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- 1 Physiological and behavioral responses of phytoplankton communities to nutrient availability
- 2 in a disturbed Mediterranean coastal lagoon

3 A. Leruste<sup>a, b\*</sup>, V. Pasqualini<sup>a</sup>, M. Garrido<sup>a, c</sup>, N. Malet<sup>d</sup>, R. De Wit<sup>b</sup>, B. Bec<sup>b</sup>

<sup>a</sup> UMR SPE CNRS/UMS Stella Mare CNRS, Université de Corse, 20250 Corte, France

<sup>b</sup> MARine Biodiversity Exploitation and Conservation MARBEC, Université de Montpellier,

6 IRD, Ifremer, CNRS. Bât. 24, Place Eugène Bataillon, 34095 Montpellier cedex 5, France,

<sup>c</sup> Environmental agency of Corsica, 7 Avenue Jean Nicoli, 20250 Corte, France

<sup>d</sup> Ifremer, Laboratoire Environnement Ressources Provence-Azur-Corse (LER/PAC), Station

9 de Bastia, Z.I. Furiani, Immeuble Agostini, 20600 Bastia, France

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<sup>\*</sup>Corresponding author: leruste-calpena\_a@univ-corse.fr

12 Running page: Phytoplankton functional response to nutrient availability

13 Keywords: growth rate, dilution experiment, eutrophication, functional traits

14 Abstract

15 Short-term bioassays were conducted in Biguglia lagoon (Corsica) to study the physiological and behavioral responses of phytoplankton to N- and P-availability. Natural communities 16 17 were collected in two stations representative of the two sub-basins, at three periods with 18 contrasting environmental characteristics to address the impact of seasonal variability. These 19 samples were separately enriched with a full N and P enrichment, and with enrichments 20 minus N or minus P. Phytoplankton size structuration, diversity, and growth of the total 21 phytoplankton, the micro-, nano- and ultraphytoplankton were evaluated using 22 spectrofluorimetry, and optical microscopy. Results showed that the communities were fueled 23 by  $NO_3^-$  in the wet periods (autumn and spring) and  $NH_4^+$  in summer. The phytoplankton 24 communities displayed highest cell size in autumn, with high abundances of nanoflagellates, 25 and smallest cell size in summer with a large dominance of phycocyanin-rich 26 picocyanobacteria. Blooms of dinoflagellates also occurred during the wet periods, coinciding 27 with high N:P ratios. The full enrichment has not stimulated phytoplankton growth in autumn, 28 suggesting the importance of other controlling factors such as light, a possible  $NH_4^+$  inhibition 29 or the use of mixotrophic abilities. In spring, communities have displayed single P-limitation 30 in the northern basin and different N and P co-limitations in the southern basin. In summer, 31 the full enrichment consistently stimulated the growth of all cell sizes. The communities 32 showed high N and P co-limitations, which is consistent with growing observations in aquatic 33 ecosystems, and reflects the different functional responses of phytoplankton communities to 34 the nutrient availability.



Graphical abstract. Chlorophyll a (Chl a) concentration, DIN:DIP ratio, main
taxa/morphotypes, response to the full nutrient enrichment, and N- and/or P-limitation during
the three bioassay periods in the two stations representatives of the Northern and the Southern
basins of Biguglia lagoon.

41

# 42 Highlights

- Freshwater inputs from the watershed strongly influenced the phytoplankton of
  Biguglia lagoon
- Nanoflagellates dominated phytoplankton communities in autumn and spring with
  high N:P ratios
- Phycocyanin-rich picocyanobacteria bloomed in the sampling in September with low
  N:P ratios
- Phytoplankton showed differential responses to nutrient enrichments and induced
   limitations

Phytoplankton was generally co-limited by N and P, with exceptions depending on seasons

54 1. Introduction

55 Coastal lagoons are very productive ecosystems at the sea-land interface hosting a high 56 degree of biodiversity and providing numerous ecosystem services for human wellbeing 57 (Barbier et al., 2011). These services confer a high economical value for the coastal lagoons, 58 e.g. as water and food supply (fishing, shellfish farming), tourism and recreation (Franco et 59 al., 2010; Rochette et al., 2010). However, because of their location in densely populated 60 littoral areas and the services they provide, coastal lagoons and their surroundings have been 61 increasingly exploited for human uses. Overexploitation combined with the hydromorphology 62 of these semi-enclosed systems and climate change have increased their vulnerability to 63 anthropogenic impacts. Hence, coastal lagoons are among the most threatened ecosystems 64 (Nixon, 2009). This threat is mainly associated with changes in their hydrology and with an 65 increase of nutrients and pollutants inputs from sewage effluents and watershed run-off 66 (Mouillot et al., 2000; Cloern, 2001). Due to shallowness and confinement, these inputs result 67 in high concentration of nutrients and pollutants in coastal lagoons, thereby exacerbating their 68 degradation (Donald et al., 2013).

69

70 An excessive or unbalanced input of nutrients disturbs the community of primary producers, 71 leading to an increase of the eutrophication processes and to drastic changes in the 72 biodiversity of the autotrophic compartment. Thus, it may promote fast-growing opportunistic 73 algae in the first place and finally phytoplankton at the expense of benthic organisms such as 74 macrophytes, and may result in an increase of the frequency of harmful algal blooms 75 (Schramm, 1999; Livingston, 2000; Le Fur et al., 2018). These changes in the composition of 76 the autotrophic communities impact all the trophic levels, altering the ecosystem functioning 77 (Cloern, 2001). High nutrient inputs can also, through an accumulation of macroalgal or 78 phytoplankton biomass, lead to the development of dystrophic crisis and hypoxia and anoxia events, having dramatic impacts on all the living organisms (Cloern, 2001). Therefore, the management of a eutrophicated coastal lagoon needs to target the reduction of nutrient loadings. In addition, knowledge is required about the availability of nutrients within lagoons and the autotrophic communities responses (Duarte et al., 2000; Glibert et al., 2015). This allows to assess the resilience of the aquatic communities, and to estimate the critical nutrient loading for the ecosystem (Pasqualini et al., 2017; Le Fur et al., 2018).

85

86 Phytoplankton represents the basis of food webs and biochemical cycles, and is generally the 87 first autotrophic compartment responding to a change of nutrient availability (Leruste et al., 88 2016). Understanding how the ecosystem functioning influences the composition of 89 phytoplankton communities and their growth mechanisms is indispensable (Livingston, 2000; 90 Paerl et al., 2003). Hence, it is particularly important to understand and predict how N and P 91 inputs affect the growth and community assembly of phytoplankton. These responses are 92 indicators of water quality, and are consequently informative for water management (Duarte 93 et al., 2000; Reed et al., 2016). Morphological and physiological traits such as cell size and 94 maximal growth rates shape the phytoplankton functional responses to nutrient availability. 95 Small-size fast-growing algae are competitive at low nutrient concentrations and low light, 96 while larger cells can generally thrive under a pulsed nutrient supply and higher light 97 intensities (Litchman et al., 2007; Bec et al., 2011; Andersen et al., 2015). The community 98 composition also reflects which nutrients are available. Diatoms are particularly competitive 99 for nitrate, while green algae and picocyanobacteria are more competitive for ammonium. The 100 latter often originates from regenerated internal sources. Dinoflagellates can develop even 101 under dissolved inorganic nutrient limitation, because of their relatively low growth rates and 102 potential mixotrophic abilities (Litchman et al., 2007; Glibert et al., 2015).

104 The general aim of this study was to explore the responses of the phytoplankton communities 105 of a disturbed Mediterranean coastal lagoon to the nutrient availability. Therefore, we choose 106 Biguglia lagoon, which is the largest lagoon on the island of Corsica, as the study site. This 107 Mediterranean lagoon has been increasingly impacted since 1980 by a strong increase of the 108 human population density, industrial and agricultural uses in its catchment and its surrounding 109 areas. This lagoon receives particularly high amounts of nitrate from runoffs, tributaries, and 110 from groundwater flows from a coastal aquifer connected with the lagoon (Erostate et al., 111 2018). Biguglia lagoon also displays a high confinement and restricted exchanges with the 112 Tyrrhenian Sea that have induced higher levels of nutrient concentration than in most other 113 French Mediterranean lagoons these last decades (Souchu et al., 2010; Pasqualini et al., 114 2017). Hence, the lagoon has experienced strong changes in its ecological state, *i.e.* increasing 115 eutrophication and pollutant levels linked with changes in the composition of its autotrophic 116 community (Mouillot et al., 2000; Pasqualini et al., 2017). Moreover, a dystrophic crisis 117 associated with a bloom of toxic cyanobacteria in 2007 has raised the awareness of the water 118 quality in the lagoon among the responsible management authorities. Therefore, they have 119 taken remediation measures to reduce the confinement in order to decrease the concentration 120 of nutrients. This included the modification of the lagoon hydrology since 2009 to increase 121 dilution processes (Cecchi et al., 2016; Garrido et al., 2016). However, so far, no efficient 122 direct management measures have been implemented to reduce the external nutrient loadings 123 that remain important and uncontrolled. Moreover, although the measures taken in 2009 may 124 have helped the development of aquatic angiosperms (Pasqualini et al., 2017), these may have 125 also promoted the occurrence of blooms of potentially toxic and or harmfull dinoflagellate 126 species (Cecchi et al., 2016).

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129 The specific objectives of this study were to describe in different seasons with contrasting 130 environmental conditions including nutrient form and origin, (1) the structure of 131 phytoplankton communities in Biguglia lagoon submitted to a strong anthropogenic impact in 132 term of size classes and species diversity, and (2) their growth rate in response to various 133 forms of nutrients inputs. These two observations illustrate phytoplankton functional 134 responses to contrasting nutrient availability, and the latter specially reflects the vulnerability 135 of the whole ecosystem to a short-term nutrient pulse. We experimentally incubated 136 phytoplankton communities under *in situ* light and temperature conditions with enrichments containing N (NO<sub>3</sub>, NH<sub>4</sub>) and P (PO<sub>4</sub>), either in combination or separately to selectively 137 138 induce either N- or P-limitation. Exploring the responses of phytoplankton communities to a 139 change in nutrient availability helped understanding the impact of these changes on the 140 ecosystem functioning.

141

142 2. Material and Methods

143 2.1. *Characterization of the study site* 

Biguglia lagoon (42°36'N; 9°28'E) is a shallow brackish coastal lagoon (average depth 1.2 m), located on the East coast of Corsica, and separated from the Tyrrhenian Sea by a sandy beach barrier (Fig. 1). This choked lagoon *sensu* Kjerfve (1994) covers 14.5 km<sup>2</sup> with a single inlet in the north to the sea that consists of a 1.5 km long, narrow and shallow natural channel. Biguglia lagoon is divided into two sub-basins by a peninsula in its middle. Each of these subbasins displays a specific hydrological functioning resulting in differences in salinity, nutrient concentrations and phytoplankton biomasses (Garrido et al., 2016).

151

152 The microtidal conditions, and the morphology of this inlet and its natural inclination to silt 153 up restrict the exchanges with the sea and thus limit marine water input in the lagoon

154 (Mouillot et al., 2000). This lagoon is highly influenced by freshwater inputs from its watershed (182 km<sup>2</sup>), and its salinity remains particularly low (Pasqualini et al., 2017). For 155 156 example, this ranged from 2.0 to 18.5 between November 2013 and December 2014. 157 Freshwater comes from rainfall, sewage plants, several rivers draining the watershed and 158 flowing in the north-west of the lagoon, the Golu River connected to the lagoon by the 159 Fossone channel in the south-western part, and from pumping stations draining the 160 agricultural plain in the southern part of the watershed (Erostate et al., 2018) (Fig. 1). 161 Therefore, Biguglia lagoon displays a salinity gradient from the north to the south (Garrido et 162 al., 2016).

163



164

Fig. 1. Location of the two stations representatives of the Northern Basin (NB) and theSouthern Basin (SB) of Biguglia lagoon.

168 Biguglia lagoon has been increasingly eutrophicated since the 1980s due to an intensification 169 of human activities and urbanization on its watershed southward of city of Bastia, including 170 four towns hosting approximately 35,000 inhabitants in winter (Lafabrie et al., 2013). During 171 summer touristic period, the population doubles, which results in increasing anthropogenic pressure. Hence, this lagoon displays very high nutrients concentrations  $(NH_4^+, NO_2^-, NO_3^-)$ 172 173 DIN, Si, TP, TN) in the water column, enhanced by the reduced exchanges with the sea 174 (Orsoni et al., 2001). The sediment compartment shows an important silting with high nutrient 175 concentrations and organic matter content compared to other French Mediterranean coastal 176 lagoons (Souchu et al., 2010). An eutrophication gradient is also observed from the south to 177 the north because of substantial anthropogenic inputs in the southern part of the lagoon.

178

The lagoon has a Mediterranean climate with a succession of a dry and warm summer and wet autumn, winter and spring seasons with sudden and plentiful rainfalls causing flash floods (Pasqualini et al., 2017). The salinity and nutrients inputs are strongly influenced by theseasonal variability of the climate (Collos et al., 2003). This generally determines three hydrological seasonal periods characterized by differences in their nutrient origin and availability, in phytoplankton biomass and size class structure, and in photosynthetic performance (Cecchi et al., 2016; Garrido et al., 2016).

186

#### 187 2.2. Sampling procedures

Water samples were collected in two stations representatives of the northern (NB) and the
southern (SB) basins (Fig. 1, 42°38'12"N, 9°27'15"E and 42°35'00"N, 9°29'18"E,
respectively).

191

192 Experiments were carried out in autumn 2013 (26 November to 5 December), spring 2014 (2 193 to 8 April), and summer 2014 (9 to 12 September). In each of the two NB and SB stations, 70 194 1 of pre-filtered water through 1000 µm mesh to remove larger debris without removing 195 zooplankton or larger phytoplankton cells (Collos et al., 2005) were sampled in sub-surface 196 (20 cm depth) wit a pump from a small boat between 7.00 am and 8.00 am, and kept in the 197 dark. Sub-surface salinity, temperature, percentage of dissolved oxygen (DO) and turbidity 198 were measured in situ with a multi parameter Water Quality Probe (YSI® 6600 V2-2). Upon 199 return to the laboratory, water samples were homogenized by gentle shaking, aliquoted and 200 immediately stored at -20°C for the characterization of phytoplankton communities. Measures of  $NH_4^+$ ,  $PO_4^{3-}$ ,  $NO_3^-$ ,  $NO_2^-$ , TN, and TP concentrations ( $\mu$ M) were performed on duplicates 201 202 of 80 ml, filtered on 20 µm meshes for the dissolved inorganic nutrients (Aminot and 203 Chaussepied, 1983).

204

#### 205 2.3. Characterization of phytoplankton biomass

206 Chlorophyll a (Chl a) concentrations ( $\mu g l^{-1}$ ) were used as a proxy for phytoplankton 207 biomass. Chl a measurements were performed on the total and the fractioned water samples. 208 This allowed assessing the contribution of the ultraphytoplankton  $<5 \mu m$ , the 209 nanophytoplankton between 5 and 20  $\mu$ m and the microphytoplankton >20  $\mu$ m to the total 210 biomass. Upon return to the laboratory, triplicates of water samples (maximum 100 ml, 211 depending on phytoplankton abundance) and of samples pre-filtered on 20 µm and on 5 µm 212 meshes were analyzed by spectrofluorimetry (Neveux and Lantoine, 1993). The biomasses of 213 the three size classes were estimated by subtracting the concentrations obtained for the 214 fraction  $<20 \ \mu m$  to the total water (microphytoplankton), and the fraction  $<5 \ \mu m$  to the fraction <20  $\mu$ m (nanophytoplankton). The Chl *a* concentration of the fraction <5  $\mu$ m 215 216 estimated the ultraphytoplankton biomass.

#### 218 2.4. Assessment of phytoplankton community composition

219 Composition and abundance of the ultraphytoplankton  $<5 \mu m$  were studied using flow 220 cytometry. Triplicates of 1 ml of sample fixed with 2 % formaldehyde (filtered on 0.2 µm, 221 final concentration) were submitted to a flash freeze in liquid nitrogen and then stored at -222 80°C until analysis. Cells counts were performed with a FACSCalibur flow cytometer 223 (Becton Dickinson), using beads for size calibration. Populations of coccoid phycoerythrin-224 rich picocyanobacteria (PE-cyanos) were identified by light diffraction (FSC) and their 225 orange fluorescence emissions, while phycocyanin-rich picocyanobacteria (PC-cyanos) were 226 distinguished by their low red fluorescence and their absence of orange fluorescence. Photosynthetic picoeukaryotes  $<3 \mu m$  were identified by their red fluorescence emissions 227 228 (Chl *a*, wavelength >650 nm).

229

230 Optical microscopy was used to describe nano- and microphytoplankton (>5 µm) composition 231 and abundance. Triplicates of 1 l of sample fixed with formaldehyde (5 % final concentration) 232 were stored at obscurity prior to analysis. A modified method of the Utermöhl protocol was 233 used (Leruste et al., 2018). Samples were examined with an optic microscope Olympus 234 AX10. At least 400 cells per sample were counted to obtain a relevant assessment of the 235 assemblage. Taxonomic resolution was realized at species level whenever possible, and 236 identification was verified according to several books (Bellinger and Sigee, 2015; Bérard-237 Therriault et al., 1999; Loir, 2004; Tomas, 1997) and database such as the World Register of 238 Marine Species (http://www.marinespecies.org/, databases available online). Taxonomic 239 diversity was explored using species richness and Shannon index (Leruste et al., 2018).

241 2.5. *Experimental procedure of bioassay experiments* 

242 To measure maximal growth rate, five dilutions of water sample (9, 17, 43, 74 and 100 %) in 243 0.2 µm filtered sample in duplicate were incubated with an enrichment containing vitamins, 244 silica, metals trace, nitrogen and phosphorus (NaH<sub>2</sub>PO<sub>4</sub>, final concentration 0.8  $\mu$ M) (Landry 245 and Hassett, 1982). Nitrogen was supplied as nitrate and/or ammonium (f/2 and h/2 media, 246 respectively, Guillard & Ryther, 1962) depending on season. In April 2014, N was supplied as 247 nitrate (20 µM final concentration), assuming that nitrate inputs from the watershed were the 248 main nitrogen source. In September 2014, N was supplied as ammonium (20 µM final 249 concentration), assuming that this may be the predominant form provided from 250 remineralization processes in the sediment (Collos et al., 2003). In November-December 251 2013, N was supplied as a mix of nitrate and ammonium (10 µM final concentration each), 252 assuming that sufficiently high temperatures allow the regeneration of ammonium from the 253 sediments, associated with flash floods bringing nitrate from the watershed. This nitrogen 254 concentration has been chosen to avoid phytoplankton growth limitation during the 255 incubation, which has been occasionally observed at 10 µM in another Mediterranean lagoon 256 (Bec et al., 2005). To highlight the potential growth limitation of phytoplankton by N and/or 257 P, two other dilution series were enriched either without nitrogen or phosphorus (Andersen et 258 al., 1991). Two bottles of sample without dilution and enrichment were incubated as control, 259 and the all bottles were simultaneously incubated for 24 h in Biguglia lagoon under in situ 260 temperature and light conditions at 30 cm depth.

261

#### 262 2.6. Estimation of maximal growth rate and N-/P-limitation

After 24 h incubation, the temporal changes of the total and size-fractionated Chlorophyll *a* in each bottle were used to calculate their apparent specific growth rate k(x) for each dilution *x*.

The relationship between the apparent growth rates k(x) in the fully-enriched bottles and dilution factor *x* were fitted to the linear equation (1):

267  $k(x) = \mu_{\max} - gx(1)$ 

Where k(x) is the phytoplankton apparent growth rate in the bottles at dilution x,  $\mu_{max}$  is the 268 269 maximal growth rate under nutrient replete conditions, and g is the grazing rate. All rates were expressed on a per day basis (d<sup>-1</sup>). The maximal growth rate  $\mu_{max}$  was obtained from the 270 271 Y axis intercept, and the phytoplankton mortality rates g as the slope of the linear regressions 272 assuming that mortality was due to grazing (Landry and Hassett, 1982). Equations were fitted 273 with the 'lmer' function in the 'lme4' library (version 1.1-10, (Bates et al., 2015)) to evaluate 274 if the linear model best fitted the trend of the relationship between the apparent growth rate 275 and the dilution factor. To check whether the linear equation (1) was acceptable, we also considered a quadratic function by adding the term  $ax^2$ . The quadratic function has a parabolic 276 277 pattern and could reflect the contribution of regenerated resources or a saturation of the 278 grazing rate in the less diluted bottles (Andersen et al., 1991). All combinations of the 279 parameters of the quadratic function were considered, and model selection was based on 280 parsimony using the small-sample corrected Akaike's information criterion (AIC<sub>c</sub>) (Burnham 281 and Anderson, 2004). Therefore, we calculated the difference between the AIC<sub>c</sub> of the linear 282 model and the model having the lowest AIC<sub>c</sub>, *i.e.* the most parsimonious model, to obtain a 283  $\Delta AIC_c$  value. The models with a  $\Delta AIC_c < 2$  were considered as having equivalent levels of 284 support (Burnham and Anderson, 2004), using the 'dredge' function of the MuMIn package 285 (Bartón, 2013). Hence for  $\triangle AIC_c < 2$  we selected the linear model as it allows calculating  $\mu_{max}$ 286 and g according to equation (1) (Landry and Hassett, 1982), while the quadratic relationship 287 did not allow calculating  $\mu_{\text{max}}$  and g.

289 Phytoplankton net growth rate in controls ( $\mu_0$ ) was calculated by adding *g* to the apparent 290 growth rate ( $k_0$ ) *i.e.*  $\mu_0 = g + k_0$ . Hence, it was assumed that grazing rate was not impacted by 291 nutrient enrichments. The  $\mu_0:\mu_{max}$  ratio assessed the impact of inorganic nutrient enrichment 292 on growth, and estimated the nutrient sufficiency for phytoplankton growth (Landry and 293 Hassett, 1982; Landry et al., 1998).

294

295 In the selectively enriched bioassays, we took account that apparent growth rate is not 296 necessarily a linear function of the dilution factor, because the instantaneous growth rate may 297 decline as internal and external nutrient pools become depleted.  $\mu_{-N}$  and  $\mu_{-P}$  were calculated as 298 followed:  $\mu_{-N} = g + k_{-N}$  and  $\mu_{-P} = g + k_{-P}$ . To estimate how much phytoplankton growth was N-299 and/or P-limited, the ratio between growth rate with the enrichments minus N or minus P ( $\mu_{-N}$ 300 or  $\mu_{-P}$ ) and maximal growth rate in theoretically non-limiting nutrient condition ( $\mu_{max}$ ) was 301 calculated. Differences between growth rates with full enrichment, enrichment minus N and 302 enrichment minus P helped distinguish different types of co-limitation (Harpole et al., 2011; 303 Burson et al., 2016).

304

#### 305 2.7. Statistical analyses

306 Data analysis was performed on R (R Core Team, 2013).

Two-way ANOVAs assessed if the total phytoplankton biomass significantly changed among the three samplings and between NB and SB. Posteriori pairwise mean comparisons between stations and samplings were made using Tukey-Kramer's post-hoc analysis (alpha level = 0.05). Permanovas assessed if the size class structure varied among stations and samplings, according to differences of biomass, temperature, salinity, dissolved inorganic nutrients, TN and TP concentrations, turbidity and DO (Anderson, 2001). Taxonomic diversity indices *i.e.* the Shannon index on the relative abundance of taxa and morphotypes (H), and the species richness (S) were estimated using Vegan package (Oksanen et al., 2018). ANOVAs assessed
if S and H significantly changed among stations and samplings. Spearman's rank correlations
between S, H, total Chl *a* biomass and environmental variables (temperature, salinity,
inorganic nutrient concentrations, TN and TP concentrations, turbidity and DO) were assessed
for the two stations and the three samplings.

319

320 Regression analyses determined which mixed models (i.e. combinations of explanatory 321 factors) best fitted the biomass of total phytoplankton and the three size classes. Among all 322 the possible fixed effects (stations, samplings, salinity, temperature, nutrients concentrations, 323 dissolved oxygen, and turbidity), the most relevant were chosen according to a correlation 324 matrix. Values of fixed effects were scaled prior to analysis. Because of pseudo-replication 325 (each triplicate was taken from the same bottle), a random effect was added as a bottle effect. 326 Models were fitted with the 'lmer' function and model selection was assessed using the AIC<sub>c</sub> 327 (see below). To estimate the relative importance of each fixed effect, the AIC<sub>c</sub>-w of every 328 model was summed (Patiño et al., 2013), and to estimate the variable force effect, we used the 329 mean of the relative coefficient weighted by the AIC<sub>c</sub>-w.

330

331 3. Results

# 332 3.1. *Environmental variables and phytoplankton biomass*

Salinities were low during the three dilution experiments, ranging from 2.0 in NB in April, to 10.9 in September 2014 (Table 1). The percentage of DO was the lowest in September 2014 for both stations, and was negatively correlated with the temperature for the three sampling periods (Table 2, Spearman's rank correlations, *p*-value <0.05). Turbidity was highest in the autumn sampling (Table 1).

Concentrations of dissolved inorganic nutrients  $(NH_4^+, NO_3^-, NO_2^-, DIN, PO_4^{3-})$  and of TN 339 340 and TP were extremely variable. In the sampling in November-December 2013, both stations 341 presented the highest  $PO_4^{3-}$  concentrations, and NB displayed the highest concentration of 342 dissolved inorganic nutrients, DIN being mainly composed of NO<sub>3</sub><sup>-</sup> (Table 1). In April 2014, 343 N was mainly represented by NO<sub>3</sub><sup>-</sup> in the two stations, and N levels were especially high in 344 SB, with 106.5 µM of TN and 95.4 µM of DIN. Inversely, in September 2014, both stations displayed low NO<sub>3</sub><sup>-</sup> concentrations ( $<0.15 \mu$ M) and NH<sub>4</sub><sup>+</sup> was the main nitrogen form. TP was 345 346 higher in SB than in NB in all three samplings (Table 1). All dissolved inorganic nutrient 347 concentrations and TN and TP concentrations were negatively correlated with the temperature. Moreover,  $NH_4^+$  concentrations were also negatively correlated with the salinity. 348 349 DIN forms were positively correlated between them, and NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations were also positively correlated with TN and TP concentrations.  $PO_4^{3-}$  concentrations were 350 351 positively correlated with the percentage of DO (Table 2). The TN:TP and DIN:DIP ratios 352 displayed highest values in April 2014, respectively 71.0 in SB and 124.5 in NB.

353

354 The biomass of the total phytoplankton significantly differed among stations and samplings, 355 and the differences among samplings also changed between the two stations (Fig. 2, ANOVA, 356 *p*-value <0.05). Chlorophyll *a* (Chl *a*) concentration generally ranged between 3.5 and 6.0  $\mu$ g  $1^{-1}$ , with the exception in SB in December 2013 when it peaked at 20.6 µg  $1^{-1}$  (Fig. 2A). Taking 357 358 all samplings collectively, we found that Chl a concentration showed a negative relationship with the salinity and  $NH_4^+$  concentrations, the latter effect changing among stations 359 (Regression analyses, force of the salinity effect: -4.5, and of the  $NH_4^+$  concentration: -8.1). 360 361 Total biomass was significantly higher (i) in SB than in NB, (ii) in the autumn sampling than 362 in April and in September 2014, as well as (iii) in April than in September samplings (Fig. 2, 363 pairwise Tukey multiple comparisons, p < 0.05). The total biomass was negatively correlated

- 364 with temperature, and positively correlated with  $NO_2^-$ ,  $NO_3^-$ ,  $PO_4^{3-}$  concentrations and with
- the percentage of DO, reflecting the peak of biomass observed in December in SB (Table 2,
- 366 Spearman's rank correlations, *p*-value <0.05).

367 Table 1. Mean values of environmental parameters in the two NB and SB stations of Biguglia lagoon in November-December 2013, April and

368 September 2014. DO: dissolved oxygen. Dissolved inorganic nitrogen is expressed as  $DIN = NH_4 + NO_3 + NO_2$ . Dissolved inorganic phosphorus 369 corresponds to  $PO_4^{3-}$  concentration.

Sampling pariods	Stations	Data	T <sup>o</sup> (C)	Colimita		Turbidity	TN	$NH_4^+$	NO <sub>3</sub> <sup>-</sup>	$NO_2^-$	DIN	TP	PO <sub>4</sub> <sup>3-</sup>	TNITD	
Sampning periods	Stations	Date	I (C)	Samily	DO (%)	(NTU)		(µM)				110.11	DIN.DIF		
New Dec 2012	NB	26/11/13	8.3	4.7	100.8	2.87	84.37	7.52	70.01	0.49	78.03	1.37	0.73	61.4	107.3
Nov -Dec 2015	SB	04/12/13	8.2	6.1	101.5	16.83	61.87	0.69	40.72	0.29	41.70	1.77	0.64	34.9	65.5
April	NB	07/04/14	15.0	2.0	113.0	0.80	34.55	2.18	17.47	0.16	19.80	0.82	0.16	41.9	124.5
2014	SB	02/04/14	15.5	6.0	93.2	4.63	106.47	1.85	93.22	0.30	95.38	1.50	0.00	71.0	-
September 2014	NB	11/09/14	24.8	10.9	97.0	1.83	38.51	0.37	0.03	0.00	0.40	0.89	0.03	43.3	13.3
-	SB	09/09/14	25.9	6.0	86.2	10.50	29.95	1.24	0.11	0.09	1.44	1.17	0.11	25.6	13.1

371 Table 2. Spearman's rank correlation between species richness (S), Shannon index (H), Chl a concentrations and environmental variables at the

372 95% confidence interval for the three sampling periods in both NB and SB stations of Biguglia lagoon. Significant correlations are in bold. DO:

	S	Н	Chl a	Temperature	Salinity	$\mathrm{NH_4}^+$	$NO_2^-$	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	TN	TP	Turbidity
Н	-0.314											
Chl a	-0.312	0.626**										
Temperature	0.469*	-0.661**	-0.900***									
Salinity	-0.051	-0.693**	-0.242	0.203								
$\mathbf{NH_4}^+$	-0.066	0.680**	0.260	-0.314	-0.928***							
$NO_2^-$	-0.513*	0.536*	0.530*	-0.657**	-0.464	0.714***						
NO <sub>3</sub> <sup>-</sup>	-0.494*	0.335	0.492*	-0.543*	-0.377	0.600**	0.943***					
PO <sub>4</sub> <sup>3-</sup>	-0.405	0.836***	0.709***	-0.880***	-0.400	0.516*	0.637**	0.395				
TN	-0.280	-0.009	0.360	-0.486*	0.058	0.257	0.771***	0.829***	0.273			
ТР	-0.809***	0.047	0.398	-0.486*	0.319	-0.086	0.600**	0.657*	0.273	0.657**		
Turbidity	-0.828***	-0.041	0.122	0.143	0.406	-0.314	0.200	0.257	0.030	0.143	0.829***	
DO	0.041	0.624**	0.774***	-0.771***	-0.348	0.257	0.200	0.086	0.698**	0.029	-0.143	-0.371

373 dissolved oxygen. \*\*\**p*-value <0.001, \*\**p*-value <0.01, \**p*-value <0.05.



Fig. 2. Mean chlorophyll *a* (Chl *a*) concentration ( $\mu$ g l<sup>-1</sup>) (A) in the three samplings at the NB and SB stations in Biguglia lagoon. Error bar indicates standard deviations. Proportions of the different size classes of phytoplankton contributing to phytoplankton biomass (B), *i.e.* microphytoplankton >20 µm (dark grey), nanophytoplankton between 5 and 20 µm (grey) and ultraphytoplankton <5 µm (light grey).

# 381

# 3.1. Phytoplankton community composition

The size class structuration of phytoplankton communities was significantly different between the NB and SB stations among the three samplings (Fig. 2B), and the differences between samplings changed between stations (Permanova, *p*-value <0.05). Taking all samplings collectively, the ultra- and the nanophytoplankton biomasses showed a positive relationship with the salinity and the phosphorus concentrations. In contrast, the microphytoplankton biomasses showed a positive relationship with NO<sub>3</sub><sup>-</sup> concentrations and a negative

388  $\mathrm{NH_4}^+$ relationship with concentrations (Regression analyses, delta-AICc <2). 389 Ultraphytoplankton was the major group in the phytoplankton communities (up to 87 % of 390 Chl a in September 2014) (Fig. 2B), except for NB in November 2013, when a higher 391 proportion of nanophytoplankton (40 %) than ultraphytoplankton (34 %) was observed. For 392 both stations, the nanophytoplankton biomass was highest in November 2013.

393

394 The taxonomic diversity indices showed different patterns at the two stations and among the 395 three samplings. Both the species richness (S) and the Shannon index (H) significantly 396 changed among stations and samplings (ANOVAs, both *p*-values <0.05). S was the lowest in 397 the autumn sampling and highest in the sampling in September for the two stations, while H 398 showed the inverse trend. Hence, S was positively correlated with temperature, and negatively 399 correlated with  $NO_2^-$ ,  $NO_3^-$ , TP concentrations and turbidity (Table 2). H was positively correlated with total biomass,  $NH_4^+$ ,  $NO_2^-$  and  $PO_4^{3-}$  concentrations and percentage of DO, 400 401 while it was negatively correlated with temperature and salinity (Table 2).

402

403 Fig. 3 gives the contributions of four size classes ( $<3 \mu m$ , between 3 and 5  $\mu m$ , between 5 and 404 20  $\mu$ m and >20  $\mu$ m) to the total abundances of cell counts in the two stations at the three 405 sampling periods, and Fig. 4 specifies the main taxonomic classes represented in each of the 406 size classes. For the three samplings, small cells dominated the phytoplankton communities 407 (Fig. 3). The NB station showed higher Bacillariophyceae abundances than SB, while the SB 408 station showed higher Chlorophyta and Cryptophyceae abundances (Fig. 4). NB has also 409 displayed higher dinoflagellates abundances than SB, except in November-December 2013 410 (Fig. 4).

412 In the sampling in November 2013, the phytoplankton community in NB showed a bloom of the dictyochophycean Apedinella radians (Lohmann) P.H. Campbell, 1973 (1.3 x 10<sup>6</sup> cells.l<sup>-1</sup>, 413 414 *i.e.* 41.6 % of total abundances, Fig. 3-4). This community also comprised high abundances of eukaryotes with flagella between 3 and 5  $\mu$ m (6.9 x 10<sup>5</sup> cells.1<sup>-1</sup>, Fig. 3-4), and of 415 416 Prorocentrum cordatum (Ostenfeld) J.D.Dodge, 1975 alias Prorocentrum minimum (Pavillard, 1916) J.Schiller, 1933 (2.9 x 10<sup>5</sup> cells.1<sup>-1</sup>, Fig. 4). Dinoflagellates dominated the 417 418 community in SB in December, mainly represented by Heterocapsa minima Pomroy, 1989  $(5.1 \times 10^6, i.e. 34.2 \% \text{ of total abundances Fig. 3})$ , associated with A. radians  $(4.8 \times 10^6 \text{ cells.})^{-1}$ 419 <sup>1</sup>, Fig. 4). Microphytoplanktonic cells were scarce (e.g. Kryptoperidinium foliaceum 420 Lindemann, 1924: 3.7 x 10<sup>3</sup> cells.l<sup>-1</sup>; *Cocconeis* sp.: 3.7 x 10<sup>3</sup> cells.l<sup>-1</sup>; *Prorocentrum micans* 421 Ehrenberg 1834:  $1.5 \times 10^3$  cells.l<sup>-1</sup>). Conversely, we observed a bloom of *Mesodinium rubrum* 422 Lohmann, 1908 (2.3 x  $10^5$  cells.l<sup>-1</sup>, *i.e.* 78 % of the microplanktonic cells, Fig. 4). 423

424

425 In the sampling in April 2014, dinoflagellates dominated the NB community mainly due to a bloom of *H. minima*  $(3.7 \times 10^6 \text{ cells.})^{-1}$ , *i.e.* 35.8 % of total abundances, Fig. 3-4). 426 427 Bacillariophyceae were also abundant and distributed among the three size classes  $>3 \mu m$  (3.1 x  $10^6$  cells.<sup>1</sup>, Fig. 4) with *e.g. Thalassiosira* sp., *Diatoma* sp., and *Chaetoceros* spp.. The SB 428 community was dominated by phycocyanin-rich picocyanobacteria (PC-cyanos, 6.4 x 10<sup>7</sup> 429 cells.1<sup>-1</sup>, *i.e.* 49.6 % of total abundances, Fig. 3-4), Picoeukaryotes (Peuk) without flagella (5.6 430 x  $10^7$  cells.l<sup>-1</sup>) and eukaryotes with flagella between 3 and 5 µm (5.9 x  $10^6$  cells.l<sup>-1</sup>, Fig. 4). 431 432 The two latest morphotypes probably belonged to the green algae according to the pigment composition (data not shown). *M. rubrum* also bloomed as in December 2013 (1.6 x  $10^5$ 433 cells. $l^{-1}$ , Fig. 4). 434

In the sampling in September 2014 the communities of the two stations were dominated by 436 PC-cyanos (9.4 x 10<sup>8</sup> cells.l<sup>-1</sup>, *i.e.* 92.7 % of total abundances in NB, 3.0 x 10<sup>8</sup> cells.l<sup>-1</sup> *i.e.* 437 91.9 % of total abundances in SB, Fig. 3-4) and Peuk without flagella (6.5 x  $10^7$  cells.l<sup>-1</sup> in 438 NB.  $1.7 \times 10^7$  cells. I<sup>-1</sup> in SB. Fig. 3-4), that most likely belonged to the green algae according 439 440 to the pigment composition (data not shown). The nanophytoplankton in NB displayed high abundances of *Tetraselmis* sp.  $(2.0 \times 10^5 \text{ cells.I}^{-1})$ , *P. cordatum*  $(8.2 \times 10^4 \text{ cells.I}^{-1})$  and *H.* 441 *minima*  $(3.2 \times 10^4 \text{ cells.})^{-1}$ . Dinoflagellates dominated the microphytoplankton with 442 *Prorocentrum* sp. *cf mexicanum* Osorio-Tafall, 1942, and *Gonvaulax* sp.  $(10^4 \text{ cells.l}^{-1})$ . We 443 also observed high abundances of Chaetoceros spp., and Ceratoneis closterium Ehrenberg, 444 1839 ( $10^4$  cells.l<sup>-1</sup>). The ultra- and the nanophytoplankton in SB also contained high 445 abundances of A. radians (1.6 x 10<sup>6</sup> cells.1<sup>-1</sup>), Pyramimonas sp. (8.9 x 10<sup>5</sup> cells.1<sup>-1</sup>), 446 *Plagioselmis* sp., *H. minima*, *P. minimum* and nanoplanktonic centric diatoms (10<sup>4</sup> cells.l<sup>-1</sup>). 447 C. closterium, and Gonyaulax sp. cf spinifera ( $10^4$  cells. $l^{-1}$ ) were the most abundant 448 449 microplanktonic cells in SB (Fig. 4).



450

451 Fig. 3. Contribution of the size classes  $<3 \mu m$ , [3-5  $\mu m$ ], [5-20  $\mu m$ ] and  $>20 \mu m$  to the total 452 abundances of cell counts in the two NB and SB of Biguglia lagoon at the three sampling 453 periods. Total abundances are specified on the top of the bars.



Fig. 4. Contribution of the main taxonomic classes to the abundances of the four size classes  $457 < 3 \mu m$ , [3-5  $\mu m$ ], [5-20  $\mu m$ ] and >20  $\mu m$  in the two stations of Biguglia lagoon at the three sampling periods.

459

### 460 3.2. *Phytoplankton net growth and mortality rates without enrichment*

461 Phytoplankton net growth rates  $(\mu_0)$  without enrichment ranged from negative values for the microphytoplankton of NB in November 2013 and April 2014, to 1.91 d<sup>-1</sup> for the 462 463 nanophytoplankton of SB in April 2014 (Table 3). In November-December 2013, the total phytoplankton displayed low net growth rates (0.45 d<sup>-1</sup> in NB and 0.48 d<sup>-1</sup> in SB), and similar 464 mortality rates (0.62  $d^{-1}$  in NB to 0.69  $d^{-1}$  in SB). In NB the ultraphytoplankton dominated by 465 466 picoeukaryotes (Peuk) displayed a two-fold higher growth rate, while its mortality rate was 467 three times higher than those of the total phytoplankton (Table 3). The nanophytoplankton, 468 mainly comprising A. radians and P. cordatum, and particularly the microphytoplankton 469 comprising diatoms and dinoflagellates species, showed a loss of biomass after 24 h 470 incubation (Table 3). In SB, only the net growth rate  $(\mu_0)$  of ultraphytoplankton could have 471 been estimated, but this size class dominated by Peuk has shown a loss of biomass after the 24 472 h incubation (Table 3, Fig. S2A).

473

In April, we were able to calculate the  $\mu_0$  only for the nanophytoplankton (Table 3, Fig. S2B). In NB, this size class dominated by *H. minima* displayed a high net growth rate ( $\mu_0 = 1.13 \text{ d}^{-1}$ ) and a relatively low mortality rate (0.28 d<sup>-1</sup>), while in SB, it was dominated by Chlorophyta and Cryptophyta cells, which displayed the highest estimated  $\mu_0$  (1.91 d<sup>-1</sup>) and a high mortality rate (0.83 d<sup>-1</sup>).

In September, the net growth rates in NB ranged between 0.50 d<sup>-1</sup> for the ultraphytoplankton 480 dominated by PC-cyanos and  $1.84 d^{-1}$  for the microphytoplankton dominated by 481 dinoflagellates and Bacillariophyceae. The nano-, dominated by Plagioselmis sp., Tetraselmis 482 sp., and P. cordatum, and the microphytoplankton showed the highest growth rates ( $\mu_0 = 1.6$ 483 and  $\mu_0 = 1.8 \text{ d}^{-1}$ , respectively) with similar mortality rates ( $g = 0.57 \text{ d}^{-1}$ ). In SB, the total 484 485 phytoplankton and the ultraphytoplankton also dominated by PC-cyanos experienced a loss of 486 biomass after 24 h incubation (Table 3). The nanophytoplankton dominated by A. radians and *Plagioselmis* sp. displayed high growth and mortality rates ( $\mu_0 = 1.4 \text{ d}^{-1}$ ;  $g = 1.1 \text{ d}^{-1}$ ). The 487 488 biomass of microphytoplankton at T0 was too low to estimate their apparent growth rate, we 489 were not able to truly calculate its specific growth and mortality rates.

491 Table 3. Net growth rate without enrichment  $(\mu_0)$  and maximal growth rates, with (i) full enrichment ( $\mu_{max}$ ), (ii) enrichment minus N ( $\mu_{-N}$ ), (iii) enrichment minus P ( $\mu_{-P}$ ) for the NB 492 493 and SB stations of Biguglia lagoon in November-December 2013, April and September 2014. 494 g represents the mortality rate. Bold values specify when the relationship between apparent 495 growth rate as a function of the dilution factor can be explained as a linear model, although 496 for the minus N and minus P enrichments other models can also fit this relationship; the 497  $\mu_0:\mu_{max}$  ratio illustrates the impact of the full enrichment on phytoplankton growth; the ratios 498 between growth rates with selectively N- or P-enrichment ( $\mu_{-N}$  or  $\mu_{-P}$ ) and  $\mu_{max}$  highlight N- or 499 P-limitations ( $\mu_{-X}$ : $\mu_{max} = 1$  no limitation,  $\mu_{-X}$ : $\mu_{max} < 1$  limitation of the lacking nutrient X,  $\mu_{-X}$ 500 : $\mu_{\text{max}} > 1$  limitation of the added nutrient).

Stations	Samplings	Fraction	$\mu_0$	g	$\mu_{ m max}$	$\mu_0:\mu_{\max}$	$\mu_{\text{-N}}$	$\mu_{-N}$ : $\mu_{max}$	$\mu_{\text{-P}}$	$\mu_{-P}$ : $\mu_{max}$
				$(d^{-1})$			$(d^{-1})$		$(d^{-1})$	
NB	Nov - Dec 2013	Total	0.45	0.62	<0	-	<0	-	0.30	<0
		Micro-	<0	1.45	<0	-	<0	-	<0	-
		Nano-	0.37	0.26	0.46	0.79	0.62	1.34	1.13	2.44
		Ultra-	0.96	1.82	<0	-	<0	-	<0	-
	April 2014	Total	<0	<0	0.25	-	0.51	1.99	0.07	0.29
		Micro-	<0	1.01	0.24	-	1.59	6.70	1.01	4.24
		Nano-	1.13	0.28	1.83	0.62	1.95	1.06	1.54	0.84
		Ultra-	<0	0.43	<0	-	<0	-	<0	-
	September 2014	Total	0.64	0.30	1.78	0.36	0.86	0.48	1.03	0.58

		Micro-	1.84	0.57	3.40	0.54	1.78	0.52	2.24	0.66
		Nano-	1.60	0.57	2.70	0.59	1.77	0.65	2.13	0.79
		Ultra-	0.51	0.45	1.12	0.45	0.51	0.46	0.52	0.46
SB	Nov - Dec 2013	Total	0.48	0.69	0.41	1.19	0.26	0.64	0.53	1.31
		Micro-	1.12	1.12	0.45	2.48	1.09	2.41	1.05	2.32
		Nano-	1.17	0.50	0.90	1.30	0.78	0.87	1.07	1.19
		Ultra-	<0	1.10	0.14	<0	1.85	13.68	2.02	14.94
	April 2014	Total	0.17	<0	0.19	0.92	0.06	0.31	0.26	1.35
		Micro-	-	-	-	-	-	-	-	-
		Nano-	1.91	0.83	2.00	0.95	1.64	0.82	1.64	0.82
		Ultra-	<0	<0	0.01	<0	0.10	8.73	0.34	27.96
	September 2014	Total	<0	0.16	1.79	<0	0.28	0.16	1.34	0.75
		Micro-	-	-	-	-	-	-	-	-
		Nano-	1.42	1.14	3.23	0.44	1.58	0.48	<0	<0
		Ultra-	<0	<0	0.87	<0	<0	<0	<0	<0

#### 502 3.3. *Bioassay experiments*

In November and December 2013, the full enrichment including  $NO_3^-$ ,  $NH_4^+$  and  $PO_4^{3-}$  did 503 504 not enhance phytoplankton growth rates in the two stations. The total phytoplankton, the 505 micro- and ultraphytoplankton in NB experienced a loss of biomass after 24 h incubation 506 compared with the controls. Only the growth rate of the ultraphytoplankton of SB was higher 507 with the enrichment, and showed a linear relationship between the apparent growth rate and 508 the dilution factor. This indicates a nutrient limitation for this class size (Table 3). In NB, the 509 enrichment minus N has only resulted in a higher growth rate of the nanophytoplankton. The 510 non-linear relationship between the apparent growth rate and the dilution factor for the 511 ultraphytoplankton (Fig. S2A) suggests the use of other resources than the externally supplied 512 nutrients, or a saturation of predation in the less diluted samples. The enrichment minus P 513 partly released the total phytoplankton growth from N-limitation, allowing a positive growth 514 rate. Ratios between  $\mu_{-N}$  or  $\mu_{-P}$  and  $\mu_{max}$  for the nanophytoplankton indicate an independent 515 co-limitation by N and P. The negative growth rates of the micro- and ultraphytoplankton, and 516 the non-linear relationship between their apparent growth rate and the dilution factor with the 517 enrichments minus N and minus P more likely suggest a co-limitation by N and P (Table 3, Fig. S2A). In SB, ratios between  $\mu_{.N}$  or  $\mu_{.P}$  and  $\mu_{max}$  of the total phytoplankton and of the nanophytoplankton, as well as their non-linear relationship between apparent growth rates and dilution factors suggest a single N-limitation (Table 3, Fig. S2B). The higher growth rate of microphytoplankton with the enrichments minus N and minus P compared with  $\mu_{max}$  (Table 3) suggests a co-limitation by N and P. Ratios between  $\mu_{.N}$  or  $\mu_{.P}$  and  $\mu_{max}$  for the ultraphytoplankton suggest an independent co-limitation by N and P (Table 3).

524

In April 2014, the full enrichment with  $NO_3^{-1}$  and  $PO_4^{-3-1}$  resulted in an increase of the growth 525 526 rate of the nanophytoplankton by 62 % and 5 % in NB and SB respectively. The non-linear 527 relationship between the apparent growth rate and the dilution factor indicates that other 528 fractions in the two stations may have been limited despite the full nutrient enrichment (Table 529 3, Fig. S3). In NB, ratios between  $\mu_{-N}$  or  $\mu_{-P}$  and  $\mu_{max}$  of the total phytoplankton and of the 530 nanophytoplankton suggest a single P-limitation. The microphytoplankton displayed an 531 independent co-limitation of N and P, which was more pronounced for P. The negative  $\mu_{\rm N}$ and  $\mu_{-P}$  of the ultraphytoplankton, and the relationship between their apparent growth rate and 532 533 the dilution factor in the enrichments minus N and minus P also suggest a co-limitation by N 534 and P (Table 3, Fig. S3A). In SB, ratios between  $\mu_{-N}$  or  $\mu_{-P}$  and  $\mu_{max}$  of the total phytoplankton 535 indicate a single N-limitation, while those of the nano- and of the ultraphytoplankton indicate 536 a simultaneous and an independent co-limitation by N and P respectively (Table 3, Fig. S3). 537 This is also supported by the non-linear relationship between their apparent growth rate and 538 the dilution factor that may highlight the use of other N and P resources to cope with the 539 nutrient limitation (Table 3, Fig. S3B).

541 In September 2014, the enrichment with  $NH_4^+$  and  $PO_4^{3-}$  led to more than the doubling of the 542 growth rate of most of phytoplankton size classes in NB (Table 3), indicating a strong nutrient

543 limitation. The microphytoplankton of NB displayed the highest  $\mu_{max}$  of the two communities 544 for the three bioassays. In SB, the full enrichment has allowed a release from nutrient 545 limitation of the entire community, especially for the ultraphytoplankton and the 546 nanophytoplankton (Table 3). In NB, ratios between  $\mu_{-N}$  or  $\mu_{-P}$  and  $\mu_{max}$  of the total 547 phytoplankton and the three size classes suggest a N and P co-limitation, that was serial for 548 the micro- and for the ultraphytoplankton, and independent for the nanophytoplankton (Table 549 3). In SB, ratios between  $\mu_{-N}$  or  $\mu_{-P}$  and  $\mu_{max}$  of the three size classes indicate an independent 550 co-limitation by N and P for the total phytoplankton, a serial co-limitation by N and P for the 551 nanophytoplankton and a simultaneous co-limitation by N and P for the ultraphytoplankton 552 (Table 3, Fig. S4B).

553

554 4. Discussion

In this study, we aimed to describe functional traits of phytoplankton communities in Biguglia lagoon submitted to a strong anthropogenic impact in different seasons with contrasting environmental conditions, and the response of these communities to various forms of nutrients inputs simulating nutrient pulses to predict their impact on ecosystem functioning.

559

560 4.1. Functional traits of phytoplankton communities in response to environmental
561 conditions

Biguglia lagoon was globally strongly influenced by freshwater inputs from the watershed and from the connection with one of the main river in Corsica in the south of the lagoon. These freshwater inputs have contributed to maintain a low salinity that prevailed in the two sub-basins during the year 2014, despite their distinct hydrological dynamics. This freshwater influence has led to the dominance of species associated with brackish or fresh waters in phytoplankton assemblages. Phycocyanin-rich picocyanobacteria (PC-cyanos) and flagellates from the Dictyochophyceae, dinoflagellates, Chlorophytes, and Cryptophytes were the main representative groups in the two stations at the three samplings. Their size structure, community composition and productivity illustrate their functional responses to lagoon variability.

572

573 Biguglia lagoon was principally enriched by high nitrogen inputs, especially during the wet 574 periods. Indeed, high nitrate inputs from a huge historical accumulation are carried to the 575 lagoon by groundwater flows during strong episodic rainfall events (Erostate et al., 2018). 576 The negative correlations, both between temperature and nutrient concentrations, and between salinity and ammonium concentration also support that freshwater discharge was the main 577 578 source for nutrient enrichment in the lagoon at the three sampling periods (Garrido et al., 579 2016; Pasqualini et al., 2017). The important precipitations in November 2013 and Marsh 580 2014 (150.4 mm and 92.6 mm respectively, https://www.infoclimat.fr/, Station of Bastia 581 Poretta) explain the high  $NO_3^-$  concentrations and high TN:TP and DIN:DIP values in the 582 autumn and spring samplings. In contrast, low dissolved inorganic nitrogen and phosphorus 583 concentrations were observed in September, although the summer period corresponds to the 584 most important period for the recycling of ammonium and phosphate in the lagoon (Collos et 585 al., 2003).

586

Phytoplankton cell size decreased with the decrease of DIN availability, which may be explained by higher uptake affinities of small cells under nutrient-limiting conditions (Litchman et al., 2007). High nanophytoplankton biomasses in Biguglia lagoon have already been related to the availability of oxidized forms of nitrogen (Cecchi et al., 2016). In this study, ultra- and nanophytoplankton biomasses were rather positively related to  $PO_4^{3-}$ availability. Dominance of fast-growing pico- and nanoflagellates can be frequent in shallow

<sup>593</sup> lagoons exposed to high nutrient loads and submitted to continuous inputs of freshwater or <sup>594</sup> discontinuous floods. These nutrient loads allow the release of the limitation of cells  $>3 \mu m$ <sup>595</sup> that can coexist with fast growing and highly competitive picoeukaryotes (Bec et al., 2011; <sup>596</sup> Coelho et al., 2015).

597

598 Nanoplanktonic dinoflagellates were particularly abundant during the samplings in the wet seasons (autumn and spring). We particularly observed blooms (>10<sup>5</sup> cells.l<sup>-1</sup>) of *P. cordatum* 599 600 in NB in November 2013, and of *H. minima* in SB in December 2013 and in NB in April 601 2014. Blooms of *P. cordatum* are frequently related to high N:P ratios in coastal transitional 602 ecosystems characterized by brackish waters (Johnson, 2015), and to freshwater inflows that 603 cause low salinities and high nitrate concentrations (Heil et al., 2005; Coelho et al., 2007; 604 Cecchi et al., 2016). Moreover, P. cordatum and some Heterocaspa species have 605 demonstrated potential mixotrophic abilities, especially under N- or P-starvation (Legrand et 606 al., 1998; Jeong et al., 2005; Johnson, 2015). The presence of potential preys such as 607 picocyanobacteria, Cryptophytes and small diatoms may thus have stimulated their 608 development despite the potential nutrient limitation.

609

Biomass of the microphytoplankton, mainly composed of Bacillariophyceae and dinoflagellates in the three samplings, has been positively related to the high  $NO_3^$ concentrations. Indeed, pulses of  $NO_3^-$  especially favor fast-growing diatoms during spring blooms in river-dominated coastal ecosystems (Paerl et al., 2010; Donald et al., 2011; Glibert et al., 2015). They were generally most abundant in the northern basin, probably due to their resistance to turbulent environments, their high affinity for nitrate, and the high freshwater inputs (Garrido et al., 2016).

618 Picophytoplankton (picocyanobacteria and picoeukaryotes) often represents an important 619 component of phytoplankton communities in brackish lagoons. Particularly, phycocyanin-rich 620 picocyanobacteria have shown blooms after floods when salinities achieved their minimal 621 values (Bec et al., 2011; Lafabrie et al., 2013). Small cells have physiological advantages that 622 confer them a high competitiveness to acquire and use nutrients under limiting conditions 623 (Raven, 1998). They are especially competitive in warmer waters and under low N-624 availability as observed during the summer period (Bec et al., 2005; Leruste et al., 2016; 625 Domingues et al., 2017). Moreover, they are able to use nitrate, ammonium as well as organic 626 N-sources in a highly efficient way (Domingues et al., 2011), and are hardly inhibited by 627 occasionally occurring high ammonium concentrations (Collos and Harrison, 2014). Bloom of 628 picophytoplankton in summer coincides with high regeneration process, and a dominance of 629 ammonium that picophytoplankton can preferentially use, while diatoms and dinoflagellates 630 preferentially use NO<sub>3</sub>, and are able to show high growth rate in nitrate-rich waters 631 (Domingues et al., 2011; Glibert et al., 2015; Reed et al., 2016).

632

633 These competitive trade-offs may thus explain the competitive exclusion of the larger cells by 634 the PC-cyanos in September. Shift between diatoms and dinoflagellates to picophytoplankton 635 from spring to late summer frequently occur in coastal aquatic systems with the shift of 636 available N forms, from  $NO_3^-$  (>88% of the DIN in the two stations in November-December 637 2013 and April 2014) to  $NH_4^+$  (>85 % of the DIN in the two stations in September 2014) 638 (Glibert et al., 2015; Domingues et al., 2017). However, ambient nutrient concentrations are 639 measures of the residual nutrients after biogeochemical activity, and may not reflect the real 640 available nutrient concentrations for phytoplankton growth. The bioassay experiments gave further insights about the relationship between their functional responses to the nutrient 641

availability. This especially specified which nutrient was potentially limiting forphytoplankton growth at the three sampling periods.

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# 645

## 4.2. Phytoplankton behavior under induced nutrient limitation

646 Temperate coastal ecosystems generally display seasonal changes of nutrient availability and 647 limitation, characterized by shifts from a P-limitation during the wet seasons after high 648 nitrate-rich freshwater inputs, to N-limitation during the dry season (Lomas and Glibert, 2000; 649 Kemp et al., 2005). In contrast, we did not observe this pattern in Biguglia lagoon, despite the 650 high DIN inputs from the watershed during the wet seasons, reflected by high TN:TP and 651 DIN:DIP<sup>-</sup> values. The results of the bioassay experiments more often show a co-limitation by 652 N and P for the phytoplankton communities. Nevertheless, some size fractions were limited 653 by one of these elements alone, *e.g.* the nanophytoplankton of the NB station limited by P in 654 April 2014, and the microphytoplankton of the SB station limited by N in December 2013. A 655 review showed that co-limitation by N and P in freshwater and marine ecosystems is more 656 common than previously thought (Elser et al., 2007). Interestingly, the single limitation for 657 some size fractions within a co-limited community, such as observed in SB in September 658 2014 may indicate niche differentiation. We also observed that the phytoplankton in SB was 659 more often N-limited than in NB despite high DIN:DIP ratios. Conclusions from the dilution 660 experiment should be drawn with caution, because incubations have been conducted on small 661 spatial and temporal scales. The responses of phytoplankton communities at these scales can 662 differ from the overall ecology of the entire ecosystem (Piehler et al., 2004; Reed et al., 663 2016). However, these results support that ambient nutrient ratios do not necessarily reflect 664 the availability of nutrients for autotrophic communities, particularly because of the known 665 variability of nutrient stoichiometry of phytoplankton. The communities of the two stations 666 showed the strongest simultaneous or serial co-limitation by N and P in summer while in the

667 wet seasons they showed single N- or P-limitations or independent co-limitation depending on 668 fractions. The figure 5 conceptualizes our observations for the three samplings, and links the 669 environmental specificities, the differences in community structure and composition, with the 670 calculated growth rates and the observed N- and P-limitation.

671

672 Picocyanobacteria, dinoflagellates and other phytoplankton groups have demonstrated 673 numerous ways to acclimate to temporally and varying N- and P-limitation (Lin et al., 2015). For example, luxury  $PO_4^{3-}$  uptake prior to sampling for the bioassays could have provided the 674 675 necessary intracellular P for phytoplankton growth during the 24 h incubation. 676 Picocyanobacteria may have used high P-storage abilities that could have allowed them to 677 maintain their bloom despite low P-availability (Sorokin and Dallocchio, 2008). Moreover, 678 they have shown high inorganic P-uptake efficiency at low levels of available soluble reactive 679 phosphorus (Collos et al., 2009). Alternatively, the high dissolved and particulate organic N 680 and P stocks in the sediment of Biguglia lagoon may have been used by the phytoplankton to 681 thrive despite low dissolved inorganic N- and P-availability. Indeed, the very low dissolved 682 inorganic phosphorus concentrations at the three samplings may have stimulated the 683 production of alkaline phosphatase, allowing the use of dissolved organic phosphorus (Piehler 684 et al., 2004; Lin et al., 2015). Moreover, high abundances of potentially mixotrophic species 685 among Prorocentrum, Heterocapsa, Gonyaulax and other genera, and of Mesodinium rubrum 686 (Collos et al., 2014; Fischer et al., 2016; Johnson, 2015) have been observed during these 687 experiments. Mixotrophy is an advantageous behavioral response under nutrient limiting 688 conditions, allowing cells to reduce direct competition for inorganic nutrients (Jeong et al., 689 2005; Mitra et al., 2014; Johnson, 2015). Hence, the high abundances of picocyanobacteria, 690 physiologically able to cope with P-limitation, and of potentially mixotrophic dinoflagellates 691 and nanoflagellates, could explain the N and P co-limitation of the communities despite high

692 DIN:DIP ratios (Burson et al., 2016). Indeed, these physiological and behavioral capacities 693 may have offset an inorganic P-limitation, either by the ingestion of starving preys that may 694 benefit from the added inorganic nutrients, or of dissolved organic substances. This may have 695 led to a N and P co-limitation rather than a single P-limitation (Burson et al., 2016; Fischer et 696 al., 2016). Mixotrophy is also stimulated by nutrient limitation, and would have been 697 particularly competitive in Biguglia lagoon during the three sampling periods. This may 698 explain the bloom of the mixotrophic Mesodinium rubrum (Tong et al., 2015; Seong et al., 699 2017) in the southern basin in the autumn and spring samplings (Fig. 5).





701

Fig. 5. Schematic representation of the environmental characteristics, phytoplankton community composition and their relationship with nutrient availability during the three bioassays periods in the two stations of Biguglia lagoon. For each sampling, the schematic representation specifies the water temperature, the salinity, the freshwater influence, and the

706 nutrients inputs whether they come from the watershed or from the sediments. Main 707 phytoplankters are specified as pictograms. Phytoplankton co-limitation by N and P is 708 inferred according to ratios between growth rate under experimentally induced N- or P-709 limitation and maximal growth rate (see Results).

710

### 4.3. Assumptions about responses of phytoplankton communities to a nutrient pulse

Bioassay experiments can provide valuable insights about relationship between nutrient
dynamics and phytoplankton physiological responses. This can simulate a nutrient pulse,
helping to predict algal-based expression of eutrophication (Xu et al., 2012).

715

716 The composition of nutrient enrichments used at the three sampling periods was based on 717 previous studies and assumptions about ecosystem functioning, and may have not always 718 reflected the real environmental conditions or the potential nutrient pulses threatening Biguglia lagoon due to its high variability. These enrichments may thus have not always been 719 720 the most suitable to involve an optimum response of the phytoplankton communities. Hence, 721 the addition of the full enrichment has not always allowed the estimation of maximal growth 722 and mortality rates probably because the underlying assumptions of this method were not 723 always fulfilled. For almost the half of the fractions (11 out of 24) the relationship between 724 the apparent growth rates and the dilution factor was non-linear. Non-linear trends may 725 indicate microzooplankton feeding threshold or saturation depending on the curve pattern 726 (Chen et al., 2014). Indeed, the shape of some of these plots (Fig. S1, red lines) may indicate a 727 strong nutrient limitation and suggests the use of other nutrient resources such as those 728 regenerated through cell lyses, grazing and mineralization that increase with the proportion of 729 undiluted sample (Andersen et al., 1991).

731 The responses of the phytoplankton communities to the nutrient enrichment in the three 732 bioassays have reflected contrasting environmental vulnerability of Biguglia lagoon to short-733 term nutrient enrichment. Nutrient limitation can have many indirect effects on phytoplankton 734 productivity, e.g. a modification of the internal cell stoichiometry that changes its nutritional 735 quality and modify predation rates (Harpole et al., 2011). In November-December 2013, the 736 addition of the full enrichment comprising a mixture of nitrate and ammonium (10 µM each) 737 seems to have had a negative impact. In certain cases, ammonium can negatively affect nitrate 738 uptake and assimilation, especially in nitrate-dominated systems (Domingues et al., 2011; 739 2017), and particularly for dinoflagellates, the least ammonium-tolerant group (Collos and 740 Harrison, 2014). Hence, the addition of ammonium when nitrogen was mainly available as 741 nitrate, and dinoflagellates were the most abundant, may have inhibited its uptake and led to a 742 decrease of growth rate (Dodds and Whiles, 2010; Glibert et al., 2015). As nutrient addition is 743 expected to increase limitation by other resources, a negative response may also reflect 744 strongly constrained N:P stoichiometry. This is suggested by high TN:TP and DIN:DIP 745 values. Rather than re-equilibrating these ratios, nutrient addition may have led to an 746 exacerbated internal stoichiometric imbalance of N and P nutrients with negative impact on 747 phytoplankton growth (Harpole et al., 2011).

748

In general, the phytoplankton maximal growth rates followed a predictable seasonal trend, with minima in autumn and maxima in summer. These changes are correlated with water temperatures and photoperiods length, providing more favorable growth conditions in summer. Lower temperatures and light availability in autumn may have altered the uptake rate of phytoplankton (Dodds and Whiles, 2010; Domingues et al., 2011; Reed et al., 2016). This is also consistent with the community composition, the picophytoplankton dominating the community in September potentially having higher growth rate than the nanophytoplankton

dominating the autumn and spring samplings (Bec et al., 2011). Phytoplankton was thus more
reactive to a nutrient addition during the summer season when the nutrient demand is the
highest. This suggests that Biguglia lagoon is the most vulnerable to nutrient inputs during
summer.

760

761 Conclusion

762 Experimental enrichments bioassays are particularly appropriate to explore cause-effect 763 relationships between nutrient availability and phytoplankton dynamics, which can reflect the 764 vulnerability of the whole ecosystem. This is an important issue for managers to draw up 765 appropriate nutrient loading budgets, and to help evaluating the efficiency of subsequent 766 nutrient reduction strategies. In our study, N inputs, particularly NO<sub>3</sub><sup>-</sup> during the wet periods 767 and  $NH_4^+$  in summer, stimulated phytoplankton growth in Biguglia lagoon emphasizing the 768 need to mitigate these inputs. The phytoplankton community was strongly influenced by 769 freshwater and nutrients inputs that have led to different physiological and behavioral 770 responses over seasons. During autumn and spring, high DIN:DIP ratios have been associated 771 with blooms of potentially harmful dinoflagellates, which are increasingly observed in 772 Biguglia lagoon.

773

The absence of a significant positive effect of the enrichment could be explained through several hypotheses. The first is the presence of sufficient amounts of dissolved inorganic nutrients in the water column to sustain the phytoplankton growth during the incubation time. The second could be a more intensive use of other resources than the external pool, such as internal or regenerated inorganic nutrients. The third could be a strong competition for available nutrient with the bacterial communities. Of increasing concern is thus the potential for organic nutrients and high abundances of small preys to promote mixotrophic species,

781 some of which are potentially harmful. It would be important to quantify the availability of 782 dissolved organic nutrients and its effect on phytoplankton growth and trophic modes. This 783 should be linked with a study about the interaction between phytoplankton and bacteria, such 784 as competition for organic and inorganic available nutrients. The impact of bacterial activity 785 on the available nutrients stocks has also to be explored. Indeed, bacterial communities can 786 play an essential role in nutrient cycling, *e.g.* through the remobilization of organic matter, 787 leading to a tight phytoplankton-bacterioplankton coupling (Caroppo, 2002). The possible use 788 of external, internal and regenerated N and P pools, as well as the mixotrophic abilities of the 789 phytoplankton communities also need to be studied.

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- 804
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- 807 6. References
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#### 1072 7. Supplementary material

1073 Table S1. Net growth rates  $(\mu_0)$  measured in incubations without enrichment and maximal growth  $(\mu_{max})$  and mortality rates (g) subsequently calculated using full N and P enrichment in 1074 1075 the two NB and SB stations of Biguglia lagoon. Values were estimated according the linear 1076 Landry and Hasset (1982) equation. This model has been compared to alternative models by 1077 mixed-effect multiple linear regressions of apparent growth rates, based on Chl a 1078 concentration from dilution experiments. The parsimony of the different models was checked 1079 by the AIC criterion. The linear model was only accepted when it presented a delta-AIC<sub>c</sub>, <2with respect to the most parsimonious model (see Methods). AIC<sub>c</sub>-w describe the strength of 1080 explanation by the model of the model. R<sup>2</sup>: coefficient of determination. The  $\mu_0:\mu_{max}$  ratio 1081 reflects the impact of the nutrient enrichment on the phytoplankton growth (1: no effect; 0: 1082 1083 high enhancement of growth), highlighting the nutrient limitation under natural conditions. \*\*\**p*-value <0.001, \*\**p*-value <0.01, \**p*-value <0.05. 1084

Stations	Samplings	Fraction	$\mu_0$	$\mu_{ m max}$	g	n	AICc	$\Delta AICc$	AICc-w	$\mathbf{R}^2$	$\mu_0$ : $\mu_{\max}$
				$(d^{-1})$							
NB	Nov - Dec 2013	Tot	0.45	<0	0.62	8	11.5	1.45	0.33	0.31.	<0
		Micro	<0	<0	1.45	7	37.1	6.05	0.04	-0.05	-
		Nano	0.37	0.46	0.26	8	6.4	3.54	0.13	0.10	0.79
		Ultra	0.96	<0	1.82	8	24.7	0.00	0.55	0.45*	<0
	April 2014	Tot	<0	0.25	<0	7	1.4	4.25	0.10	0.19	<0
		Micro	<0	0.24	1.01	8	24.0	2.39	0.19	0.13	<0
		Nano	1.13	1.83	0.28	8	-5.2	0.00	0.50	0.53*	0.62
		Ultra	<0	<0	0.43	8	9.9	2.26	0.21	0.23	-
	September 2014	Tot	0.64	1.78	0.30	9	-10.7	0.00	0.68	0.64**	0.36
		Micro	1.84	3.40	0.57	9	3.2	0.00	1.00	0.58*	0.54
		Nano	1.60	2.70	0.57	9	-4.1	0.00	1.00	0.76**	0.59
		Ultra	0.51	1.12	0.45	8	2.20	0.86	0.39	0.48*	0.45
SB	Nov - Dec 2013	Tot	0.48	0.41	0.69	10	1.7	0.00	0.54	0.65**	1.19
		Micro	1.12	0.45	1.12	9	45.3	4.40	0.09	-0.09	2.48
		Nano	1.17	0.90	0.50	10	20.3	2.62	0.18	0.05	1.30
		Ultra	<0	0.14	1.10	8	9.3	0.00	0.93	0.70**	<0
	April 2014	Tot	0.17	0.19	<0	7	15.1	5.18	0.07	0.08	0.92
		Micro	-	-	-	-	-	-	-	-	-
		Nano	1.91	2.00	0.83	7	12.8	0.37	0.33	0.59*	0.95
		Ultra	<0	0.01	<0	8	14.3	4.46	0.09	-0.01	<0
	September 2014	Tot	<0	1.79	0.16	7	-21.1	0.45	0.44	0.88**	<0
		Micro	0.39	10.08	0.39	9	27.9	4.80	0.05	-0.09	0.04
		Nano	1.42	3.23	1.14	10	11.6	0.69	0.40	0.65**	0.44
		Ultra	<0	0.87	<0	10	9.6	0.67	0.32	0.38*	<0





1086 Fig. S1. Apparent growth rate as a function of the dilution factor in bioassays with full N and 1087 P enrichment in NB and SB station of Biguglia lagoon in (A) November-December 2013, (B) April 2014, and (C) September 2014. From the left to the right, total phytoplankton, 1088 1089 microphytoplankton >20  $\mu$ m, nanophytoplankton between 5 and 20 μm, and 1090 ultraphytoplankton <5  $\mu$ m. The line corresponds to the linear equation  $k(x) = \mu_{max} - gx$ , with k 1091 the apparent growth rate, x the fraction of undiluted plankton,  $\mu_{max}$  (as Y axis intercept) the 1092 maximum growth rate, and g the grazing rate. Blue lines highlight when the linear model 1093 best-fitted the relationship between the apparent growth rates and the dilution factors.

1094	Table S2. Net growth $(\mu_{-N})$ and mortality rates $(g)$ subsequently calculated using enrichment
1095	minus N in the two NB and SB stations of Biguglia lagoon. Values were estimated according
1096	the equations from Andersen et al. (1991). The linear model has been compared to alternative
1097	models by mixed-effect multiple linear regressions of apparent growth rates, based on Chl a
1098	concentration from dilution experiments. The parsimony of the different models was checked
1099	by the AIC criterion. The linear model was only accepted when it presented a delta-AIC <sub>c</sub> , $<2$
1100	with respect to the most parsimonious model (see Methods). AIC <sub>c</sub> -w describe the strength of
1101	explanation by the model of the model. $R^2$ : coefficient of determination. ***p-value <0.001,
1102	** <i>p</i> -value <0.01, * <i>p</i> -value <0.05.

Stations	Samplings	Fraction	$\mu_{\text{-N}}$	8	n	AIC <sub>c</sub>	$\Delta AIC_{c}$	AIC <sub>c</sub> -w	$R_2$
				$(d^{-1})$		-			
NB	Nov - Dec 2013	Total	<0	0.19	8	-5.3	1.75	0.30	0.28
		Micro-	<0	1.70	6	-	-	-	-0.13
		Nano-	0.62	0.29	8	-9	0,00	0.93	0.70**
		Ultra-	<0	1.82	8	24.7	0,00	0.55	0.45*
	April 2014	Total	0.51	0.71	9	-5	0,00	1,00	0.84***
		Micro-	1.59	2.57	8	19.4	0,00	0.93	0.70**
		Nano-	1.95	0.83	10	-6.1	0,00	1,00	0.86***
		Ultra-	-1.59	0.13	8	-	-	-	-0.03
	September 2014	Total	0.86	0.71	10	-1.3	0,00	0.99	0.73**
		Micro-	1.78	0.55	9	10	0.02	0.50	0.33 .
		Nano-	1.77	0.99	10	12.6	0,00	0.93	0.56**
		Ultra-	0.51	0.73	9	1.3	0,00	0.98	0.73**
SB	Nov - Dec 2013	Total	0.26	0.39	10	-9.7	0,00	0.98	0.65**
		Micro-	1.09	2.14	9	35.8	0.38	0.45	0.30.
		Nano-	0.78	0.15	9	-	-	-	-0.08
		Ultra-	1.85	0.82	10	3.1	0,00	0.99	0.69**
	April 2014	Total	0.06	0.03	9	-	-	-	-0.14
		Micro-	-	-	-	-	-	-	-
		Nano-	1.64	0.42	10	-	-	-	0.08
		Ultra-	0.10	0.50	9	4.1	0,00	0.71	0.45*
	September 2014	Total	0.28	1.06	10	1.2	0,00	1.00	0.83***
		Micro-	9.80	1.11	10	8.7	0,00	0.99	0.70**
		Nano-	1.58	1.01	10	-3.6	0,00	1,00	0.87***
		Ultra-	<0	1.25	10	7.1	0,00	1.00	0.78***

1103	Table S3. Net growth $(\mu_{-P})$ and mortality rates $(g)$ subsequently calculated using enrichment
1104	minus P in the two NB and SB stations of Biguglia lagoon. Values were estimated according
1105	the equations from Andersen et al. (1991). The linear model has been compared to alternative
1106	models by mixed-effect multiple linear regressions of apparent growth rates, based on Chl a
1107	concentration from dilution experiments. The parsimony of the different models was checked
1108	by the AIC criterion. The linear model was only accepted when it presented a delta-AIC <sub>c</sub> , $<2$
1109	with respect to the most parsimonious model (see Methods). $AIC_c$ -w describe the strength of
1110	explanation by the model of the model. $R^2$ : coefficient of determination. *** <i>p</i> -value <0.001,
1111	** <i>p</i> -value <0.01, * <i>p</i> -value <0.05.

Stations	Samplings	Fraction	$\mu_{ ext{-P}}$	g	n	AIC <sub>c</sub>	$\Delta AIC_{c}$	AIC <sub>c</sub> -w	$\mathbb{R}^2$
				$(d^{-1})$		-			
NB	Nov - Dec 2013	Total	0.30	0.24	9	-1.9	1.16	0.36	0.24
		Micro-	<0	<0	10	-	-	-	-0.12
		Nano-	1.13	0.44	10	1.9	0,00	0.74	0.40*
		Ultra-	<0	<0	10	-	-	-	-0.12
	April 2014	Total	0.07	0.41	8	1.9	0,00	0.65	0.50*
		Micro-	1.01	1.46	4	-	-	-	0.27
		Nano-	1.54	0.44	8	8.2	1.35	0.34	0.31
		Ultra-	<0	0.53	6	7.4	1.14	0.36	0.71*
	September 2014	Total	1.03	0.24	10	-4.8	0.15	0.48	0.26 .
		Micro-	2.24	0.58	10	12.3	0.01	0.50	0.27
		Nano-	2.13	0.29	10	-5.2	0,00	0.67	0.37*
		Ultra-	0.52	0.31	9	-0.1	0.12	0.49	0.32 .
SB	Nov - Dec 2013	Total	0.53	0.78	10	-0.6	0,00	1.00	0.75***
		Micro-	1.05	2.65	9	25.7	0,00	0.95	0.65**
		Nano-	1.07	0.48	10	3.5	0,00	0.74	0.40*
		Ultra-	2.02	0.83	10	5.2	0,00	0.98	0.65**
	April 2014	Total	0.26	0.57	9	-9.5	0,00	1.00	0.86***
		Micro-	-	-	-	-	-	-	-
		Nano-	1.64	0.52	9	3	0,00	0.83	0.53*
		Ultra-	0.34	0.69	9	-7	0,00	1.00	0.87***
	September 2014	Total	1.34	0.03	9	-	-	-	-0.14
		Micro-	11.72	-0.17	10	-	-	-	-0.06
		Nano-	<0	2.45	8	-	-	-	0.15
		Ultra-	<0	0.19	9	-4.6	1.65	0.30	0.19



1113

1114 Fig. S2. Apparent growth rate as a function of the dilution factor in bioassays with full FH enrichment minus N and minus P, in the NB (A) and SB (B) stations of Biguglia lagoon in 1115 1116 November-December 2013. From the left to the right, total phytoplankton, 1117 microphytoplankton >20 µm, nanophytoplankton between 5 and 20 µm, and 1118 ultraphytoplankton <5  $\mu$ m. The line corresponds to the linear equation  $k(x) = \mu - gx$ , with k 1119 the apparent gross growth rate, x the fraction of undiluted plankton,  $\mu$  (as Y axis intercept) the 1120 gross growth rate, and g the grazing rate. However, we assume that this model can not best-fit 1121 the relationship between apparent growth rate and fraction of undiluted plankton in nutrient 1122 limiting conditions, due to the potential use of alternative resources (Andersen et al., 1991).



1124

1125 Fig. S3. Apparent growth rate as a function of the dilution factor in bioassays with full F 1126 enrichment minus N and minus P, in the NB (A) and SB (B) stations of Biguglia lagoon in 1127 April 2014. From the left to the right, total phytoplankton, microphytoplankton >20 µm 1128 (absent in SB), nanophytoplankton between 5 and 20  $\mu$ m, and ultraphytoplankton <5  $\mu$ m. The line corresponds to the linear equation  $k(x) = \mu - gx$ , with k the apparent gross growth rate, x 1129 1130 the fraction of undiluted plankton,  $\mu$  (as Y axis intercept) the gross growth rate, and g the 1131 grazing rate. However, we assume that this model can not best-fit the relationship between 1132 apparent growth rate and fraction of undiluted plankton in nutrient limiting conditions, due to 1133 the potential use of alternative resources (Andersen et al., 1991).



1135

1136 Fig. S4. Apparent growth rate as a function of the dilution factor in bioassays with full H enrichment minus N and minus P, in the NB (A) and SB (B) stations of Biguglia lagoon in 1137 1138 September 2014. From the left to the right, total phytoplankton, microphytoplankton >20  $\mu$ m, 1139 nanophytoplankton between 5 and 20 µm, and ultraphytoplankton <5 µm. The line corresponds to the linear equation  $k(x) = \mu - gx$ , with k the apparent growth rate, x the 1140 1141 fraction of undiluted plankton,  $\mu$  (as Y axis intercept) the growth rate, and g the grazing rate. 1142 However, we assume that this model can not best-fit the relationship between apparent growth 1143 rate and fraction of undiluted plankton in nutrient limiting conditions, due to the potential use 1144 of alternative resources (Andersen et al., 1991).