236 based on a linearization of the problem that allow to estimate slopes and intercepts before and after  
237 a breakpoint, as well as a confidence interval for the breakpoint (Muggeo, 2003) which is  
238 implemented in the *{segmented}* package (Muggeo, 2008) in the R software. The relationships  
239 between total phosphorus and cyanobacterial abundance, and between total phosphorus and land  
240 use areas were explored with correlations and linear models. Due to the lack of sufficient available  
241 data points and to the heterogeneity in the methodological approaches applied to estimate nitrogen  
242 in water, this nutrient was not included in the analysis.

243

244 To evaluate the effect of the unequal sample size and the increasing sampling effort with time on  
245 the estimation of cyanobacterial abundance (Hallegraeff et al., 2021) we conducted different  
246 strategies (Supplementary material 1); i) aggregating the information as yearly average both  
247 including and excluding the periods with less data (between 1995 and 2003), ii) aggregating the  
248 information every 5-years and performing unweighted and weighted regressions (weights  
249 proportional to the number of data points per period), and iii) using bootstrapping type of re-  
250 sampling to produce 250 smaller samples of 15 cases and then producing 250 temporal linear  
251 models with the yearly average in each period. In iii) the number of significant models with positive  
252 slopes was registered. The models with similar response variable were compared using AIC  
253 (Supplementary material 2).

254

255 Temporal trends in temperature (average, maximum, minimum), precipitation average, river flow  
256 (accumulated and maximum), and  $\log_{10}$  cyanobacterial abundance were explored for each data set.  
257 The presence of white noise, was evaluated in the residuals of the segmented regression on yearly  
258 clustered information, using Ljung-Box and turning points tests. The results did not allowed to

259 discard white noise and therefore specific time series analyses were not necessary. Spearman non-  
260 parametric rank correlation and the variance inflation factor (VIF) were used to evaluate  
261 multicollinearity among variables, including climatic, hydrological and land use.

262

263 Causal path models were constructed to evaluate the relative contribution as well as the interactions  
264 of the different potential driving factors of the annual average of  $\log_{10}$  (cyanobacterial  
265 abundance+1) ( $\log_{10}Cya$ ) using {"Lavaan"} package. This model framework is specially designed to  
266 evaluate competing models representing hypothesis based on theoretical arguments (Petraitis et al.,  
267 1996). We *a priori* introduced eight path models, including the effect of climate (precipitation: PP,  
268 maximum temperature: Tmax), hydrology (accumulated river flow: Qacum), and land use (area of  
269 land with annual crops and pastures and natural area) (Figure 2). Covariates were standardized to 0-  
270 1 prior to the analysis. To evaluate the existence of multicollinearity among the explaining  
271 variables, we inspected the VIF. No significant multicollinearity was retrieved as VIF values were  
272 in all cases lower than four. Competing models were compared based on the AIC and paths  
273 coefficients were estimated including their standard error (SE), z statistics and the associated p  
274 value. The Fisher's C statistic was calculated to evaluate whether the data follow the causal  
275 hypothesis specified in the dyacyclic aggregated paths. For the selected model, the root mean square  
276 error of approximation (RMSEA), the explained variance ( $R^2$ ) and the comparative fit index (*cfi*)  
277 were estimated. Models were performed for the 1985-2019 period using land use covariates from  
278 the Brazilian catchment area.

279

## 280 **RESULTS**

### 281 *Temporal Changes in cyanobacterial abundance and composition*

282 Total abundance of cyanobacterial cells in the Salto Grande reservoir and downstream increased by  
283 4 to 5 orders of magnitude from 1963-1970 (average  $33 \text{ cell L}^{-1}$ , maximum =  $53 \text{ cell L}^{-1}$ ) to 2010-  
284 2020 (average  $21281 \text{ cell L}^{-1}$ , maximum =  $1.69 \times 10^6 \text{ cell L}^{-1}$ ; Figure 3) and phytoplankton  
285 communities shifted from either diatom- or chlorophyte-dominated to cyanobacteria-dominated.

286 The dominant cyanobacterial genera shifted from the first decades when *Merismopedia*, *Lyngbya*,  
287 *Planktothrix* and other genera from the Oscillatoriales order dominated, to *Dolichospermum* and  
288 *Microcystis* after the 2000s. In recent years, blooms were in most cases dominated by members of  
289 the *Microcystis aeruginosa* complex.

290

291 After 2000, blooms became highly frequent along with an exponential increase in both maximum  
292 and average cell abundance (Figure 3). Surface scums (i.e., water discoloration events) became  
293 frequent from 2000 to 2007, particularly in the lower Uruguay river (Figure 1). Further, the risk  
294 level alerts also increased with time, from no risk to vigilance (cell abundance higher than 200 cell  
295  $\text{mL}^{-1}$ ) in the period between 1963 to 2005, then from vigilance to at least one level 1 alert (2,000 -  
296 100,000 cell  $\text{mL}^{-1}$ ) per year, and since 2006 at least one level 2 alert (100,000 cell  $\text{mL}^{-1}$ ) per year  
297 was observed. During the last decade (2010-2020), 155 and 46 alerts of level 1 and 2, were  
298 respectively reported. In addition, the temporal persistence of blooms throughout the year increased  
299 from isolated occasions in summer to continuous presence, including even the cold seasons (winter  
300 and autumn). Between 1960 and 2000 no winter or autumn blooms occurred; between 2000 and  
301 2010, 24 cases of level 1 alert and one case level 2 alter were observed in winter. In the last decade,  
302 65 level 1 and 16 level 2 alerts developed during winter and autumn.

303

304 Temporal dynamics of yearly average cyanobacterial abundance, presented two different periods  
305 and was well characterized by a segmented log-linear model: I) a first period (1963-2000) where  
306 average cell abundance remained constant with time (slope [95%CI]= -0.02[-0.05 to 0.01]), a  
307 breakpoint after year 2000 [CI95%=1995 to 2005], and II) a second period characterized by an  
308 exponential increase (slope [95%CI]= 0.21[0.13 to 0.30]) (Figure 3). The results remained similar  
309 either by including or excluding years with less data (between 1995 and 2003) or aggregating the  
310 information into 5-year periods. Linear weighted regressions also provided similar trends and  
311 coefficients and thus we decided to keep the simpler approach. In addition, when data were  
312 aggregated in 5-year bins and the lineal model was performed 250 times, the fitted temporal models

313 indicated a positive trend of cyanobacterial abundance increase in 100% of the cases, being  
314 significant in 93% of the cases (Supplementary material 2).

315

### 316 *Climate and hydrological changes observed in the Uruguay river basin*

317 Average, maximum and minimum annual temperature at the watershed level were significantly  
318 correlated (minimum vs mean temperature:  $r=0.93$ ; maximum vs mean temperature:  $r=0.84$ ;  
319 minimum vs maximum temperature:  $r=0.58$ ;  $N=60$ , all  $p < 0.01$ ) (Figure 4A). Minimum annual  
320 temperatures presented significant positive temporal linear trends since the beginning of the time  
321 series ( $T_{\min} = -22.11 + 0.018 \text{ year}$ ;  $N=60$ ,  $R^2=0.32$ ,  $p < 0.01$ ). A simple linear trend was chosen with  
322 respect to the more complex segmented model as the difference in AIC was low ( $\Delta\text{AIC} < 2$ ).

323

324 Precipitation (PP) in the watershed showed an apparent linear temporal trend in the period 1963-  
325 2020 but this trend was not significant ( $p > 0.05$ ;  $R^2=0.05$ ) and no breakpoints were detected (Figure  
326 4B). Neither the accumulated flow nor the annual maxima of the Uruguay river flow presented  
327 temporal trends ( $p > 0.05$ ;  $R^2=0.004$  and  $p > 0.05$ ;  $R^2=0.025$  respectively) (Figure 4C).

328

329 Correlation analyses between climatic variables for the period 1963-2020 indicated that average  
330 annual precipitation and minimum temperature had a weak positive linear correlation ( $r=0.52$ ,  
331  $p < 0.01$ ). Precipitation presented a strong correlation with water flow, either accumulated ( $r=0.83$ ,  $p$   
332  $< 0.01$ ) or maximum flow ( $r=0.73$ ,  $p < 0.01$ ). Minimum temperature exhibited a weak but significant  
333 correlation with yearly precipitation and water flow ( $r_{\text{PP}}=0.52$ ;  $r_{\text{Qmax}}=0.42$ ;  $r_{\text{Qacum}}=0.4$ ; all  $p$  values  
334  $< 0.01$ ) while maximum yearly temperature displayed a negative weak correlation with accumulated  
335 water flow ( $r_{\text{Qacum}}=-0.35$ ). Maximum and accumulated water flow were strongly correlated  
336 (Supplementary material 3).

337

### 338 *Changes in indicators of human and cattle effects*

339 From 1987 to 2020 the collected FIB exhibited a variability of six orders of magnitude, ranging  
340 from undetectable to  $2 \times 10^6$  UFC  $100\text{mL}^{-1}$ , a median value of 160 and a mean of 5,875 UFC  
341  $100\text{mL}^{-1}$  (Figure 5). A negative temporal trend in FIB concentration was described by a log-linear  
342 model (slope[95%CI]= -0.017 [-0.02 to -0.013]) with a low but significant explained variance  
343 ( $R^2=0.04$ ,  $p<0.001$ ,  $N=2119$ ).

344

#### 345 *Temporal trends in land use change in the Uruguay river basin*

346 The Uruguay river watershed displayed a marked transition in land use from 2000 to 2019 (Figure 1  
347 and Figure 6). The most conspicuous patterns were a 17% increase in land use dedicated to crops  
348 and pastures ( $23,842 \text{ km}^2$ ), a 97% increase in the forestation area ( $10,047 \text{ km}^2$ ), a decrease in 6% of  
349 natural forest ( $3,004 \text{ km}^2$ ) and a decrease in 17% of natural grasslands ( $23,842 \text{ km}^2$ ). This last  
350 change represents a loss of ~8% of natural grasslands replaced with intensive agricultural land use,  
351 including crops and forestation. Pastures and crops, as well as forestation gains occurred along the  
352 entire basin, with larger proportions in the middle and lower areas (Figure 1). In the upper basin the  
353 loss of pastures and crops were mainly detected in the littoral zones of the river (Figure 1). Data  
354 recorded in the upper and middle basins, available for 1985 to 2020, presented the same trend and  
355 were highly correlated with the changes registered in the lower basin for the 2000-2020 period  
356 (Table 2; Supplementary material 4). In addition, the proportion of land uses remained similar  
357 throughout most of the study, therefore representing a proxy for total watershed changes (Table 2).

358

359 A major increase in land use dedicated to soybean production occurred along with a large decrease  
360 in other crops together with depleted pastures and the class “mosaic of pastures and annual crops”.  
361 Soybeans were first introduced in 2000 but nowadays accounts for nearly 45% ( $4,114 \text{ km}^2$ ) of the  
362 annual cropland area (Figure 7). Pastures, other crops and the “mosaic of pastures and annual  
363 crops” decreased by 34, 24 and 48%, respectively.

364

#### 365 *Importance of drivers explaining cyanobacterial abundance*

366 The causal path model indicated that changes in the area dedicated to crops and pastures (CP)  
367 displayed the strongest and significant positive relationship with cyanobacterial abundance (logCya)  
368 (Table 3 and Figure 8). Model 8 presented the larger difference in AIC ( $\Delta AIC > 35$ ) with the  
369 remaining models and included only the path between CP and logCya as significant (Table 3,  
370 Figure 8). A positive relationship between annual precipitation and accumulated flow was also  
371 observed, but this relationship did not affect cyanobacterial abundance (Table 3). The selected  
372 model Fisher's *C* statistic was not significant ( $C=1.50$ ,  $df=2$ ,  $p=0.47$ ) supporting the hypothesis that  
373 data were generated following the causal relations specified in the dyacyclic aggregated path  
374 (Model 8, Table 4). The RMSE of approximation was low (95% confidence interval [ $\sim 0-0.36$ ],  
375  $p=0.49$ ) while the comparative fit index was high ( $cfi=1.00$ ) as well as the explained variance  
376 ( $R^2=0.52$ ). Precipitation accounted for a high amount of variance from the accumulated water flow  
377 ( $R^2=0.79$ ). Similar results were obtained when Tmin was included in the model instead of Tmax  
378 (Supplementary material 5).

379

### 380 *Relationship with total phosphorus concentration in water*

381 An increase in average total annual phosphorus in water (TP) was observed with time (TP<sub>mean</sub>=  
382  $5.98 + 0.003 \text{ year}$ ;  $R^2=0.66$ ,  $p < 0.005$ ). Cyanobacterial cells abundance (Cya) was significantly  
383 related with TP values higher than  $0.2 \text{ mgL}^{-1}$  ( $N= 46$ , Pearson correlation:  $0.46$ ;  $\log_{10}(Cya+1) =$   
384  $2.22 * TP \text{ mgL}^{-1} + 1.96$ ;  $df = 44$ ,  $R^2 = 0.18$ ,  $p < 0.005$ ) (Figure 9). For TP values lower than  $0.2 \text{ mgL}^{-1}$   
385 no significant correlation was obtained. Finally, we also obtained a positive relationship between  
386 average annual TP concentration in water and area dedicated to annual crops and pastures (TP mg  
387  $\text{mL}^{-1} = 1.79 * \log_{10}(\text{Annual crops and pasture area}) - 8.93$ ,  $R^2 = 0.54$ ,  $p < 0.05$ ; Figure 10A) and a  
388 negative relationship with natural grassland area (TP mg  $\text{mL}^{-1} = -1.70 * \log_{10}(\text{Grasslands area}) +$   
389  $8.79$ ,  $R^2 = 0.63$ ,  $p < 0.05$ ; Figure 10B).

390

## 391 **DISCUSSION**

392 In this manuscript we evidenced the exponential increase in cyanobacterial abundance and number

393 of blooms in a relevant subtropical aquatic ecosystem of South America. A comprehensive  
394 geographical long-term analysis confirmed that the increase of intensive agriculture land use was  
395 the main driver. Climatic and hydrological effects on cyanobacterial abundance, otherwise, were  
396 rather marginal. Moreover, results indicate that models not including the effect of land use change  
397 would incorrectly point to temperature as a relevant variable, but under a causal path analysis we  
398 demonstrated that its effect was negligible when compared with land use change. Anthropogenic  
399 drivers of eutrophication other than land use, such as sewage or contamination by cattle were  
400 discarded as the temporal trend of fecal contamination was opposite to that observed for  
401 cyanobacterial abundance. Previous field studies suggest that anthropogenic factors (e.g. fertilizer,  
402 gross domestic product, population) are stronger than climatic drivers (e.g. temperature,  
403 rainfall)(Fang et al., 2022). Also nutrients and not temperature were identified as the key drivers for  
404 cyanobacterial biomass in lakes from America (Bonilla et al., 2023).

405

406 The global trend towards the replacement of natural areas by cropping and pastures was validated  
407 for the Uruguay basin. A drastic change in the type of farming and crops occurred in the new  
408 millennium (Giller et al., 2021; Schindwein et al., 2021) with a large expansion of soybean  
409 production (Figure 7). These practices can trigger large phosphorous releases to water bodies, due  
410 to increased phosphate fertilization and no-till practices, stratification of phosphorous content in  
411 surface soil horizons and facilitation of phosphorous runoff (Carver et al., 2022). These mechanisms  
412 were confirmed here with linear positive relations between total phosphorous and area of crops and  
413 sown pastures, as well as between total phosphorous and cyanobacterial abundance. In future  
414 analyses nitrogen inputs should also be evaluated (Paerl et al., 2016; Wurtsbaugh et al., 2019).

415

416 In the middle and lower Uruguay basin, soybean expansion was at expense of the deterioration area  
417 of natural grasslands previously devoted to cattle ranching, which shows lower phosphorous exports  
418 (Goyenola et al., 2020; Modernel et al., 2016; Viglizzo et al., 2001). This resulted in increased  
419 nutrient loads and a reduced capacity of natural vegetation for filtering the contaminated water

420 runoff from land (Goyenola et al., 2020). The gain in secondary forest in the northern basin (Rosa et  
421 al., 2021) was not able to counteract the increment in agriculture area promoting cyanobacterial  
422 abundance and toxic blooms.

423

#### 424 *Climatic and hydrological variables affecting cyanobacteria*

425 In general terms, temperature, precipitation and river flow modulate ecosystems eutrophication  
426 (Meerhoff et al., 2022; Salk et al., 2022). In this study, these variables did not present a significant  
427 contribution to explain major historical changes in cyanobacterial abundance in the Uruguay river.  
428 Temperature values were never limiting for cyanobacterial growth (Reynolds et al., 2002; Segura et  
429 al., 2018), and blooms occurred throughout the year including the coldest months (Kruk et al.,  
430 2017). Furthermore, it has been recently shown that different toxic cyanobacterial ecotypes  
431 exhibiting different temperature optima exist (Martínez de la Escalera et al., 2022) sustaining  
432 potential toxicity production along the entire year in the studied system.

433

434 The obtained positive relationship between precipitation and water flow is coincident with studies  
435 evaluating larger periods (1900-2020) in the lower reaches of the basin (Saurral et al., 2008; Barros  
436 et al., 2015; Díaz et al., 2020; Ungerovich et al., 2021). Also, previous studies described how water  
437 flow modulates cyanobacterial blooms (Mitrovic et al., 2011; Ferrari et al., 2011; O'Farrell &  
438 Izaguirre, 2014; Adloff et al., 2018). However, our results evidenced that in the long run (years-  
439 decades), land use change and its effect on eutrophication were hierarchically more important than  
440 hydrology. Hydrological variability is expected to modulate cyanobacteria at shorter time scales  
441 (days-months), promoting blooms by biomass accumulation (Bowling et al., 2013) and long-range  
442 transport (Kruk et al., 2021).

443

444 An increase in more than four orders of magnitude in the abundance of cyanobacteria took place  
445 between 1963-2000 and 2000-2020. This type of catastrophic shifts, are commonly observed in  
446 elsewhere aquatic ecosystems (Scheffer et al., 1993) and specifically in reservoirs (Yang et al.,

447 2017). Phytoplankton community shifts driven by human activities, particularly nutrient  
448 enrichment, changes rapidly from species rich communities experiencing good water quality into  
449 cyanobacteria dominated communities with toxin production (Scheffer et al., 1993). These drastic  
450 shifts are non-linear and reversing such a process could take far longer than it takes to arrive to  
451 degraded quality ecosystems. A clear evidence of a regime shift can be traced to the extreme of the  
452 closure of three potabilization plants (years 2005, 2006 and 2008) (OSE, 2009), the persistence of  
453 blooms throughout the year, the dramatic increase in the number of health alerts from bi-national  
454 authorities (especially after 2000), and the occurrence of acute human intoxications in the river and  
455 downstream (Giannuzzi et al., 2011; Vidal et al., 2017). These bloom events reached a climax in  
456 2019, when an unprecedented large and persistent bloom of *Microcystis* reached the Río de la Plata  
457 (Aubriot et al., 2020; Kruk et al., 2021).

458

#### 459 ***Future scenarios and land use changes on demand***

460 Land use changes are affected mainly by economical drivers (Bueno et al., 2021; Modernel et al.,  
461 2016; Schlindwein et al., 2021). Brazil, Argentina and Uruguay are major producers of agricultural  
462 commodities that increased substantially in the last years to supply the demand of the world market  
463 (Bueno et al., 2021; Schlindwein et al., 2021). As a result, the expansions of monocultures such as  
464 soybean or eucalyptus were performed in absence of adequate conservation strategies and  
465 environmental impact assessments (Bueno et al., 2021; Modernel et al., 2016; Viglizzo & Frank,  
466 2006). Along with this escalation in world demand of crops, warming, increased precipitation and  
467 increased river flow are expected (Díaz et al., 2013; Vera et al., 2006) with synergistic effects  
468 modulating water quality, pollution and biodiversity loss (Wilkinson et al., 2022; Zabel et al.,  
469 2019). Some of the areas that currently experience restrictions on cultivation will become  
470 productive regions depending on supplementary irrigation by constructing dams. Dams modulate  
471 river discharge and sequester sediments (Bonachea et al., 2010; Depetris & Pasquini 2007a), thus  
472 favoring eutrophication and allowing reproduction and accumulation of cyanobacteria, increasing  
473 their toxin production and diversity (Martínez de la Escalera et al., 2017, 2022) as reported for the

474 upper reaches (Debastiani Júnior et al., 2016) and at the lower stretches (O’Farrell & Izaguirre,  
475 2014) of the river. Increases in extreme precipitation events, allow rapid nutrient transport from  
476 soils into rivers (Ockenden et al., 2017) and then to dams, where blooms will continue to increase in  
477 frequency and magnitude. Extreme events will help transport blooms to coastal and marine  
478 ecosystems along with their negative effects (Alcántara et al., 2022; Kruk et al., 2021; Preece et al.,  
479 2017).

480

481 The first and more important step to limit the increase in cyanobacterial blooms is to reduce the  
482 external anthropogenic loading of nutrients at the entire basin level as well as limiting dam  
483 construction. Otherwise, local actions will have little chance of being effective (Chorus & Welker,  
484 2021). Major changes in agricultural practices will be required to mitigate phosphorus losses and  
485 cyanobacterial blooms under a climate change scenario (Ockenden et al., 2017) specially at the  
486 catchment-to-coast transboundary continuum (Paerl et al., 2018; Wurtsbaugh et al., 2019). Large  
487 river basins include international territories over several countries and their management requires  
488 coordinated regional actions in terms of river water use and land use policies, which are still far  
489 from being implemented in the current settings (Saguier et al., 2021). As current results suggests,  
490 the increase of surface area dedicated to high nutrient input agricultural practices and the  
491 intensification of these practices in the basin will intensify the present situation of toxic  
492 cyanobacterial blooms in this subtropical river.

493

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502

## 503 **REFERENCES**

- 504 Adloff, C. T., Bem, C. C., Reichert, G., & Azevedo, J. C. R. de. (2018). Analysis of the  
505 phytoplankton community emphasizing cyanobacteria in four cascade reservoirs system of the  
506 Iguazu River, Paraná, Brazil. *RBRH*, 23(0). <https://doi.org/10.1590/2318-0331.0318170050>
- 507 Alcántara, I., Somma, A., Chalar, G., Fabre, A., Segura, A., Achkar, M., Arocena, R., Aubriot, L.,  
508 Baladán, C., Barrios, M., Bonilla, S., Burwood, M., Calliari, D. L., Calvo, C., Capurro, L.,  
509 Carballo, C., Céspedes-Payret, C., Conde, D., Corrales, N., ... García-Rodríguez, F. (2022). A  
510 reply to “Relevant factors in the eutrophication of the Uruguay River and the Río Negro.”  
511 *Science of The Total Environment*, 818, 151854.  
512 <https://doi.org/10.1016/j.scitotenv.2021.151854>
- 513 Aubriot, L., Zabaleta, B., Bordet, F., Sienna, D., Risso, J., Achkar, M., & Somma, A. (2020).  
514 Assessing the origin of a massive cyanobacterial bloom in the Río de la Plata (2019):  
515 Towards an early warning system. *Water Research*, 181, 115944.  
516 <https://doi.org/10.1016/j.watres.2020.115944>
- 517 Azevedo, S. M. F. O., Carmichael, W. W., Jochimsen, E. M., Rinehart, K. L., Lau, S., Shaw, G. R.,  
518 & Eaglesham, G. K. (2002). Human intoxication by microcystins during renal dialysis  
519 treatment in Caruaru—Brazil. *Toxicology*, 181–182, 441–446. [https://doi.org/10.1016/S0300-](https://doi.org/10.1016/S0300-483X(02)00491-2)  
520 [483X\(02\)00491-2](https://doi.org/10.1016/S0300-483X(02)00491-2)
- 521 Baeza, S., Vélez-Martin, E., De Abelleira, D., Banchero, S., Gallego, F., Schirmbeck, J., Veron, S.,  
522 Vallejos, M., Weber, E., Oyarzabal, M., Barbieri, A., Petek, M., Guerra Lara, M., Sarrailhé, S.,  
523 S., Baldi, G., Bagnato, C., Bruzzone, L., Ramos, S., & Hasenack, H. (2022). Two decades of  
524 land cover mapping in the Río de la Plata grassland region: The MapBiomass Pampa initiative.  
525 *Remote Sensing Applications: Society and Environment*, 28, 100834.  
526 <https://doi.org/10.1016/j.rsase.2022.100834>

527 Bakker, E. S., & Hilt, S. (2016). Impact of water-level fluctuations on cyanobacterial blooms:  
528 Options for management. *Aquatic Ecology*, 50(3), 485–498. [https://doi.org/10.1007/s10452-](https://doi.org/10.1007/s10452-015-9556-x)  
529 015-9556-x

530 Barros, V. R., Boninsegna, J. A., Camilloni, I. A., Chidiak, M., Magrín, G. O., & Rusticucci, M.  
531 (2015). Climate change in Argentina: Trends, projections, impacts and adaptation: Climate  
532 change in Argentina. *Wiley Interdisciplinary Reviews: Climate Change*, 6(2), 151–169.  
533 <https://doi.org/10.1002/wcc.316>

534 Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human Impact on Erodeable Phosphorus  
535 and Eutrophication: A Global Perspective. *BioScience*, 51(3), 227.  
536 [https://doi.org/10.1641/0006-3568\(2001\)051\[0227:HIOEPA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2)

537 Bonachea, J., Bruschi, V. M., Hurtado, M. A., Forte, L. M., da Silva, M., Etcheverry, R.,  
538 Cavallotto, J. L., Dantas, M. F., Pejon, O. J., & Zuquette, L. V. (2010). Natural and human  
539 forcing in recent geomorphic change; case studies in the Rio de la Plata basin. *Science of The*  
540 *Total Environment*, 408(13), 2674–2695. <https://doi.org/10.1016/j.scitotenv.2010.03.004>

541 Bonilla, S., Aguilera, A., Aubriot, L., Huszar, V., Almanza, V., Haakonsson, S., Izaguirre, I.,  
542 O'Farrell, I., Salazar, A., Becker, V., Cremella, B., Ferragut, C., Hernandez, E., Palacio, H.,  
543 Rodrigues, L. C., Sampaio da Silva, L. H., Santana, L. M., Santos, J., Somma, A., ...  
544 Antoniadis, D. (2023). Nutrients and not temperature are the key drivers for cyanobacterial  
545 biomass in the Americas. *Harmful Algae*, 121, 102367.  
546 <https://doi.org/10.1016/j.hal.2022.102367>

547 Bordet, F., Fontanarrosa, M. S., & O'Farrell, I. (2017). Influence of light and mixing regime on  
548 bloom-forming phytoplankton in a subtropical reservoir. *River Research and Applications*,  
549 33(8), 1315–1326. <https://doi.org/10.1002/rra.3189>

550 Bowling, L. C., Merrick, C., Swann, J., Green, D., Smith, G., & Neilan, B. A. (2013). Effects of  
551 hydrology and river management on the distribution, abundance and persistence of  
552 cyanobacterial blooms in the Murray River, Australia. *Harmful Algae*, 30, 27–36.  
553 <https://doi.org/10.1016/j.hal.2013.08.002>

554 Bueno, C., Alves, F. L., Pinheiro, L. M., Perez, L., Agostini, V. O., Fernandes, E. H. L., Möller, O.  
555 O., Weschenfelder, J., Pinho, G. L. L., Wallner-Kersanach, M., Moura, R. R., Durán, J. M.,  
556 Etchevers, I., Costa, L. D. F., Werlang, C. C., Bortolin, E., Machado, E., Figueira, R. C. L.,  
557 Ferreira, P. A. L., ... García-Rodríguez, F. (2021). The effect of agricultural intensification  
558 and water-locking on the world's largest coastal lagoonal system. *Science of The Total*  
559 *Environment*, 801, 149664. <https://doi.org/10.1016/j.scitotenv.2021.149664>

560 CARU. Comisión Administradora del Río Uruguay, 2019. Medio Ambiente: informe de vigilancia  
561 de playas. CARU, Paysandú, Entre Ríos <https://www.caru.org.uy/web/medio-ambiente/>.

562 Carver, R. E., Nelson, N. O., Roozeboom, K. L., Kluitenberg, G. J., Tomlinson, P. J., Kang, Q., &  
563 Abel, D. S. (2022). Cover crop and phosphorus fertilizer management impacts on surface  
564 water quality from a no-till corn-soybean rotation. *Journal of Environmental Management*,  
565 301, 113818. <https://doi.org/10.1016/j.jenvman.2021.113818>

566 Chakraborty, S., Tiwari, P. K., Sasmal, S. K., Misra, A. K., & Chattopadhyay, J. (2017). Effects of  
567 fertilizers used in agricultural fields on algal blooms. *The European Physical Journal Special*  
568 *Topics*, 226(9), 2119–2133. <https://doi.org/10.1140/epjst/e2017-70031-7>

569 Chorus, I., & Welker, M. (Eds.). (2021). *Toxic cyanobacteria in water: A guide to their public*  
570 *health consequences, monitoring and management (Second edition)*. CRC Press, an imprint of  
571 Informa.

572 de Vasconcelos, A. C. F., Schlindwein, S. L., Lana, M. A., Fantini, A. C., Bonatti, M., D'Agostini,  
573 L. R., & Martins, S. R. (2014). Land use dynamics in Brazilian La Plata Basin and  
574 anthropogenic climate change. *Climatic Change*, 127(1), 73–81.  
575 <https://doi.org/10.1007/s10584-014-1081-8>

576 Debastiani Júnior, J. R., Naliato, D. A. de O., Perbiche-Neves, G., & Nogueira, M. G. (2016).  
577 Fluvial lateral environments in Río de La Plata basin: Effects of hydropower damming and  
578 eutrophication. *Acta Limnologica Brasiliensia*, 28(0). [https://doi.org/10.1590/s2179-](https://doi.org/10.1590/s2179-975x5516)  
579 [975x5516](https://doi.org/10.1590/s2179-975x5516)

580 Depetris, P.J., Pasquini, A.I., 2007a. The geochemistry of the Paraná river: an overview, In: Parma  
581 M.J. (eds), *Limnology of a subtropical wetland*. Springer-Verlag Berlin Heidelberg. Pp, 144-  
582 174.

583 Depetris, P.J., Pasquini, A.I., 2007b. Discharge trends and flow dynamics of southern  
584 southamerican rivers draining the southern Atlantic seabord: an overview. *J. Hidrol.* 333, 385-  
585 399. doi: 10.1016/j.hydrol.2006.09005.

586 Di Persia, D. H. & Neiff, J. J. (1986): The Uruguay River system. – In: Davies, B. R. & Walker K.  
587 F. (eds.): *The ecology of River Systems*, pp. 599–621. Dr. W. Junk Publ., Dordrecht,  
588 Netherlands.

589 Díaz, A., Maciel, F., & Saurral, R. (2013). Multi-annual variability of streamflow in La Plata Basin.  
590 Part II: Simulations for the twenty-first century. *International Journal of River Basin*  
591 *Management*, 11(4), 361–371. <https://doi.org/10.1080/15715124.2014.880708>

592 Díaz, N., Barreiro, M., & Rubido, N. (2020). Intraseasonal Predictions for the South American  
593 Rainfall Dipole. *Geophysical Research Letters*, 47(21).  
594 <https://doi.org/10.1029/2020GL089985>

595 Doubek, J. P., Carey, C. C., & Cardinale, B. J. (2015). Anthropogenic land use is associated with N-  
596 fixing cyanobacterial dominance in lakes across the continental United States. *Aquatic*  
597 *Sciences*, 77(4), 681–694. <https://doi.org/10.1007/s00027-015-0411-x>

598 Doughty, C. E. (2010). The development of agriculture in the Americas: An ecological perspective.  
599 *Ecosphere*, 1(6), art21. <https://doi.org/10.1890/ES10-00098.1>

600 Downing, J. A., Watson, S. B., & McCauley, E. (2001). Predicting Cyanobacteria dominance in  
601 lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(10), 1905–1908.  
602 <https://doi.org/10.1139/f01-143>

603 Fang, C., Song, K., Paerl, H. W., Jacinthe, P., Wen, Z., Liu, G., Tao, H., Xu, X., Kutser, T., Wang,  
604 Z., Duan, H., Shi, K., Shang, Y., Lyu, L., Li, S., Yang, Q., Lyu, D., Mao, D., Zhang, B., ...  
605 Lyu, Y. (2022). Global divergent trends of algal blooms detected by satellite during 1982–  
606 2018. *Global Change Biology*, 28(7), 2327–2340. <https://doi.org/10.1111/gcb.16077>

607 Ferrari, G., del Carmen Pérez, M., Dabezies, M., Míguez, D., & Saizar, C. (2011). Planktic  
608 Cyanobacteria in the Lower Uruguay River, South America. *Fottea*, 11(1), 225–234.  
609 <https://doi.org/10.5507/fot.2011.021>

610 Giannuzzi, L., Sedan, D., Echenique, R., & Andrinolo, D. (2011). An Acute Case of Intoxication  
611 with Cyanobacteria and Cyanotoxins in Recreational Water in Salto Grande Dam, Argentina.  
612 *Marine Drugs*, 9(11), 2164–2175. <https://doi.org/10.3390/md9112164>

613 Giller, K. E., Delaune, T., Silva, J. V., Descheemaeker, K., van de Ven, G., Schut, A. G. T., van  
614 Wijk, M., Hammond, J., Hochman, Z., Taulya, G., Chikowo, R., Narayanan, S., Kishore, A.,  
615 Bresciani, F., Teixeira, H. M., Andersson, J. A., & van Ittersum, M. K. (2021). The future of  
616 farming: Who will produce our food? *Food Security*, 13(5), 1073–1099.  
617 <https://doi.org/10.1007/s12571-021-01184-6>

618 Goyenola, G., Graeber, D., Meerhoff, M., Jeppesen, E., Teixeira-de Mello, F., Vidal, N., Fosalba,  
619 C., Ovesen, N. B., Gelbrecht, J., Mazzeo, N., & Kronvang, B. (2020). Influence of Farming  
620 Intensity and Climate on Lowland Stream Nitrogen. *Water*, 12(4), 1021.  
621 <https://doi.org/10.3390/w12041021>

622 Graesser, J., Ramankutty, N., & Coomes, O. T. (2018). Increasing expansion of large-scale crop  
623 production onto deforested land in sub-Andean South America. *Environmental Research*  
624 *Letters*, 13(8), 084021. <https://doi.org/10.1088/1748-9326/aad5bf>

625 Guerrero, R. A., Acha, E. M., Framin~an, M. B., & Lasta, C. A. (1997). Physical oceanography of  
626 the Río de la Plata Estuary, Argentina. *Continental Shelf Research*, 17(7), 727–742.  
627 [https://doi.org/10.1016/S0278-4343\(96\)00061-1](https://doi.org/10.1016/S0278-4343(96)00061-1)

628 Hallegraeff, G. M., Anderson, D. M., Belin, C., Bottein, M.-Y. D., Bresnan, E., Chinain, M.,  
629 Enevoldsen, H., Iwataki, M., Karlson, B., McKenzie, C. H., Sunesen, I., Pitcher, G. C.,  
630 Provoost, P., Richardson, A., Schweibold, L., Tester, P. A., Trainer, V. L., Yñiguez, A. T., &  
631 Zingone, A. (2021). Perceived global increase in algal blooms is attributable to intensified  
632 monitoring and emerging bloom impacts. *Communications Earth & Environment*, 2(1), 117.  
633 <https://doi.org/10.1038/s43247-021-00178-8>

- 634 Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M.  
635 (2018). Cyanobacterial blooms. *Nature Reviews Microbiology*, 16(8), 471–483.  
636 <https://doi.org/10.1038/s41579-018-0040-1>
- 637 Kim, H., Jo, B. Y., & Kim, H. S. (2017). Effect of different concentrations and ratios of ammonium,  
638 nitrate, and phosphate on growth of the blue-green alga (cyanobacterium) *Microcystis*  
639 *aeruginosa* isolated from the Nakdong River, Korea. *ALGAE*, 32(4), 275–284.  
640 <https://doi.org/10.4490/algae.2017.32.10.23>
- 641 Kosten, S., Huszar, V. L. M., Bécares, E., Costa, L. S., Donk, E., Hansson, L.-A., Jeppesen, E.,  
642 Kruk, C., Lacerot, G., Mazzeo, N., Meester, L., Moss, B., Lürling, M., Nöges, T., Romo, S.,  
643 & Scheffer, M. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes.  
644 *Global Change Biology*, 18(1), 118–126. <https://doi.org/10.1111/j.1365-2486.2011.02488.x>
- 645 Kruk, C., Martínez, A., Martínez de la Escalera, G., Trinchin, R., Manta, G., Segura, A. M., Piccini,  
646 C., Brena, B., Yannicelli, B., Fabiano, G., & Calliari, D. (2021). Rapid freshwater discharge  
647 on the coastal ocean as a mean of long distance spreading of an unprecedented toxic  
648 cyanobacteria bloom. *Science of The Total Environment*, 754, 142362.  
649 <https://doi.org/10.1016/j.scitotenv.2020.142362>
- 650 Kruk, C., Piccini, C., Devercelli, M., Nogueira, L., Accattatis, V., Sampognaro, L., & Segura, A. M.  
651 (2021). A trait ~~based approach and prediction of microbial~~  
652 invasive species. *Oikos*, 130(4), 571–586. <https://doi.org/10.1111/oik.07694>
- 653 Kruk, C., Piccini, C., Segura, A. M., & Martínez de la Escalera, G. (2015). Herramientas para el  
654 monitoreo y sistema de alerta de floraciones de cianobacterias nocivas: Río Uruguay y Río de  
655 la Plata. *INNOTEC*, 10. <https://doi.org/10.26461/10.02>
- 656 Kruk, C., Segura, A. M., Nogueira, L., Alcántara, I., Calliari, D., Martínez de la Escalera, G.,  
657 Carballo, C., Cabrera, C., Sarthou, F., Scavone, P., & Piccini, C. (2017). A multilevel trait-  
658 based approach to the ecological performance of *Microcystis aeruginosa* complex from  
659 headwaters to the ocean. *Harmful Algae*, 70, 23–36. <https://doi.org/10.1016/j.hal.2017.10.004>

660 MacDonald, G. K., Bennett, E. M., Potter, P. A., & Ramankutty, N. (2011). Agronomic phosphorus  
661 imbalances across the world's croplands. *Proceedings of the National Academy of Sciences*,  
662 108(7), 3086–3091. <https://doi.org/10.1073/pnas.1010808108>

663 Martínez de la Escalera, G., Kruk, C., Segura, A. M., Nogueira, L., Alcántara, I., & Piccini, C.  
664 (2017). Dynamics of toxic genotypes of *Microcystis aeruginosa* complex (MAC) through a  
665 wide freshwater to marine environmental gradient. *Harmful Algae*, 62, 73–83.  
666 <https://doi.org/10.1016/j.hal.2016.11.012>

667 Martínez de la Escalera, G., Segura, A. M., Kruk, C., Ghattas, B., Cohan, F. M., Iriarte, A., &  
668 Piccini, C. (2022). Genotyping and Multivariate Regression Trees Reveal Ecological  
669 Diversification within the *Microcystis aeruginosa* Complex along a Wide Environmental  
670 Gradient. *Applied and Environmental Microbiology*, 88(3), e01475-21.  
671 <https://doi.org/10.1128/aem.01475-21>

672 Massey, I. Y., Al osman, M., & Yang, F. (2020). An overview on cyanobacterial blooms and toxins  
673 production: Their occurrence and influencing factors. *Toxin Reviews*, 1–21.  
674 <https://doi.org/10.1080/15569543.2020.1843060>

675 Meerhoff, M., Audet, J., Davidson, T. A., De Meester, L., Hilt, S., Kosten, S., Liu, Z., Mazzeo, N.,  
676 Paerl, H., Scheffer, M., & Jeppesen, E. (2022). Feedback between climate change and  
677 eutrophication: Revisiting the allied attack concept and how to strike back. *Inland Waters*,  
678 12(2), 187–204. <https://doi.org/10.1080/20442041.2022.2029317>

679 Milliman, J. D., Farnsworth, K. L., Jones, P. D., Xu, K. H., & Smith, L. C. (2008). Climatic and  
680 anthropogenic factors affecting river discharge to the global ocean, 1951–2000. *Global and  
681 Planetary Change*, 62(3–4), 187–194. <https://doi.org/10.1016/j.gloplacha.2008.03.001>

682 Mitrovic, S. M., Hardwick, L., & Dorani, F. (2011). Use of flow management to mitigate  
683 cyanobacterial blooms in the Lower Darling River, Australia. *Journal of Plankton Research*,  
684 33(2), 229–241. <https://doi.org/10.1093/plankt/fbq094>

685 Modernel, P., Rossing, W. A. H., Corbeels, M., Dogliotti, S., Picasso, V., & Tiftonell, P. (2016).  
686 Land use change and ecosystem service provision in Pampas and Campos grasslands of

687 southern South America. *Environmental Research Letters*, 11(11), 113002.  
688 <https://doi.org/10.1088/1748-9326/11/11/113002>

689 Muggeo, V.M.R. (2003). Estimating regression models with unknown break-points. *Statist. Med.*,  
690 22, 3055–3071.

691 Muggeo, V.M.R., 2008. Segmented: An R Package to Fit Regression Models with Broken-Line  
692 Relationships. *R News*, 8/1, 20-25. URL <https://cran.r-project.org/doc/Rnews/>.

693 Mulligan, M., van Soesbergen, A., & Sáenz, L. (2020). GOODD, a global dataset of more than  
694 38,000 georeferenced dams. *Scientific Data*, 7(1), 31. [https://doi.org/10.1038/s41597-020-](https://doi.org/10.1038/s41597-020-0362-5)  
695 0362-5

696 MVOTMA-OAN, 2019. Calidad de agua. Montevideo: MVOTMA. [12 de marzo de 2019].  
697 <https://www.dinama.gub.uy/oan/datos-abiertos/calidad-agua/>.

698 Nagy GJ, Gómez-Erache M, Lopez CH, Perdomo AC (2002). Distribution patterns of nutrients and  
699 symptoms of eutrophication in the Río de la Plata River Estuary System. *Hydrobiologia*  
700 475/476, 125-139.

701 Nagy, G. J., Gómez-Erache, M., López, C. H., & Perdomo, A. C. (2002). Distribution patterns of  
702 nutrients and symptoms of eutrophication in the Rio de la Plata River Estuary System.  
703 *Hydrobiologia*, 475/476, 125–139. <https://doi.org/10.1023/A:1020300906000>

704 O’Farrell, I., & Izaguirre, I. (2014). Phytoplankton of the middle and lower stretches of the Uruguay  
705 River. *Advances in Limnology*, 65, 113–126. [https://doi.org/10.1127/1612-166X/2014/0065-](https://doi.org/10.1127/1612-166X/2014/0065-0037)  
706 0037

707 Ockenden, M. C., Hollaway, M. J., Beven, K. J., Collins, A. L., Evans, R., Falloon, P. D., Forber,  
708 K. J., Hiscock, K. M., Kahana, R., Macleod, C. J. A., Tych, W., Villamizar, M. L., Wearing,  
709 C., Withers, P. J. A., Zhou, J. G., Barker, P. A., Burke, S., Freer, J. E., Johnes, P. J., ...  
710 Haygarth, P. M. (2017). Major agricultural changes required to mitigate phosphorus losses  
711 under climate change. *Nature Communications*, 8(1), 161. [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-017-00232-0)  
712 017-00232-0

713 OSE (2009). Informe del comportamiento de las cianobacterias en el agua bruta de Fray Bentos.  
714 Montevideo: Obras Sanitarias del Estado, 26 pp. Informe del comportamiento de las  
715 cianobacterias en el agua bruta de Fray Bentos. PLANILLA CIANOBACTERIAS CON  
716 RESULTADO POSITIVO 1963-2008.

717 Paerl, H. W., & Huisman, J. (2008). Blooms Like It Hot. *Science*, 320(5872), 57–58.  
718 <https://doi.org/10.1126/science.1155398>

719 Paerl, H.W. and J. Huisman (2009). Climate Change: A Catalyst for Global Expansion of Harmful  
720 Cyanobacterial Blooms. *Environmental Microbiology Reports* 1(1):27-37.

721 Paerl, H. W., J.T. Scott, M.J. McCarthy, S.E. Newell, W.S. Gardner, K.E. Havens, D.K. Hoffman,  
722 S.W. Wilhelm and W.A. Wurtsbaugh. (2016). It takes two to tango: When and where dual  
723 nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems.  
724 *Environmental Science & Technology* 50: 10805–10813.

725 Paerl, H. W., Otten, T. G., & Kudela, R. (2018). Mitigating the Expansion of Harmful Algal  
726 Blooms Across the Freshwater-to-Marine Continuum. *Environmental Science & Technology*,  
727 52(10), 5519–5529. <https://doi.org/10.1021/acs.est.7b05950>

728 Petraitis, P.S., Dunham, A.E. & Niewiarowski, P.H. (1996). Inferring Multiple Causality: The  
729 Limitations of Path Analysis. *Functional Ecology*, 10, 421.

730 Pérez, L., Barreiro, M., Etchevers, I., Crisci, C., & García-Rodríguez, F. (2021a). Centennial  
731 hydroclimatic and anthropogenic processes of South East South America modulate  
732 interannual and decadal river discharge. *Science of The Total Environment*, 781, 146733.  
733 <https://doi.org/10.1016/j.scitotenv.2021.146733>

734 Pérez, L., Crisci, C., Lüning, S., de Mahiques, M. M., & García-Rodríguez, F. (2021b). Last  
735 millennium intensification of decadal and interannual river discharge cycles into the  
736 Southwestern Atlantic Ocean increases shelf productivity. *Global and Planetary Change*, 196,  
737 103367. <https://doi.org/10.1016/j.gloplacha.2020.103367>

738 Preece, E. P., Hardy, F. J., Moore, B. C., & Bryan, M. (2017). A review of microcystin detections in  
739 Estuarine and Marine waters: Environmental implications and human health risk. *Harmful*  
740 *Algae*, 61, 31–45. <https://doi.org/10.1016/j.hal.2016.11.006>

741 Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L., & Melo, S. (2002). Towards a functional  
742 classification of the freshwater phytoplankton. *Journal of Plankton Research*, 24(5), 417–428.  
743 <https://doi.org/10.1093/plankt/24.5.417>

744 Rosa, M. R., Brancalion, P. H. S., Crouzeilles, R., Tambosi, L. R., Piffer, P. R., Lenti, F. E. B.,  
745 Hirota, M., Santiami, E., & Metzger, J. P. (2021). Hidden destruction of older forests  
746 threatens Brazil’s Atlantic Forest and challenges restoration programs. *Science Advances*,  
747 7(4), eabc4547. <https://doi.org/10.1126/sciadv.abc4547>

748 Saguier, M., Gerlak, A. K., Villar, P. C., Baigún, C., Venturini, V., Lara, A., & dos Santos, M. A.  
749 (2021). Interdisciplinary research networks and science-policy-society interactions in the  
750 Uruguay River Basin. *Environmental Development*, 38, 100601.  
751 <https://doi.org/10.1016/j.envdev.2020.100601>

752 Salk, K. R., Venkiteswaran, J. J., Couture, R., Higgins, S. N., Paterson, M. J., & Schiff, S. L.  
753 (2022). Warming combined with experimental eutrophication intensifies lake phytoplankton  
754 blooms. *Limnology and Oceanography*, 67(1), 147–158. <https://doi.org/10.1002/lno.11982>

755 Saurral, R. I., Barros, V. R., & Lettenmaier, D. P. (2008). Land use impact on the Uruguay River  
756 discharge: LAND USE AND THE URUGUAY RIVER DISCHARGE. *Geophysical Research*  
757 *Letters*, 35(12), n/a-n/a. <https://doi.org/10.1029/2008GL033707>

758 Scheffer, M., Hosper, S. H., Meijer, M.-L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria  
759 in shallow lakes. *Trends in Ecology & Evolution*, 8(8), 275–279.  
760 [https://doi.org/10.1016/0169-5347\(93\)90254-M](https://doi.org/10.1016/0169-5347(93)90254-M)

761 Schindler, D. W. (1977). Evolution of Phosphorus Limitation in Lakes. *Science*, 195(4275), 260–  
762 262. <http://www.jstor.org/stable/1743244>

763 Schlindwein, S. L., Feitosa de Vasconcelos, A. C., Bonatti, M., Sieber, S., Strapasson, A., & Lana,  
764 M. (2021). Agricultural land use dynamics in the Brazilian part of La Plata Basin: From

765 driving forces to societal responses. *Land Use Policy*, 107, 105519.  
766 <https://doi.org/10.1016/j.landusepol.2021.105519>

767 Segura, A. M., Piccini, C., Nogueira, L., Alcántara, I., Calliari, D., & Kruk, C. (2017). Increased  
768 sampled volume improves *Microcystis aeruginosa* complex (MAC) colonies detection and  
769 prediction using Random Forests. *Ecological Indicators*, 79, 347–354.  
770 <https://doi.org/10.1016/j.ecolind.2017.04.047>

771 Segura, A. M., Sarthou, F., & Kruk, C. (2018). Morphology-based differences in the thermal  
772 response of freshwater phytoplankton. *Biology Letters*, 14(5), 20170790.  
773 <https://doi.org/10.1098/rsbl.2017.0790>

774 Silvarrey Barruffa, A., Faggian, R., Sposito, V., & Duarte Guigou, M. (2021). Using models to  
775 inform water policy in a changing climate: Comparing the Australian and Uruguayan  
776 experiences. *Marine and Freshwater Research*, 72(2), 275. <https://doi.org/10.1071/MF19266>

777 Souza, C. M., Z. Shimbo, J., Rosa, M. R., Parente, L. L., A. Alencar, A., Rudorff, B. F. T.,  
778 Hasenack, H., Matsumoto, M., G. Ferreira, L., Souza-Filho, P. W. M., de Oliveira, S. W.,  
779 Rocha, W. F., Fonseca, A. V., Marques, C. B., Diniz, C. G., Costa, D., Monteiro, D., Rosa, E.  
780 R., Vélez-Martin, E., ... Azevedo, T. (2020). Reconstructing Three Decades of Land Use and  
781 Land Cover Changes in Brazilian Biomes with Landsat Archive and Earth Engine. *Remote  
782 Sensing*, 12(17), 2735. <https://doi.org/10.3390/rs12172735>

783 Sukenik, A., & Kaplan, A. (2021). Cyanobacterial Harmful Algal Blooms in Aquatic Ecosystems:  
784 A Comprehensive Outlook on Current and Emerging Mitigation and Control Approaches.  
785 *Microorganisms*, 9(7), 1472. <https://doi.org/10.3390/microorganisms9071472>

786 Svirčev, Z., Lalić, D., Bojadžija Savić, G., Tokodi, N., Drobac Backović, D., Chen, L., Meriluoto,  
787 J., & Codd, G. A. (2019). Global geographical and historical overview of cyanotoxin  
788 distribution and cyanobacterial poisonings. *Archives of Toxicology*, 93(9), 2429–2481.  
789 <https://doi.org/10.1007/s00204-019-02524-4>

- 790 Trimbee, A. M., & Prepas, E. E. (1987). Evaluation of Total Phosphorus as a Predictor of the  
791 Relative Biomass of Blue-green Algae with Emphasis on Alberta Lakes. *Canadian Journal of*  
792 *Fisheries and Aquatic Sciences*, 44(7), 1337–1342. <https://doi.org/10.1139/f87-158>
- 793 Tucci, C. E. M. (2001). Some scientific challenges in the development of South America's water  
794 resources. *Hydrological Sciences Journal*, 46(6), 937–946.  
795 <https://doi.org/10.1080/02626660109492887>
- 796 Tucci, C. E. M., & Clarke, R. T. (1998). Environmental Issues in the la Plata Basin. *International*  
797 *Journal of Water Resources Development*, 14(2), 157–173.  
798 <https://doi.org/10.1080/07900629849376>
- 799 Ungerovich, M., Barreiro, M., & Masoller, C. (2021). Influence of Madden–Julian Oscillation on  
800 extreme rainfall events in Spring in southern Uruguay. *International Journal of Climatology*,  
801 41(5), 3339–3351. <https://doi.org/10.1002/joc.7022>
- 802 Utermöhl H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitteilungen.*  
803 *Internationale Vereinigung fuer Theoretische und Angewandte Limnologie*, 9, 1–38.
- 804 Vera, C., Silvestri, G., Liebmann, B., & González, P. (2006). Climate change scenarios for seasonal  
805 precipitation in South America from IPCC-AR4 models. *Geophysical Research Letters*,  
806 33(13), L13707. <https://doi.org/10.1029/2006GL025759>
- 807 Vidal, F., Sedan, D., D'Agostino, D., Cavalieri, M., Mullen, E., Parot Varela, M., Flores, C.,  
808 Caixach, J., & Andrinolo, D. (2017). Recreational Exposure during Algal Bloom in Carrasco  
809 Beach, Uruguay: A Liver Failure Case Report. *Toxins*, 9(9), 267.  
810 <https://doi.org/10.3390/toxins9090267>
- 811 Viglizzo, E. F., & Frank, F. C. (2006). Land-use options for Del Plata Basin in South America:  
812 Tradeoffs analysis based on ecosystem service provision. *Ecological Economics*, 57(1), 140–  
813 151. <https://doi.org/10.1016/j.ecolecon.2005.03.025>
- 814 Viglizzo, E. F., Lértora, F., Pordomingo, A. J., Bernardos, J. N., Roberto, Z. E., & Del Valle, H.  
815 (2001). Ecological lessons and applications from one century of low external-input farming in

816 the pampas of Argentina. *Agriculture, Ecosystems & Environment*, 83(1–2), 65–81.  
817 [https://doi.org/10.1016/S0167-8809\(00\)00155-9](https://doi.org/10.1016/S0167-8809(00)00155-9)

818 Vollenweider, R. & J. Kerekes, 1982. *Eutrophication of waters, monitoring, assessment and control*.  
819 OECD, Paris.

820 Wilkinson, J. L., Boxall, A. B. A., Kolpin, D. W., Leung, K. M. Y., Lai, R. W. S., Galbán-Malagón,  
821 C., Adell, A. D., Mondon, J., Metian, M., Marchant, R. A., Bouzas-Monroy, A., Cuni-  
822 Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M.,  
823 Luarte, T., ... Teta, C. (2022). Pharmaceutical pollution of the world's rivers. *Proceedings of*  
824 *the National Academy of Sciences*, 119(8), e2113947119.  
825 <https://doi.org/10.1073/pnas.2113947119>

826 Withers, P., Neal, C., Jarvie, H., & Doody, D. (2014). Agriculture and Eutrophication: Where Do  
827 We Go from Here? *Sustainability*, 6(9), 5853–5875. <https://doi.org/10.3390/su6095853>

828 Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful  
829 algal blooms along the freshwater to marine continuum. *WIREs Water*, 6(5).  
830 <https://doi.org/10.1002/wat2.1373>

831 Yang, J. R., Lv, H., Isabwe, A., Liu, L., Yu, X., Chen, H., & Yang, J. (2017). Disturbance-induced  
832 phytoplankton regime shifts and recovery of cyanobacteria dominance in two subtropical  
833 reservoirs. *Water Research*, 120, 52–63. <https://doi.org/10.1016/j.watres.2017.04.062>

834 Zabel, F., Delzeit, R., Schneider, J. M., Seppelt, R., Mauser, W., & Václavík, T. (2019). Global  
835 impacts of future cropland expansion and intensification on agricultural markets and  
836 biodiversity. *Nature Communications*, 10(1), 2844. [https://doi.org/10.1038/s41467-019-](https://doi.org/10.1038/s41467-019-10775-z)  
837 [10775-z](https://doi.org/10.1038/s41467-019-10775-z)

838 Zhang, F., Lee, J., Liang, S., & Shum, C. (2015). Cyanobacteria blooms and non-alcoholic liver  
839 disease: Evidence from a county level ecological study in the United States. *Environmental*  
840 *Health*, 14(1), 41. <https://doi.org/10.1186/s12940-015-0026-7>

841 **Table 1.** Uruguay river (UR) basin and sub-basins areas, countries, MapBiomass (MB) collections  
842 and temporal period of the information used (MapBiomass Pampa: Baeza et al. 2022, Atlantic Forest  
843 Tri-national initiatives: Souza et al., 2020). Refer to Figure 1 for visual location. Land uses and land  
844 cover were grouped into six classes: natural forest, forest plantation, grasslands and wetlands,  
845 farming (annual crops and sown pastures), non-vegetated areas (that include bare soil and urban  
846 infrastructure) and water bodies and rivers.

<b>Basin</b>	<b>Area (km<sup>2</sup>)</b>	<b>Country</b>	<b>Information origin</b>	<b>Temporal period</b>	<b>Type of information</b>
Total UR basin	329.003	Brazil, Argentina and Uruguay	MB Brazil Collection 5, MB Atlantic Forest Collection 1 and MB Pampa Collection 1	2000-2019	Six classes
Upper and middle	167.130	Brazil	MB Brazil Collection 5 and 6	1985-2019	Six classes and composition of “annual crops and pasture”
Lower	161.875	Argentina and Uruguay	MB Atlantic Forest Collection 1 and MB Pampa Collection 1	2000-2019	Six classes

847

848 **Table 2.** Correlation between annual values of land use area from 2000 to 2019 between the lower  
 849 area (LOW) and the combination of upper and middle areas (UM) of the Uruguay river basin. Land  
 850 uses included were: crops and sown pastures (CP), natural forests (Nat), grasslands and wetlands  
 851 (Gra), and forest plantation (For). The symbol indicates the significance level of Spearman  
 852 correlation (\* p <0.05, \*\* p <0.01).

	<b>CP Low</b>	<b>CP UM</b>	<b>Gras Low</b>	<b>Gras UM</b>	<b>For Low</b>	<b>For UM</b>	<b>Nat Low</b>
<b>CP Low</b>							
<b>CP UM</b>	0.73**						
<b>Gra Low</b>	-0.84**	-0.91**					
<b>Gra UM</b>	-0.72**	-0.98**	0.96**				
<b>For Low</b>	0.56*	0.86**	-0.91**	-0.94**			
<b>For UM</b>	0.67**	0.94**	-0.94**	-0.98**	0.96**		
<b>Nat Low</b>	-0.45*	-0.58**	0.53*	0.59**	-0.61**	-0.59**	
<b>Nat UM</b>	-0.54*	-0.77**	0.49*	0.68**	-0.40	-0.67**	0.52*

853

854 **Table 3.** List of the causal path models constructed to explain cyanobacterial abundance (logCya)  
855 sorted according to Akaike information criteria (AIC). Significant paths and their coefficients  
856 ( $p < 0.05$ ) are shown for each model. We recall that temperature was significant only when land uses  
857 were not included as a possible path in the model. ns: path included but with non-significant  
858 coefficients, “-”: path not included in the model. CP: crops and pastures area, Tmax: maximum  
859 annual temperature, PP: precipitation, Qacum: accumulated water flow, Nat: natural class.

Model	$\Delta AIC$	paths					
		CP→ logCya	Tmax→ logCya	PP→ Qacum	Qacum→ logCya	PP→ logCya	Nat→ logCya
8: all except Nat	0	0.61	ns	0.89	ns	ns	-
1: all variables	2	0.55	ns	0.89	ns	ns	ns
5: CP and Tmax	41	0.61	ns	-	-	-	-
6: only CP	41	0.70	-	-	-	-	-
4: CP and Nat	43	0.72	-	-	-	-	ns
2: all except effect of PP in Qacum	47	0.55	ns	-	ns	ns	ns
7: only Tmax	52	-	0.46	-	-	-	-
3: climatic	55	-	0.46	ns	ns	ns	-

860 **Table 4.** Coefficients estimated in the path analysis of the selected model to explain cyanobacterial  
861 abundance with climatic, land use and hydrologic variables. Their standard error (se), the z statistics  
862 (z) and the associated p values are included. The significant variable was crops and pastures area  
863 (CP in bold) and the relationship between precipitation (PP) and accumulated flow (Qacum). The  
864 remaining variables showed non-significant relationships ( $p>0.05$ ). Tmax: maximum annual  
865 temperature, PP: precipitation, Qacum: accumulated water flow, Nat: natural forests class.

<b>Variable</b>	<b>Coefficient</b>	<b>se</b>	<b>z</b>	<b>p</b>
PP	-0.14	0.30	-0.47	0.64
Tmax	0.23	0.16	1.39	0.17
Qacum	0.13	0.30	0.42	0.67
<b>CP</b>	<b>0.55</b>	0.22	2.32	0.02
Nat	-0.06	0.22	-0.27	0.79
<b>PP~Qacum</b>	<b>0.89</b>	0.03	9.55	<0.001

866