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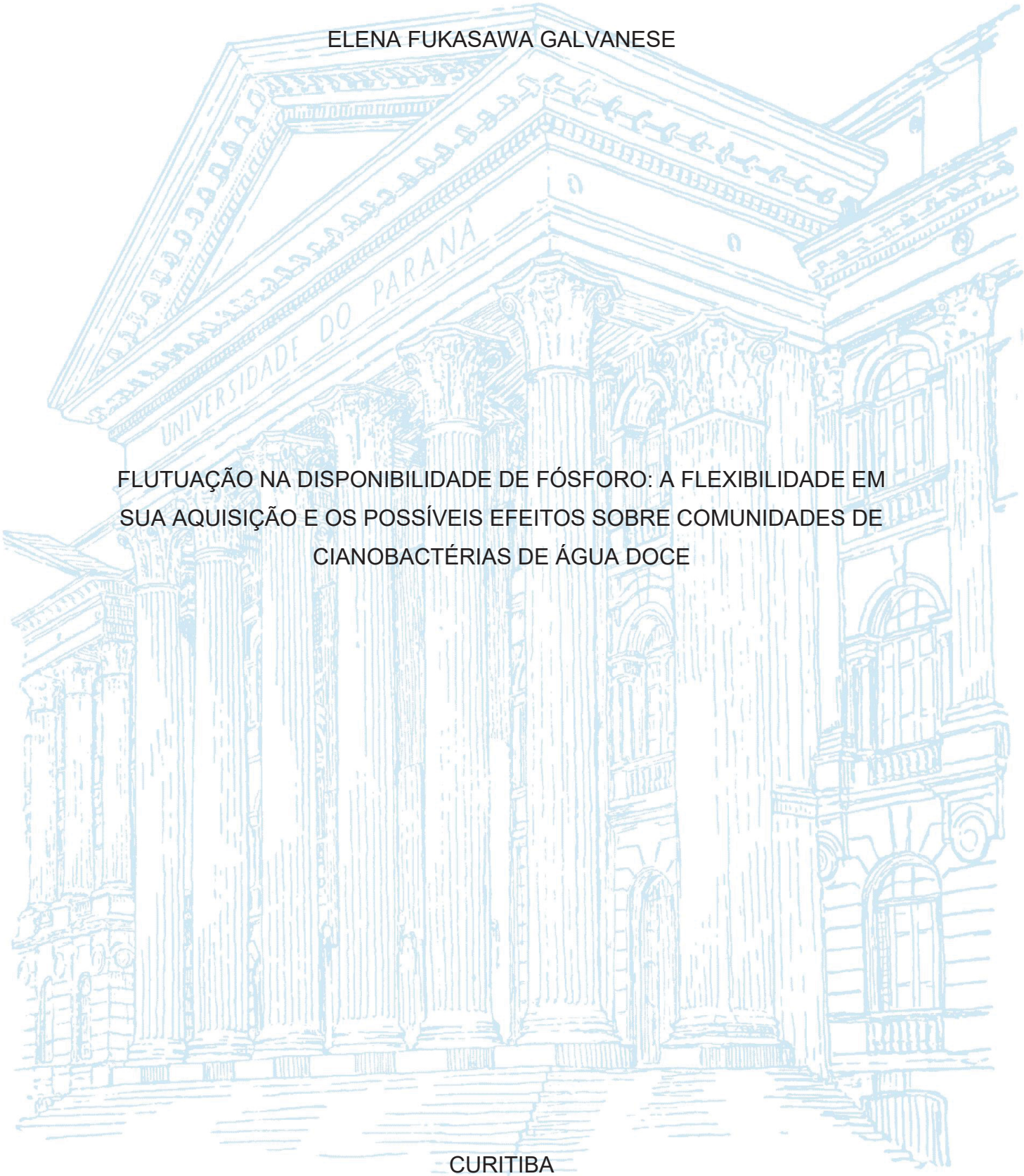
ELENA FUKASAWA GALVANESE



FLUTUAÇÃO NA DISPONIBILIDADE DE FÓSFORO: A FLEXIBILIDADE EM SUA AQUISIÇÃO E OS POSSÍVEIS EFEITOS SOBRE COMUNIDADES DE CIANOBACTÉRIAS DE ÁGUA DOCE

CURITIBA

2021

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FLUTUAÇÃO NA DISPONIBILIDADE DE FÓSFORO: A FLEXIBILIDADE EM SUA AQUISIÇÃO E OS POSSÍVEIS EFEITOS SOBRE COMUNIDADES DE CIANOBACTÉRIAS DE ÁGUA DOCE

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Orientador: Prof. Dr. André Andrian Padial
Co-orientador: Prof. Dr. Luis Aubriot

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A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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ANDRE ANDRIAN PADIAL
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Dedico este singelo trabalho a todos que acreditam e tentam arduamente nos libertar da pré-história da humanidade.

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RESUMO

Competição é considerado o principal mecanismo atuando sobre a composição das comunidades, uma vez que os recursos disponíveis aos organismos são limitados. Entretanto, a relação entre disponibilidade de recurso e crescimento das populações não é sempre diretamente proporcional. Consequentemente, os resultados das interações e os padrões de coexistência entre espécies podem variar, não sempre culminando em exclusão competitiva. Um bom exemplo são as cianobactérias de água doce. Estes organismos podem estar sujeitos à limitação por fósforo (P), e através dos diferentes ajustes do sistema de absorção sob distintos padrões de disponibilidade de P (pulsos), diferentes equilíbrios energéticos podem ser alcançados e a absorção desse nutriente pode cessar em diferentes concentrações externas. Considerando que as concentrações nas quais se interrompe a absorção podem impactar o crescimento e a disponibilidade de P para outras espécies, o objetivo desta tese foi avaliar como diferentes padrões de disponibilidade de P podem afetar a coexistência de populações de cianobactérias de água doce. No capítulo 1, são apresentados os resultados de experimentos com monocultivos e cultivos mistos de *Aphanizomenon gracile* e *Dolichospermum* sp, adicionando P em um pulso (1P) ou dez pulsos (10P). A maior probabilidade de coexistência ocorreu em 10P, provavelmente devido a uma desvantagem de *A. gracile*. *Aphanizomenon gracile* também teve maior atividade de fosfatases alcalinas, assim como 10P em comparação com 1P nos cultivos mistos. No capítulo 2, além de *A. gracile* e *Dolichospermum circinale*, também foi avaliada *Raphidiopsis raciborskii*. Foram aplicados 1P e 10P sob condições saturadas e não saturadas, e foi usado ^{33}P para seguir a concentração de P no meio. Foram encontrados efeitos dos pulsos e ausência de completa exclusão competitiva, e diferenças na atividade da fosfatase alcalina mesmo sob saturação. Em cultivos mistos, *A. gracile* foi favorecida em 1P e *R. raciborskii* em 10P, embora ambas espécies tenderam a ter maiores taxas de crescimento em 1P em monocultivos. Os parâmetros de absorção de P também mudaram de acordo com os pulsos e sugerem maior eficiência em 1P, corroborando estudos anteriores. No capítulo 3, é feita uma breve revisão da teoria de competição e do P enquanto nutriente essencial. E, considerando os resultados experimentais obtidos, é proposto que usar a razão entre as formas orgânica e inorgânica de P seria mais útil para entender as dinâmicas de população e comunidade, a usar apenas a parcela inorgânica.

Palavras-chave: Competição. Ecofisiologia. Fósforo orgânico. Recurso limitante.

ABSTRACT

Competition is thought to be the main driver of community composition, as the resources available to organisms are limited. However, the relationship between resource availability and population growth is not always directly proportional. Consequently, the outcomes of species interactions and patterns of coexistence can vary, not always resulting in competitive exclusion. A good example are freshwater species of cyanobacteria. These organisms might be subjected to phosphorus (P) limitation, and by adjusting differently its phosphorus uptake system when under distinct patterns of P availability (pulses), they can achieve distinct energetic equilibriums and cease their P uptake at different external concentrations. Considering that these different external concentrations at which uptake ceases might impact growth and P availability to other species, the aim of this thesis was to assess how different patterns of P availability can affect the coexistence of freshwater Cyanobacteria populations. In chapter 1, are presented results of experiments with mono and mixed cultures of *Aphanizomenon gracile* and *Dolichospermum* sp, adding P in single (1P) or ten pulses (10P). Higher probability of coexistence occurred in 10P, probably due a disadvantage of *A. gracile*. *Aphanizomenon gracile* also had higher alkaline phosphatase activity, and the same occurred in 10P in mixed cultures in comparison to 1P. In chapter 2, besides *A. gracile* and *Dolichospermum circinale*, it was also assessed *Raphidiopsis raciborskii*. One and 10P were applied under saturated and non-saturated conditions, and ^{33}P was used to follow the P concentration in the medium. Effects of pulses and absence of complete species exclusion were found, and also different alkaline phosphatase activity even under saturated condition. In mixed cultures, *A. gracile* was favored in 1P and *R. raciborskii* in 10P, although both species tended to have higher growth rates in 1P in monocultures. The P uptake parameters also changed according P pulses and suggest higher efficiency in 1P, corroborating previous studies. In chapter 3, the competition theory and the approach of P as an essential resource are briefly reviewed. And, taking into consideration the experimental results obtained, it is proposed that the use of organic P:inorganic P ratio would be more useful to understand population and community dynamics, than to use only inorganic P.

Key-words: Competition. Ecophysiology. Organic phosphorus. Limiting resource.

SUMÁRIO

APRESENTAÇÃO

A diversidade biológica ainda é um surpreendente fato para os ecólogos. A surpresa decorre de um outro fato simples: os recursos disponíveis às populações são limitados**.** Logo**,** a força das interações competitivas seria forte o suficiente para limitar o número de espécies dependentes dos mesmos recursos. Essa “luta pela existência” (sensu Charles Robert Darwin) levando à permanência do competidor superior é o que conhecemos como princípio da exclusão competitiva (GAUSE, 1934), e junto com ela vem a premissa de que quanto menos similares os organismos, maior sua probabilidade de coexistência. De fato, muitos estudos evidenciaram a exclusão competitiva em experimentos (e.g. LITCHMAN, 2003)**,** e também a partir da distribuição geográfica de espécies (MACARTHUR, 1972). Porém, outros estudos mostraram que a similaridade entre espécies foi positivamente relacionada à sua probabilidade de coexistência (NARWANI et al., 2017) ou ainda, que a similaridade no uso de recursos variou no tempo (DE LEÓN et al., 2014). Ainda que processos de maior escala espacial e temporal, como dispersão (GLEASON, 1926; LEIBOLD et al., 2004) e adaptação (VELLEND, 2010; BERNHARDT; KRATINA; et al., 2020), possam ser importantes para explicar a presença das espécies numa determinada área, ambos processos são dependentes de populações estabelecidas em uma certa área. Assim, o entendimento da diversidade biológica necessariamente passa também pelo entendimento da relação entre populações compartilhando recursos.

Talvez o mais emblemático exemplo da inesperada alta diversidade é descrita por Hutchinson (1961), o chamado ‘Paradoxo do Plâncton’. Para Hutchinson (1961, pg 137) “o problema que é apresentado pelo fitoplâncton é

essencialmente como é possível para um número de espécies coexistir em um ambiente isotópico ou desestruturado todas competindo pelos mesmos tipos de recursos. O problema é particularmente agudo porque há adequada evidência de experimentos de enriquecimento que águas naturais, pelo menos no verão, apresentam um ambiente de impressionante deficiência de nutrientes, assim a competição é provavelmente extremamente severa". Hutchinson (1961) descarta que as hipóteses de não equilíbrio e heterogeneidade espacial explicam essa alta diversidade. A primeira porque a extinção de espécies seria um processo importante e o registro fóssil mostra uma situação oposta, de co-ocorrência de mais de uma espécie de diatomácea em um ambiente razoavelmente estável; e no caso da segunda, porque à escala espacial dos organismos haveria homogeneidade das condições na região do epilímnio (i.e. camada mais próxima à superfície, estabelecida pela diferença de temperatura com as camadas mais profundas).

Partindo da relação entre disponibilidade de recursos e competição, diversos estudos já mostraram experimentalmente que a diferença na aquisição de recursos, quantitativamente e qualitativamente, de fato pode explicar padrões de coexistência ou co-ocorrência. No primeiro caso, espera-se que a espécie que possui a menor demanda por um recurso compartilhado ou uma aquisição mais rápida, seja competitivamente superior (TILMAN, 1977). Assim, os padrões de coexistência e ausência de determinada espécie, seriam explicados pela disponibilidade dos recursos compartilhados e as características das espécies. No segundo caso, i.e. uso qualitativamente diferente de recursos, o uso pelas espécies de diferentes parcelas (no caso da luz, diferentes espectros de absorção da luz; STOMP et al., 2007) ou formas (no caso do fósforo, formas

inorgânica e orgânica; SCHOFFELEN et al., 2018) dos recursos, explicaria sua coexistência. Uma terceira situação, misturando os dois casos, é quando espécies potencialmente competidoras estão limitadas por recursos diferentes. Nesse cenário intermediário, cada espécie está limitada pelo recurso em que é o competidor superior, e assim a coexistência é possível (TILMAN, 1977). De modo geral, estudos analisando a superioridade competitiva na aquisição de nutrientes por uma espécie utilizam as equações de Monod ou Droop, as quais usam o arcabouço de reações enzimáticas (WAGNER; FALKNER, 2001). E, os parâmetros calculados para as espécies isoladamente são usados para prever os resultados de cenários com mistura de espécies.

Considerando que a disponibilidade de recursos varia porque o ambiente está continuamente flutuando, e é a disponibilidade de recursos que dirige a força da interação competitiva entre espécies, é possível considerar que essencialmente o ambiente modula a interação competitiva entre espécies. De fato, isso não é uma ideia nova. Hutchinson (1953) discutiu o papel que distúrbios podem ter em evitar que a exclusão competitiva ocorra, a depender da relação entre a frequência das flutuações e o tempo de geração das espécies. Mais recentemente, Giordano (2013) também discutiu a relação entre e a duração de um distúrbio ou estresse, e o tempo de geração de uma população. Porém, Giordano (2013) discute essa relação levando em conta as respostas dos organismos, que podem ser homeostase ou aclimatação. A opção por uma ou outra resposta está relacionada aos custos metabólicos e ao máximo aproveitamento que o organismo pode ter em sua aptidão. Nesse sentido, a antecipação das perturbações, e.g. mudanças sazonais, tem um amplo e

reconhecido papel na preparação dos organismos (BERNHARDT; O'CONNOR; et al., 2020).

Os efeitos das flutuações ambientais e a resposta fisiológica a elas é especialmente importante em organismos sésseis e microrganismos, devido à reduzida ou mesmo ausência de mobilidade. Um interessante grupo biológico para abordar essas questões são as cianobactérias de água doce, que podem ter respostas bem variadas à limitação de nutrientes-chave como fósforo (P). Como já apontado por Hutchinson (1961), em ambientes aquáticos há séria limitação por nutrientes, ao mesmo tempo que processos como mistura de água de diferentes profundidades, e rompimento de células (por morte celular e herbivoria) podem disponibilizar P e outros nutrientes aos microrganismos. Então, durante a duração de seu ciclo de vida, microrganismos podem passar por cenários de limitação e frequentes, porém imprevisíveis, *inputs* de P. Claro, uma vez sob limitação, é esperado que a aquisição do nutriente seja muito rápida, podendo chegar a poucos minutos no caso desses organismos (e.g. RIGLER, 1956). Porém, a aquisição de P não pode seguir indefinidamente, porque não só há um custo cada vez maior para o transporte de P conforme sua concentração ambiental diminui, como também a formação de polifosfatos (i.e. cadeias longas de fosfato) dentro das células não pode ser infinita. Os organismos então alcançam um equilíbrio, em que se integram as limitações para adquirir e acumular P, e sua demanda futura de crescimento e manutenção.

A capacidade de coordenação de diferentes sistemas internos com a concentração externa de P pode ser ainda mais complexa e flexível. Isso porque esse equilíbrio, que é representado pela concentração externa constante de P, pode variar. Além disso, essa variação não é apenas dependente da

concentração de P, mas também da forma de sua oscilação e do tamanho da população. Por exemplo, a adição de uma mesma quantidade de P em uma única vez ou em múltiplas adições em intervalos regulares poderá resultar em diferentes equilíbrios (FALKNER et al., 2006). No mesmo sentido, populações com diferentes tamanhos populacionais, porém supridas como a mesma quantidade proporcional de P, poderão culminar em diferentes limiares (i.e. concentração na qual não há mais aquisição de P; WAGNER et al., 2000). A implicação dessa flexibilidade não é só para os organismos individualmente, mas também para o restante da comunidade. Se alguns organismos podem cessar a aquisição de P a uma concentração mais alta, haverá maior disponibilidade desse nutriente a outros organismos. Assim, como sugerido por alguns autores, alguns ajustes e equilíbrios alcançados pelos organismos poderiam favorecer a coexistência de espécies de fitoplâncton (WAGNER et al., 2000 e AUBRIOT et al., 2011).

Nesta tese de doutoramento, busquei entender como o ambiente pode modular interações competitivas e, por conseguinte, a coexistência. Como Hutchinson (1961) e Giordano (2013), considerei os efeitos das flutuações ambientais sobre os organismos, mas diferentemente desses autores, busquei entender como diferentes respostas fisiológicas, resultantes de flutuações de recursos, podem interferir na interação entre espécies. O objetivo geral foi comparar diferentes padrões de disponibilidade de P sobre as espécies, individualmente, e sobre as interações entre populações de cianobactérias de água doce. Dessa forma, foi possível avaliar como pulsos de recursos modulam a coexistência de espécies. A tese está dividida em três capítulos em formato de artigos científicos em inglês, sendo os dois primeiros capítulos fenomenológicos,

mas usando diferentes níveis de descrição do fenômeno, e o último capítulo uma crítica teórica e síntese.

No capítulo 1, as espécies *Aphanizomenon gracile* Lemmermann 1907 e *Dolichospermum* sp (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, 2009 foram utilizadas, após serem isoladas do mesmo lago. Sob limitação de P, monocultivos foram avaliados em situação de redução do P total concomitante com redução da biomassa total, e em situação de não redução do P total e da biomassa. Cultivos mistos foram avaliados em relação à adição de P em uma única vez ou dividida em dez adições (i.e. pulsos), ambas adições totalizando a mesma quantidade de P.

No capítulo 2, é apresentado o resultado da colaboração com o laboratório da Prof Dra Michele Burford do Australian Rivers Institute, Griffith University, na Austrália. Nos experimentos realizados, as espécies *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, 2009, *A. gracile* e *Raphidiopsis raciborskii* (Woloszynska) Aguilera & al. 2018 foram analisadas, em monocultivos e cultivos mistos, sob situações de saturação e não saturação de P. Em ambos casos, P foi adicionado numa única vez ou dividido em dez adições regulares. Como foi utilizado o isótopo ^{33}P, também foi avaliada a quantidade remanescente de P no meio e taxas de absorção quando a adição de P não foi saturante.

Por fim, no capítulo 3, é feita uma breve revisão dos conceitos fundamentais sobre competição e uso de recursos, e sobre a aquisição de P por microrganismos aquáticos. E, a partir da analogia entre a aquisição de luz e de P, propomos uma abordagem teoricamente mais ampla do P enquanto nutriente limitante às populações. Apontamos que considerar ao mesmo tempo as

múltiplas formas de P que os organismos podem adquirir pode ajudar a aprofundar o conhecimento sobre as espécies e sobre as interações entre as espécies.

CAPÍTULO 1

CAN RESOURCE PULSES SHAPE FRESHWATER CYANOBACTERIA COMMUNITIES?

Can resource pulses shape freshwater Cyanobacteria communities?

Elena Fukasawa Galvanese, André Andrian Padial, Luis Aubriot

1.1 ABSTRACT

Natural cyanobacterial populations undergo phosphorus (P) limitation frequently. Given P is a macronutrient, there are several studies aimed to assess growth and species interaction under P limitation. In this study, we investigated the role of P concentration and P pulses on previously limited *Aphanizomenon gracile* and *Dolichospermum* sp. populations, and we aimed to test whether there are: a) a directly proportional relationship between growth rates and P concentration and, b) differences in the outcomes of mixed cultures under P pulses. Using nitrogen-free batch cultures of two populations isolated from the same water sample of a lake, we conducted experiments submitting monocultures to 0.25 mgP L^{-1} and 0.5 mgP L^{-1}, and reducing half the initial biomass and light, accordingly. Also, we mixed both cultures in unequal initial abundance and added P in one (1P) or ten pulses (10P), in either case adding a total of 0.5 mgP L^{-1}. We followed the growth using light attenuation, collected samples to count organisms, and measured initial and final soluble reactive phosphorus (SRP), total nitrogen (TN) and particulate P (PP). Alkaline phosphatase activity (APA) was measured as a proxy of organic P usage. Under 0.25 mgP L^{-1}, the growth rates were higher than at 0.5 mgP L^{-1}, as also was APA. The no directly proportional relationship of P concentration and growth rate reinforces the flexibility of P use, which might involve the alleviation of inorganic P limitation by organic P usage. In mixed cultures, APA increased along the time, and difference between P pulses was found in *Dolichospermum* sp. dominated cultures, but it was not related to SRP. Total nitrogen did not vary among P treatments in both experiments, and it might indicate the use of organic P to attend the N fixing demand, but not in direct response to SRP. No complete species exclusion occurred, and species reached different final abundances depending on 1P or 10P. However, *Dolichospermum* sp. was less able to invade *A. gracile* dominated cultures, indicating that factors not directly related to resource acquisition played a role in this interaction.

Keywords: alkaline phosphatase activity, coexistence, competition, P uptake

1.2 INTRODUCTION

The patterns in diversity have been the focus of Ecologists for many generations, and it is still one of the main questions in the field. Even though there are many theories to explain these patterns (VELLEND, 2010), consensus exists in that the important processes governing diversity are selection, dispersal, speciation and drift (VELLEND, 2010). At local scales, where field samplings and individual interactions take place, there is no definitive consensus about the mechanisms that maintain the diversity. Both neutral and niche related hypothesis are probably relevant to explain species coexistence (LEIBOLD; MCPEEK, 2006).

The niche theory, synthesized in the seminal work of Hutchinson (1957), predicts at least one different dimension in the n-dimensional hypervolume to species coexistence be possible in a certain place and time. The premise is that, if the interspecific competition by the limiting resource is not controlled, the species will not be able to coexist (i.e. competitive exclusion principle; HARDIN, 1960). In summary, studies concerning the niche and competitive interactions have found that: the niche difference was determinant to species diversity (LEVINE; HILLERISLAMBERS, 2009); the niche partitioning was variable in time and space (MATICH et al., 2017); the niche difference in resource use was variable in time (DE LEÓN et al., 2014); the predicted competitive exclusion based on single populations characteristics was the opposite when the populations were grown together (DE NOBEL et al., 1997) and yet, the negative co-occurrences (i.e. absence of one species when the other is present) were not associated with competition (BRAZEAU; SCHAMP, 2019). These findings exemplify the many ways individuals can acquire and use resources, and the complexity of species interactions. Thus, understanding a certain observed pattern requires analyzing different processes at different scales.

Regarding the spatial and temporal changes in resource use, aquatic autotrophic microbial communities are an interesting biological model. Nutrient limitation by phytoplankton might occur frequently, for example due to the stratification of water column in summer and(or) periods of high water residence time. Processes that can make available nutrients to organisms include mixing of water column, rainfall, sudden runoff, cell lysis and decomposition of organic matter. Therefore, microorganisms under nutrient limitation can be subjected to

rapid nutrients fluctuations due to the sudden rise in ambient concentration, when under active nutrient uptake (RIGLER, 1956). In this regard, Cyanobacteria is a recognized group that take advantage of phosphorus fluctuations, besides being able to grow and even dominate under very low phosphate concentrations (POSSELT; BURFORD and SHAW, 2009). It has been demonstrated that this group, in order to sustain growth under such fluctuating conditions, regulate the P uptake according to nutrient availability and during ambient short-term P pulses (FALKNER; WAGNER and SMALL, 1995). Moreover, this adjustment to P concentration is not a direct function of the ambient concentration: when organisms are P starved and subsequently are exposed to higher concentration of external P, the cells will not necessarily exhaust the external P (AUBRIOT; BONILLA, 2012). The capacity to adjust the P uptake kinetics depends on the time required by cells to perceive the change in external nutrient concentration (exposure time) and the time to react accordingly (reaction time; PLAETZER et al., 2005; AUBRIOT; BONILLA and FALKNER, 2011; AUBRIOT; BONILLA, 2012). This is important because the cells have a limited space, and would collapse if a given amount of P is constantly taken up at maximum velocity without any anticipatory mechanism (WAGNER; FALKNER, 2001). Besides that, the constant uptake could change the homeostasis of the cells, disrupting the balance of the growth (WAGNER; FALKNER, 2001). In this sense, two situations could provide enough exposure time to ambient P to allow the uptake system to adjust its kinetic and energetic properties: the slower reduction of external P during uptake due to low population size (FALKNER; PRIEWASSER and FALKNER, 2006), or the combination between pulse concentration and a given uptake rate (AUBRIOT; BONILLA, 2012). In this last case, both one or multiple pulses could be enough to achieve sufficient exposure time to drive changes in P uptake parameters (AUBRIOT; BONILLA and FALKNER, 2011). And, the resulting new equilibrium could also impact positively the growth rate (AMARAL; BONILLA and AUBRIOT, 2014).

Another mechanism to acquire P is the release of phosphate from organic compounds, mainly resultant from alkaline phosphatase activity (APA; JANSSON; OLSSON and PETTERSSON, 1988). In general, under lower phosphate concentration, APA is increased (CHRÓST; OVERBECK, 1987) and therefore it is used to indicate P limitation. However, the independent relationship

between soluble reactive phosphorus and APA (HINO, 1988), and high APA under P sufficiency (ISTVÁNOVICS et al., 1992), rises controversy about its use for such end and questions of to what extent organic compounds would be an important P source to organisms (HEATH, 1986).

Considering the above-mentioned flexibility in P uptake, if each organism of a given phytoplankton community is capable of performing short-term adjustments of uptake kinetics to the same shared ambient P levels, then the whole community could achieve a similar adjustment, and the community could work as a meta-organism (WAGNER; FALKNER and FALKNER, 1995; AUBRIOT; BONILLA and FALKNER, 2011). In this sense, it is plausible to assume that the coexistence and species diversity in phytoplankton community could be better understood considering the flexible physiology of organisms, and the environmental experiences modulating these physiological responses along the time. Using Cyanobacteria as a biological model, in the present work we intended to evaluate: a) the directly proportional dependence of growth rate on nutrient concentration and b) the possible outcomes of different P pulses to mixed cultures of two diazotrophic (N_2 fixers) cyanobacterial species isolated from the same water sample. Experimentally, we manipulated population densities and nutrient concentration and expected i) higher growth rate under concomitant lower P concentration and reduced population size; and ii) no species exclusion because in at least one of treatments an efficient uptake would be induced and the coexistence be favored.

1.3 MATERIALS AND METHODS

1.3.1 Species

Aphanizomenon gracile Lemmermann 1907 and *Dolichospermum* sp. (Ralfs ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, 2009 were isolated from the same water sample obtained from Lake Javier, Uruguay (34°51'S, 56°20'W, Fig. S1). Lake Javier is a meso-eutrophic lake (BONILLA et al., 2016; AGUILERA et al., 2017), known by the cyanobacterial dominance of the phytoplankton community (VIDAL; KRUK, 2008; BONILLA et al., 2016). During the isolation process, the cultures were kept within Erlenmeyer glasses of 250 mL with modified BG-11 medium, without N (-N), 1 mgP L^{-1}, and ferric ammonium citrate

reduced to sixty percent to avoid P precipitation by excess of Fe. The light:dark photoperiod was 16h:8h under light intensity of 80 µmol photon m^{-2} s^{-1} and temperature of 26 ±1 °C.

Both cultures were non-axenic and periodically checked at the microscope to ensure that cultures were monospecific before the beginning of the experiments. The toxin anatoxin-a was detected in *A. gracile* cultures (data not published) as well as strikingly geosmin production (organoleptic testing) by *Dolichospermum* sp.

1.3.2 Experimental design

We performed two experiments in batch cultures (Fig. 1). The first was designed to assess the relationship between the nutrient concentration and population abundance on growth rate in monocultures (objective a); and the second to assess the effect of the pattern of P availability on coexistence probability in mixed cultures (objective b). In the two experiments we used cultures from the same flasks and the experiments were run in parallel. Twenty-seven days before the start of the first experiment, the cultures were acclimated to constant light. Ten days before the first experiment, the total P content in the cultures was reduced to 0.5 mg L^{-1}. Three days before the start of the first experiment the soluble reactive phosphorus (SRP) concentrations in the culture medium were low (0.011 and 0.015 mg L^{-1} in *A. gracile* and *Dolichospermum* sp, respectively). The rapid decrease of soluble reactive phosphorus after addition is another way to confirm the P limited condition, because it indicates the activity of high affinity uptake systems (WAGNER, FALKNER and FALKNER, 1995). In this regard, approximately 60 min after the start of the first experiment, the SRP in the medium of half diluted cultures (see below) was as low as 0.022 mg L^{-1}.

In the first experiment (hereafter “monocultures experiment”), the cultures were submitted to two treatments: not diluted and half P diluted. First, in the not diluted treatment, we renewed the medium with 0.5 mg P L^{-1} (optical densities at 750 nm were *A. gracile* - 0.146, *Dolichospermum* sp. - 0.185), and the cultures were distributed in three replicates of 50 mL each placed in 250 mL culture flasks (Greiner) and incubated at 80 µmol photon m^{-2} s^{-1}. The half diluted treatment was performed with the same original cultures but half diluted with BG-

166 11 -N without P (-P), achieving the final total P concentration of 0.25 mgP L^{-1}
167 (optical densities at 750 nm were *A. gracile* – 0.081, *Dolichospermum* sp. –
168 0.081). The three replicates of 50 mL culture were incubated at a light intensity
169 of 40 µmol photon m^{-2} s^{-1}. The average room temperature was 25.8 ±1 °C, and
170 the experiment duration was 10 days. Every day cultures were gently shaken and
171 repositioned randomly in the experimental trays.
172 In the second experiment (hereafter “mixed experiment”), we performed
173 a factorial combination of two initial biomass proportions of each species and two
174 patterns of P availability, but achieving the same total P final concentration of 0.5
175 mg L^{-1}. The initial biomass proportion of each species varied by mixing different
176 volumes of each monoculture, changing the dominant species. We varied the
177 initial dominance instead of mixing equal culture volumes because, according to
178 the theory of coexistence (see CHESSON, 2000, 2018), one pivotal assumption
179 to the stable coexistence is the capability of species recovery from low
180 abundance (i.e. when rare) in presence of all other species in equilibrium (i.e.
181 invasibility criterion; CHESSON, 2000; SIEPIELSKI; MCPEEK, 2010). The *A.*
182 *gracile* dominant treatment (‘Dominance *A. gracile*‘) was achieved mixing 300 mL
183 of *A. gracile* monoculture with 100 mL of *Dolichospermum* sp. The dominance of
184 *Dolichospermum* sp. (‘Dominance *Dolichospermum* sp‘) was achieved mixing
185 250 mL of *Dolichospermum* sp. and 100 mL of *A. gracile* culture. The volume of
186 350 mL in both cultures was achieved removing 50 mL of *A. gracile* dominant
187 culture. Then, 175 mL of each mixed culture was subdivided in two flasks and
188 fresh BG-11 -N -P medium was added to achieve the final volume of 400 mL (final
189 optical densities at 750nm were *A. gracile* dominant – 0.134, *Dolichospermum*
190 sp. dominant – 0.138). The patterns of P availability were one (1P) of 0.5 mgP L^{-1}
191 or ten pulses (10P) of 0.05 mgP L^{-1} added every six minutes, both totaling final
192 P concentration of 0.5 mg L^{-1}, as mentioned before.
193 After the pulses, 100 mL of the cultures were incubated in triplicates in
194 cell culture flasks of 250 mL. The light intensity was 80 µmol photon m^{-2} s^{-1} and
195 the average room temperature 25.8±1 °C. This experiment lasted for 13 days,
196 and as for monocultures, every day the flasks were gently shaken and randomly
197 repositioned in the experimental trays.
198 To follow the growth, we daily measured light attenuation through the
199 culture Greiner flasks (flat walls) with a LI-COR radiometer (model LI-250A,

equipped with LI-192 Underwater Quantum Sensor) in a dark box equipped with a light source irradiance of approximately 234.25 µmol photon m^{-2} s^{-1}. As the attenuation is inversely proportional to the biomass, the attenuation data are shown as $attenuation^{-1}$. This data was also used to calculate the maximum growth rates of each triplicate and treatment using the equation (WOOD; EVERROAD and WINGARD, 2005):

$$\text{Growth rate} = \frac{\ln (\text{attenuation}^{-1})_{\text{tf}} - \ln (\text{attenuation}^{-1})_{\text{ti}}}{\text{tf} - \text{ti}},$$

where “tf“ and “ti” the final and initial days under exponential growth, respectively.

1.3.3 P and N analysis

We quantified the SRP, total nitrogen (TN) and particulate P (PP) in the medium at the beginning of the first experiment. These nutrients and fractions were also quantified at the end of the experiment. In mixed cultures, at days zero, five and 13, SRP, PP and TN were quantified.

We measured SRP using the molybdate blue method proposed by Murphy and Riley (1962); for PP, samples were filtered in GF/F filters and then analyzed according to the digestion method of Valderrama (1981) and determined as SRP according to Murphy and Riley (1962); for TN, whole samples were used and also followed digestion method of Valderrama (1981) and nitrate was measured according to Müller and Wiedemann (1955).

1.3.4 Alkaline phosphatase activity (APA)

To evaluate the use of organic P by the cultures we measured the alkaline phosphatase activity following the protocol of fluorogenic technique proposed by Ammerman (1993). The method uses 4-Methylumbelliferyl Phosphate (4-MUP) as substrate for the activity of alkaline phosphatases, which has lower fluorescence than its form without the P. Therefore, as the alkaline phosphatases acts upon the 4-MUP substrate, the fluorescence signal increases. We diluted the 4-MUP ‘ready to use solution’ (M3168, Sigma-Aldrich) with deionized water to achieve a solution at 100 µM. This solution was added to the samples to achieve a final concentration of 100nM. Immediately after its addition, 3 ml was

distributed in 4 ml cuvette and 1 mL of borate buffer was added, as the maximum fluorescence of MUF is under pH ≥ 10.8. The fluorescence reading was done with a Trilogy® fluorometer Turner Designs using the CDOM/Ammonium module (λexcitation = 350/80 nm, λemission = 410-450 nm). The fluorescence signal was recorded every minute, for at least eight minutes. In both experiments, the APA was measured at first day of the experiments. Also, it was measured in the final day of monoculture experiments, and fifth and final days of mixed culture experiments. The APA was measured in all replicates.

The APA was expressed by the maximum rate of MUF formation in the samples. We calculated this rate by first selecting the linear slope of the fluorescence curve. We used the final data of the linear slope to estimate the MUF concentration applying the equation derived from the calibration curve with MUF (M1381, Sigma-Aldrich). This MUF concentration was then divided by the minutes of the linear slope and afterwards normalized by biovolume.

1.3.5 Counts and calculation of biovolume

Aliquots of each replicate were fixed with acid Lugol and counted with an optic microscope (Opton) to estimate the population size, using an acrylic Sedgwick-Rafter chamber (minimum of 100 trichomes counted). For data presenting inconsistency between OD750nm and number of trichomes, linear regressions between OD750nm and number of trichomes were used to correct the data (Fig. S2). This was made only for *Dolichospermum* sp. in monocultures experiments. Measurements of maximum linear dimension and width were performed using an inverted microscope (IX70, Olympus). In each triplicate, ten individuals were measured. In samples from the beginning of experiments, 30 individuals were measured. These data were used to calculate the biovolume according to HILLEBRAND et al. (1999). In one replicate of *Dolichospermum* sp. of half diluted treatment (i.e. 0.25 mgP L^{-1}), adequate counts and measurements were not possible due disruption of trichomes, though the cells were intact. Therefore, data normalized by biovolume (APA, TN and PP) did not include this replicate, and the average included only the other two replicates.

1.3.6 Data analysis

265 To assessed the effects of P treatments on the cultures, we compared the growth
266 rates, APA, SRP, PP and TN between treatments using ANOVA with
267 permutations (functions 'aov' and 'lmp' from packages stats and lmPerm
268 (WHEELER; TORCHIANO, 2016), respectively). Furthermore, we assessed the
269 relationship between APA and dissolved P using also linear regression with
270 permutations. All statistical analysis and graphs were performed in R software,
271 version 4.0.2 (R Core Team 2021).

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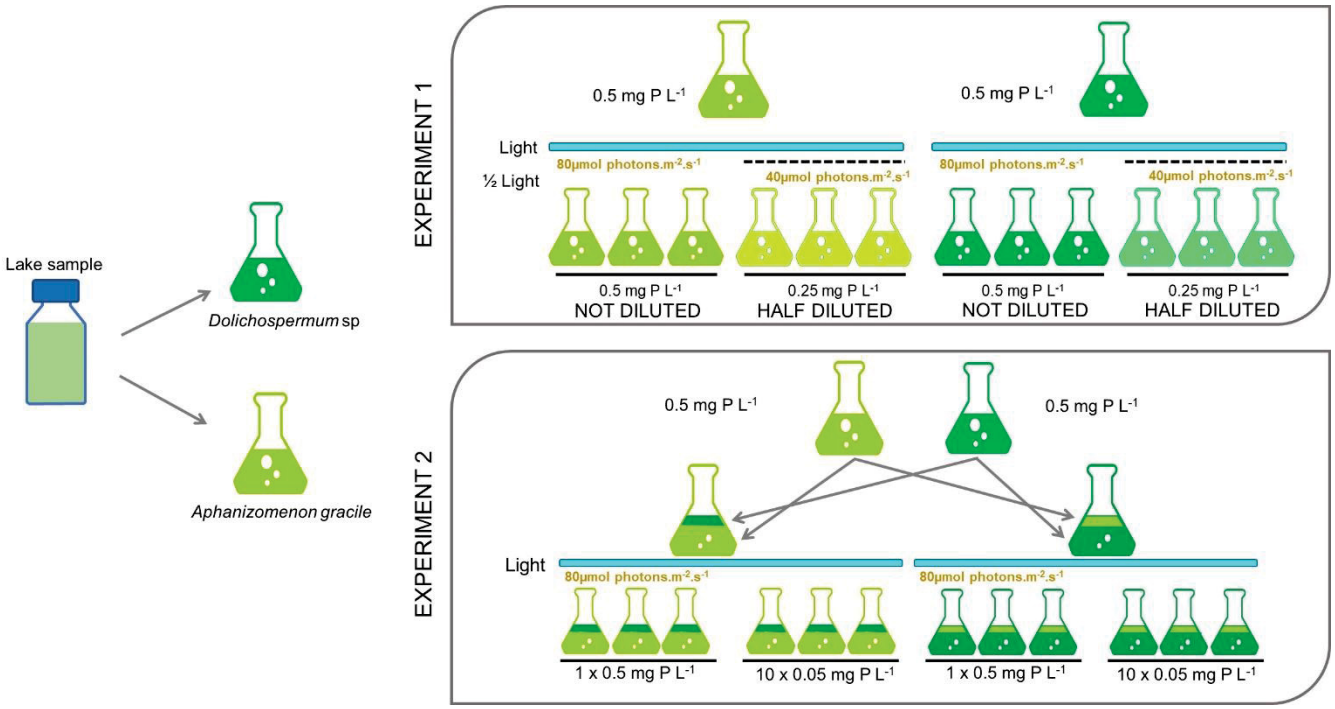

Figure 1 Experimental design of the experiments conducted. Experiment 1 – monocultures experiment, experiment 2 – mixed cultures experiment.

1.4 RESULTS

1.4.1 Growth rates in monocultures

The growth rate in not diluted treatment was lower than in half diluted, in both species (*A. gracile* – $F_{1,4} = 64.82$, $P = 0.001$; *Dolichospermum* sp. – $F_{1,4} = 8.984$, $P = 0.04$). The average growth rates of *A. gracile* were 0.022±0.002 d^{-1} and 0.104±0.017 d^{-1}, and 0.047±0.017 d^{-1} and 0.09±0.018 d^{-1}, for *Dolichospermum* sp., in not diluted and half diluted treatments, respectively. There were no growth rate differences between species under the same treatment. According to attenuation data, the growth curve in half diluted treatment followed the expected pattern (i.e. lag, exponential, stationary and decay), except by the absence of a stationary phase in *A. gracile* (Fig. 2).

The biovolume *A. gracile* increased, in average, from 50.93 to 165.80 mm^3 L^{-1} in not diluted treatment (3.25 times increase), and from 29.30 to 71.54 mm^3 L^{-1} in half diluted treatment (2.44 times increase). In number of individuals, the final population of *A. gracile* not diluted treatment was 2.01 times higher than the initial, and 2.54 times enhanced in half diluted. In *Dolichospermum* sp. cultures, the average biovolume enhancement was 108.13 to 147.65 mm^3 L^{-1} in not diluted (1.37 times), and from 41.87 to 93.18 mm^3 L^{-1} in half diluted treatment (2.22 times increase). Regarding the population changes, it enhanced 1.79 times during the experiment in not diluted and 2.07 in half diluted treatment.

At day 10, some contamination of *Dolichospermum* sp. with trichomes of *A. gracile* in replicates from the not diluted treatment was detected. The contamination represented <0.7 % of total biovolume, which confirms that the biomass was residual due recent isolation, and any effect on the results is likely negligible.

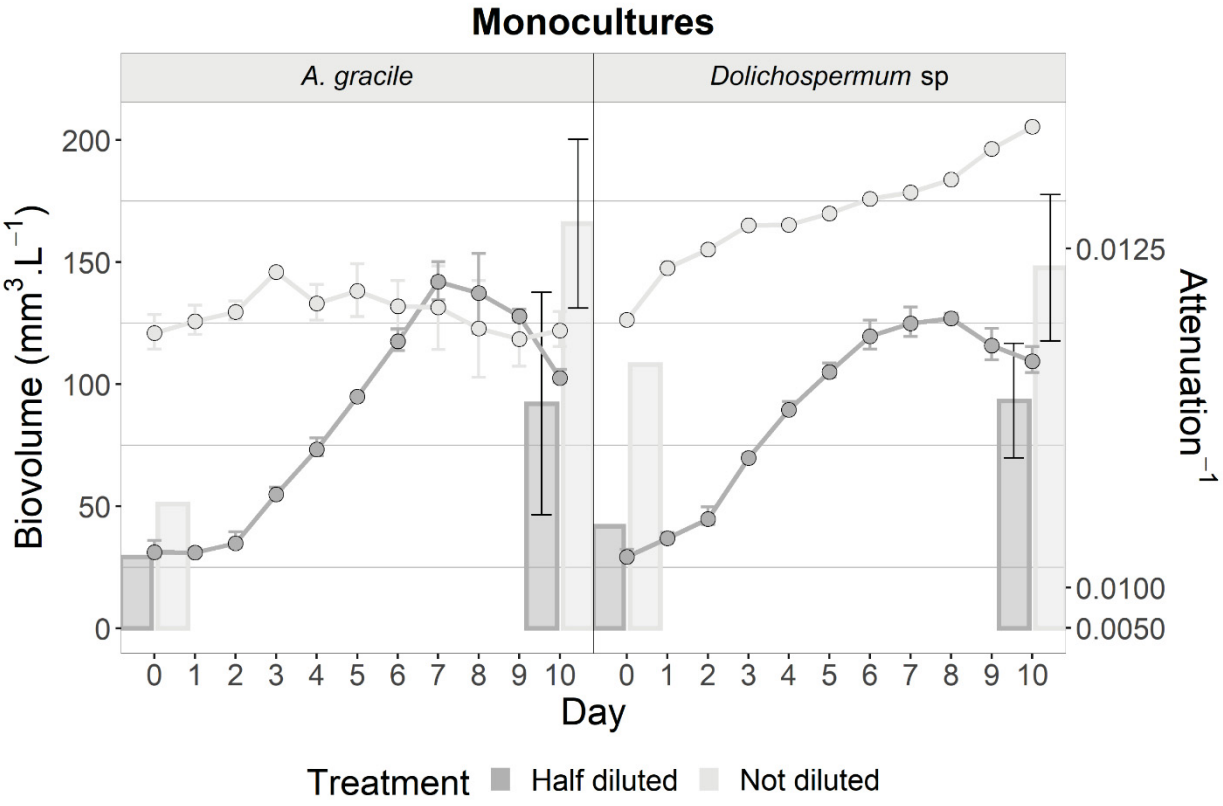

Figure 2 Growth curve and initial and final biovolumes of monocultures experiment. Growth curves were obtained from light attenuation data. Dots and bars are averages from triplicates. Standard deviation bars are also shown.

1.4.2 Growth rates in mixed cultures

The growth rates in cultures dominated by *A. gracile* were 0.091±0.009 d^{-1} and 0.087±0.016 d^{-1}, in 1P and 10P treatments respectively. In the case of *Dolichospermum* sp. dominated cultures, the growth rates were 0.117±0.007 d^{-1} and 0.111±0.017 d^{-1} (1P and 10P, respectively; Fig. 3). The global growth rates did not differ when contrasting the P pulses treatments, only when contrasting the cultures in 1P treatment ($F_{1,4}$ = 14.77, P = 0.0184), and in this case the cultures dominated by *Dolichospermum* sp. had higher growth rate.

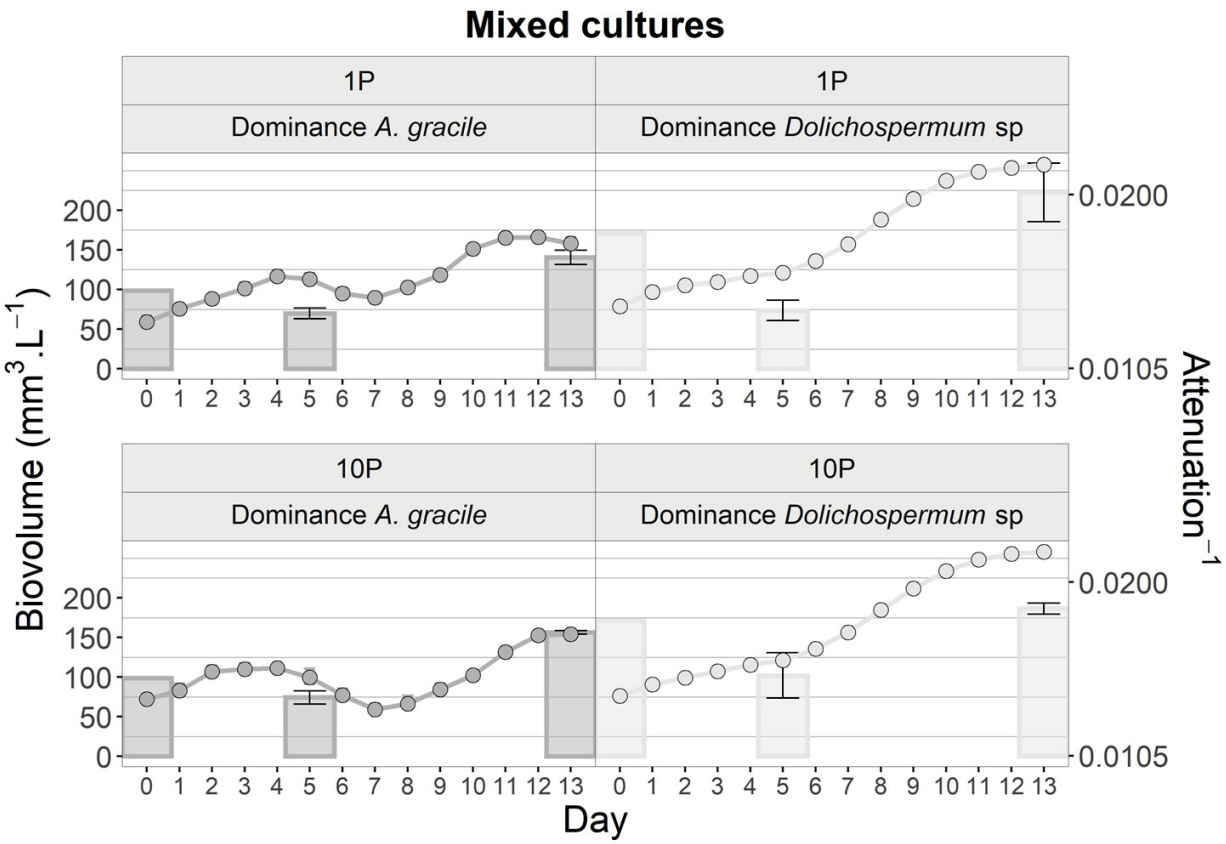

Figure 3 Growth curves and global biovolume from mixed cultures experiment. For growth curves, light attenuation data were used. The data are averages of triplicates and standard deviation bars are also shown.

The relative biovolume of each species is shown in Figure 4. At the end of the experiment, there was no species exclusion and both species persisted until the end of the experiment. However, *Dolichospermum* sp. was not as good invader as *A. gracile*. The final biovolume of *Dolichospermum* sp. was always lower than initial, in contrast to *A. gracile*, which final biovolume achieved 5.69 times the initial in dominance of *Dolichospermum* sp. at 1P (Table 1). Moreover, interestingly the variation in relative biovolumes between replicates was higher than the variation in the total biovolume. In contrast, the total number of organisms was more variable among replicates (Fig. S3).

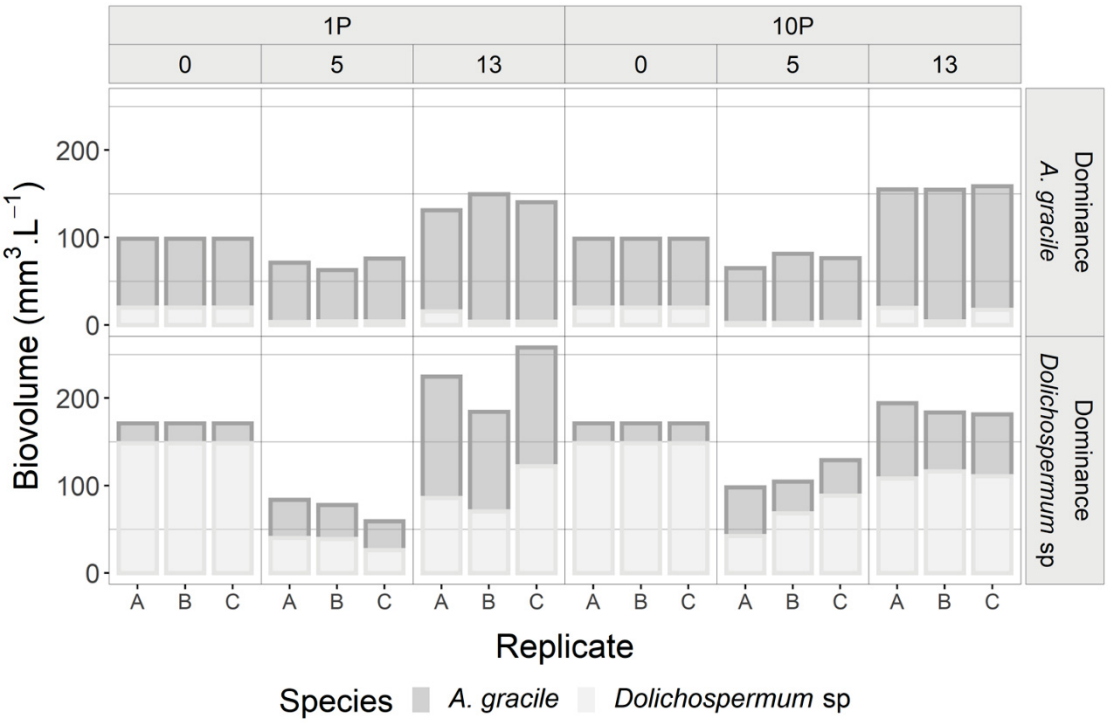

Figure 4 Relative biovolume of species in mixed cultures experiments, in each triplicate.

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Table 1 Average biovolume change of *A. gracile* and *Dolichospermum* sp. during mixed cultures experiment ($mm^3.L^{-1}$).

	1P		**10P**	
	Dominance of		Dominance of	
	Dolichospermum sp.	*A. gracile*	*Dolichospermum* sp.	*A. gracile*
A. gracile initial	22.75	78.75	22.75	78.75
A. gracile final	129.45±13.87	132.66±15.36	74.60±9.91	142.40±7.48
Final/initial	5.69	1.68	3.28	1.81
Dolichospermum sp. initial	146.88	20.10	146.88	20.10
Dolichospermum sp. final	91.04±28.74	7.38±6.17	111.76±4.09	13.17±7.63
Final/initial	0.62	0.37	0.76	0.65

1.4.3 Alkaline phosphatase activity (APA), SRP and PP

Alkaline phosphatase activity increased along the time in both cultures and species of the monocultures experiment (Fig. 5, left panel). No significative difference between treatments or between species was found, despite the clear trend of increased APA in half diluted cultures. Regarding the SRP at day 10, higher values were found in not diluted than half diluted, in *Dolichospermum* sp. cultures ($F_{1,4}$ = 13.68, P = 0.0209). Also, *Dolichospermum* sp. In not diluted treatment had higher SRP in comparison to *A.* gracile ($F_{1,4}$ = 14.22, P = 0.0196). Finally, no linear relationship was found between SRP and APA (Fig. S4).

In mixed cultures experiment (Fig. 5, right panel), the APA also enhanced during the experiment, in both treatments and cultures. Alkaline phosphatase activity was different according P pulses only in cultures dominated by *Dolichospermum* sp. in day 13, higher in 10P than 1P ($F_{1,4}$ = 35.86, P = 0.00391). Differences between cultures in the same day and treatment were more common, and cultures dominated by *A. gracile* always had higher APA (day 5 10P - $F_{1,4}$ = 10.05, P = 0.0339, day 13 1P - $F_{1,4}$ = 12.94, P = 0.0228, day 13 10P - $F_{1,4}$ =17.34, P = 0.0141). Interestingly, in the mixed cultures experiment, the APA reached higher levels than the monocultures experiment at the same total P of not diluted treatment.

Contrasting the SRP between 1P and 10P in the same cultures, SRP in 1P was higher than 10P only in day 5 (Dominance *A. gracile* - $F_{1,4}$ = 21.25, P = 0.00995; Dominance *Dolichospermum* sp. - $F_{1,4}$ = 17.36, P = 0.0141). When comparing the cultures in the same day and treatment, differences were found only in 10P treatment, on days 5 ($F_{1,4}$ = 44.26, P = 0.00265) and 13 ($F_{1,4}$ = 12.36, P = 0.0245). In the two cases, cultures initially dominated by *Dolichospermum* sp. had higher SRP. No linear relationship was found between APA and SRP (Fig. S5).

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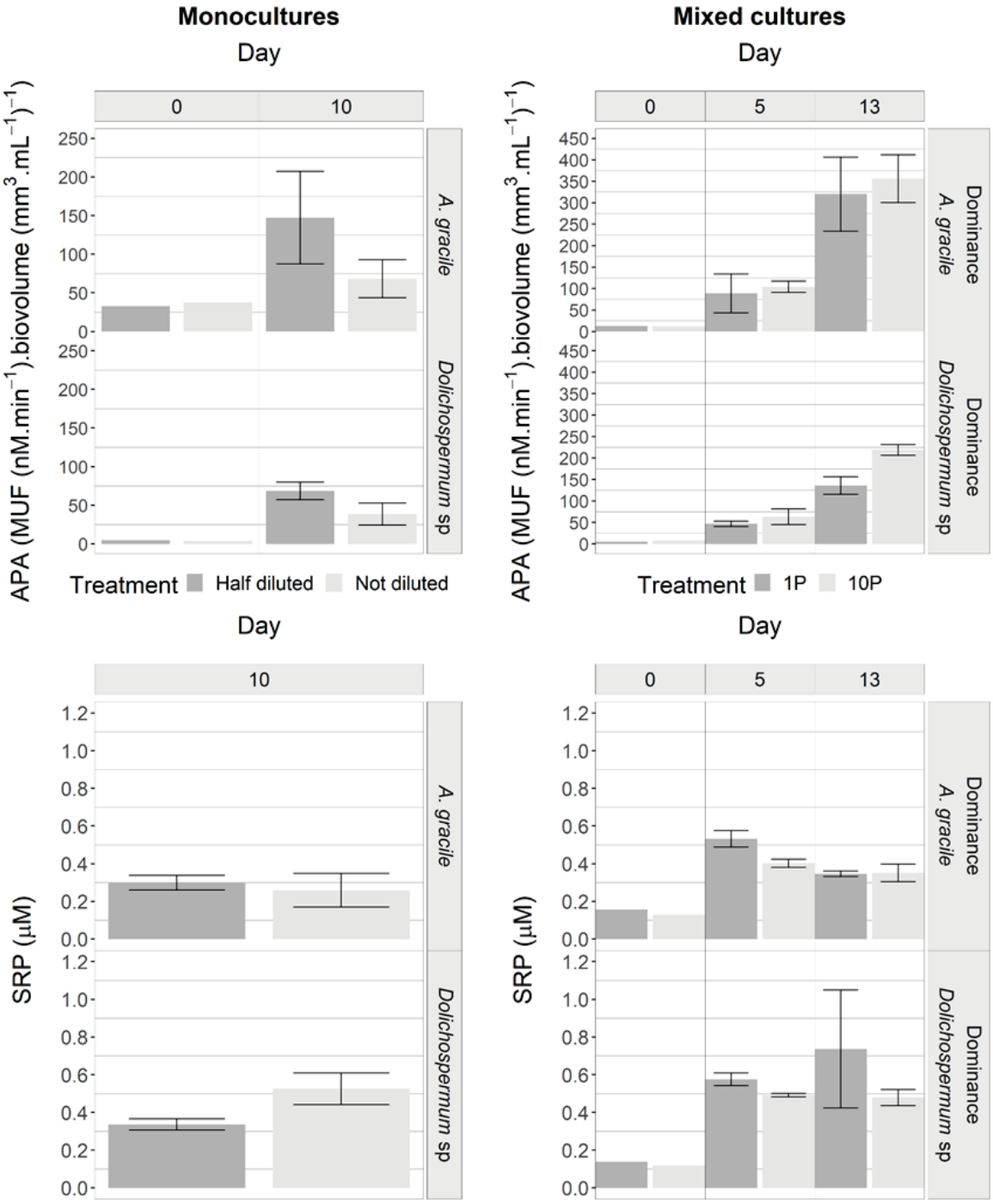

Figure 5 APA and SRP in monocultures and mixed cultures experiments. APA is standardized by biovolume. Data are averages of triplicates with standard deviation bars. Note the different scales in APA.

353

354 Particulate P decreased along the time in monocultures experiment, and
355 the treatments did not differ (Fig. 6, left). Also, no directly proportional relationship
356 between APA and PP was found (Fig. S6). In mixed cultures, the PP increased

at day 5, and decreased at day 13. It did not vary between 1P and 10P. Particulate P was higher in cultures dominated by *A. gracile* in day 13 1P ($F_{1,4}$ = 15.62, *P* = 0.0168), in comparison to cultures dominated by *Dolichospermum* sp. (Fig. 6, right). Finally, PP and APA were negatively related in cultures dominated by *A. gracile*, both 1P ($F_{1,5}$= 7.123, *P* = 0.0444) and 10P ($F_{1,5}$=12.38, *P* = 0.01696; Fig. S7).

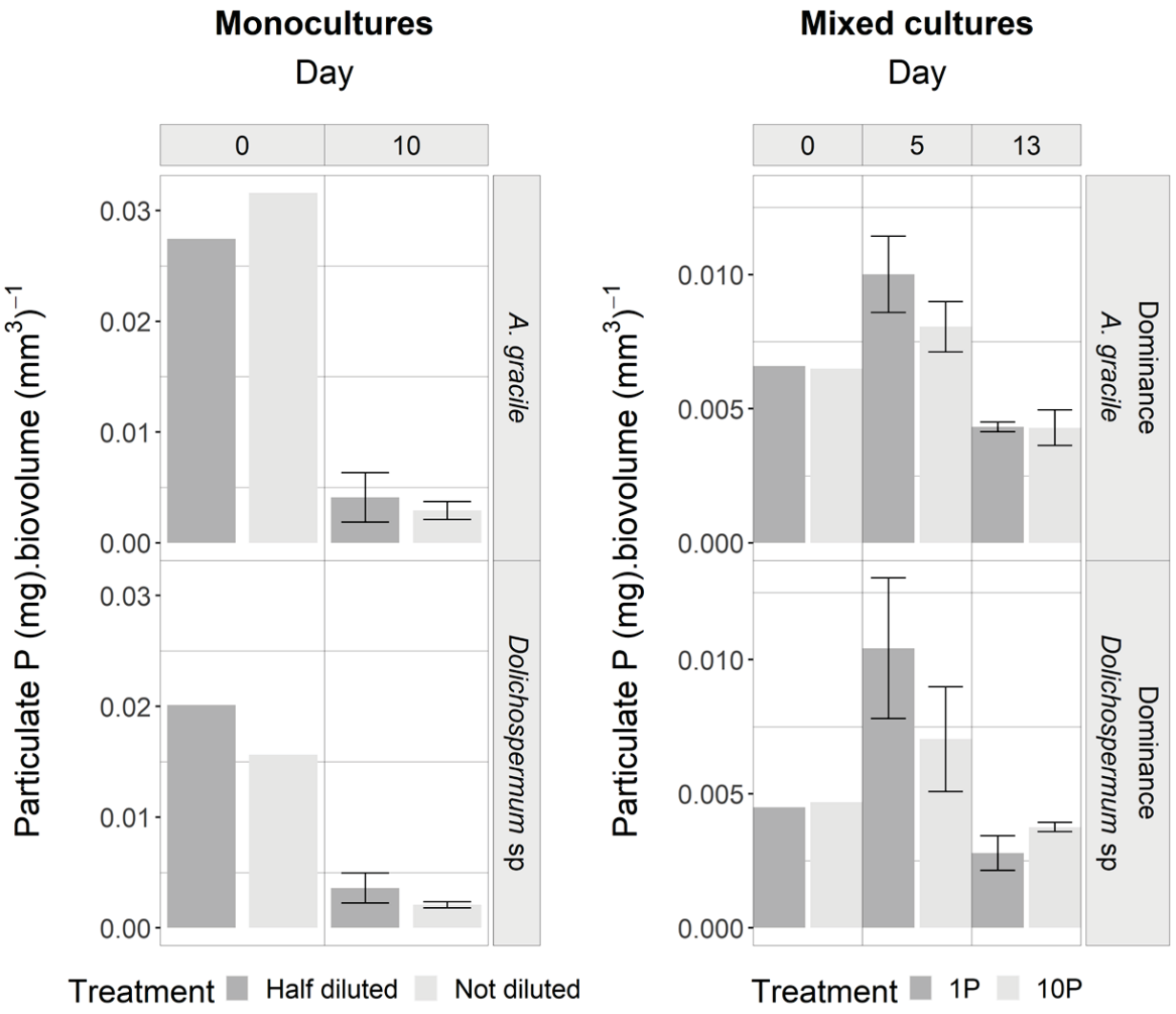

Figure 6 Particulate P standardized by biovolume un monocultures and mixed cultures. Data are average of triplicates; standard deviation bars are also indicated. Note the different scales.

1.4.4 Total nitrogen

In monocultures, the TN decreased along the time in both P treatments, and no difference between treatments and species was found (Fig. 7). In the case of mixed cultures, TN was higher in cultures dominated by *A. gracile* in day 13 10P ($F_{1,4}$ = 11.35, P = 0.0281). If using the number of trichomes to standardize the TN rather than biovolume, more differences between treatments and cultures appear (Fig. S8) in both monocultures and mixed cultures, and in these cases *Dolichospermum* sp. usually have the higher N content.

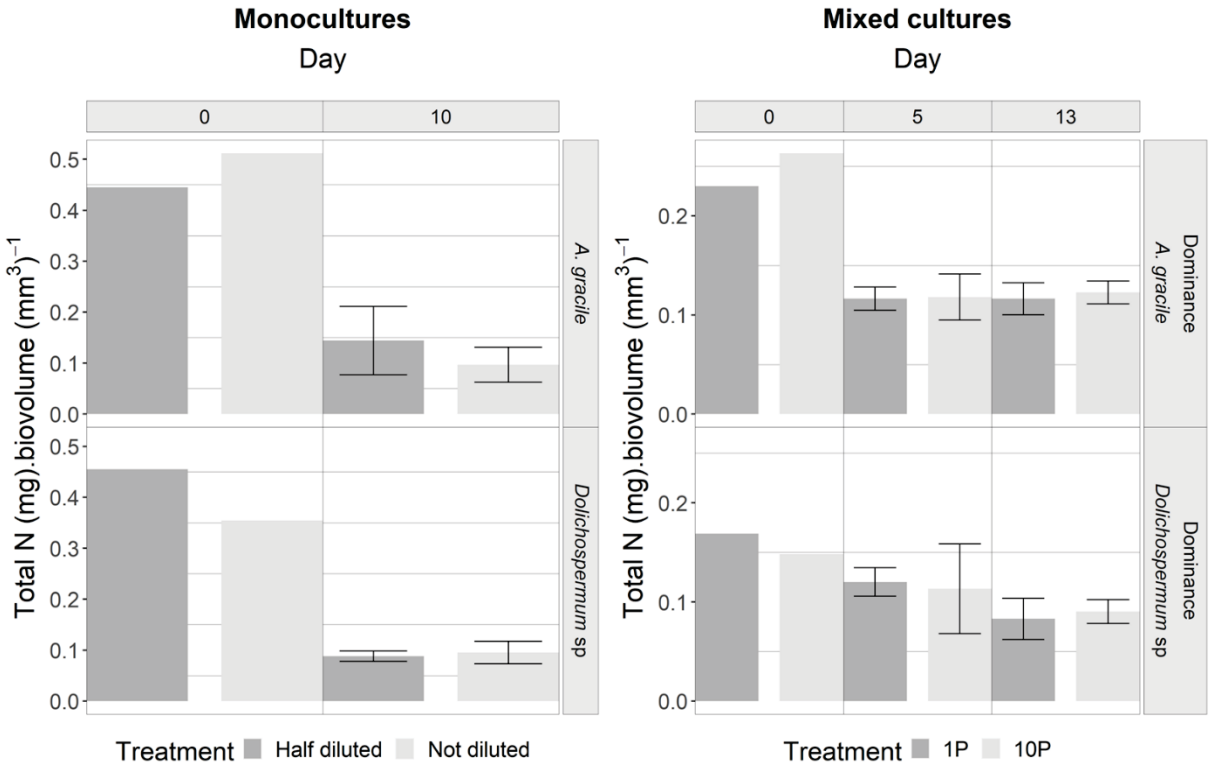

Figure 7 Total nitrogen in monocultures and mixed cultures standardized by biovolume. Data are average of triplicates. Standard deviation bars are also indicated.

Regarding the APA and TN relationship in mixed cultures, a temporal pattern can be identified (Fig. 8). Along the time, there is an increase in APA for the same range of TN.

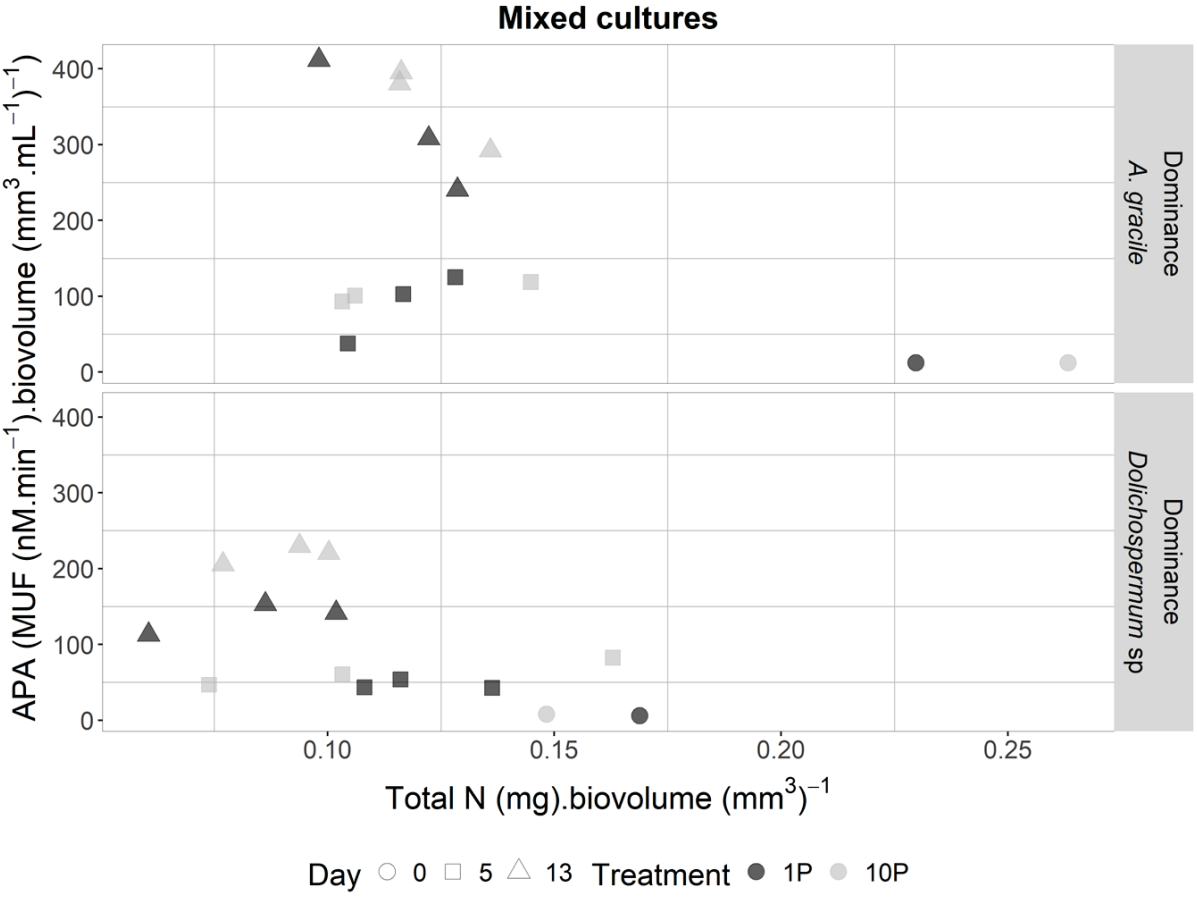

Figure 8 Scatter plot of APA and TN in mixed cultures. Data are identified according the day in the experiment. Note the high APA for the same range in TN.

1.5 DISCUSSION

In this study, we generated evidence that differential ecophysiological responses might be partially responsible for coexistence of freshwater cyanobacterial species in a fluctuating ecosystem. The pulses triggered distinct physiological adjustments, as evidenced by SRP and APA, leading to different proportions of each species at the end of the experiment. However, there was hierarchy in species' performance, indicating particular physiological adjustments and density dependent effects. Therefore, in natural environment, where the fluctuations are occurring constantly, we should expect a scenario with a myriad of possible combinations of species.

In monoculture experiments, regarding the relationship between P concentration and population size, we confirmed our hypothesis of higher growth rate under proportional lower P and population size. However, depending on using number of organisms or biovolume to assess the increase in population size, different results can be obtained, especially in the case of *A. gracile*. Also, despite similar levels of TN and PP between the species, they greatly differed in APA, and *A. gracile* had the higher APA. In the case of mixed cultures, the P pulse did not affect the global growth of the cultures, and no complete species exclusion took place. However, the capability of growing when rare was distinct among species and P pulses. Higher probability of coexistence occurred in 10P as the relative biovolumes were more similar in 10P. Although, there was a strong influence of the initial biovolume on species performance. The initial population size had a greater role in *Dolichospermum* sp. success, although allelopathic and other chemical interactions not assessed in this study cannot be discarded. Moreover, APA and PP were usually higher in cultures dominated by *A. gracile*, and APA did not change with P pulse treatments, in contrast to *Dolichospermum* sp. dominated cultures. Possibly, *Dolichospermum* sp. is more sensible to SRP fluctuations. Importantly, our results reinforce the absence of direct proportional relationship between P concentration and growth, and the complexity of APA, being in line with other studies reporting the independency of APA from external inorganic P concentration (HINO, 1988), as found here in *A. gracile*.

The higher growth rates found in monocultures subjected to lower P could be explained by dense-dependent adjustments of P uptake systems. The

425 reduced population depletes P slower in comparison with higher population size,
426 which extends the exposure time to P in the medium and allows the cells to adjust
427 the uptake systems in a balance between the exposure and reaction times
428 (WAGNER; FALKNER and FALKNER,1995; FALKNER; WAGNER and
429 FALKNER 1996; AUBRIOT; BONILLA and FALKNER, 2011). In this sense, a
430 more efficient P uptake could be expected in reduced biomass and P
431 concentration. Moreover, if the P uptake is more efficient, one could also expect
432 a lower APA considering it is an energy consuming mechanism involved in P
433 acquisition (CHRÓST, 1991). Our results actually showed unexpected higher
434 APA in half diluted. A possible explanation is that the cultures were in distinct
435 growth phases. If half diluted cultures started the growth earlier and faster than
436 the not diluted, then they might have been P limited sooner. This interpretation is
437 in accordance with greater increase in biovolume, the lower level of SRP and
438 elevated PP, as PP might be reflecting polyphosphate granule accumulation that
439 occur at the early stationary phase (KULAEV; KULAKOVSKAYA, 2000;
440 SOLOVCHENKO; GORELOVA and KARPOVA, 2020). The contamination of few
441 trichomes of *A. gracile* only in *Dolichospermum* sp. cultures in 0.5 mgP L^{-1} is
442 another evidence of P limitation in half diluted cultures, considering the
443 disadvantage of a small initial *A. gracile* population.
444 In the case of *A. gracile*, the absence of difference in SRP might be
445 attributed to the major usage of organic P (Heath; Cooke, 1975; SCHOFFELEN
446 et al., 2018), thus decoupling the SRP and APA; or to an early expression of
447 phosphatases (e.g. APA at SRP concentration of 0.681 mgP L^{-1} in Lake Stefanksi
448 in Poland, during *Aphanizomenon flos-aquae* bloom, Chen et al., 2020).
449 Interestingly, the biovolume produced by *A. gracile* in not diluted treatment was
450 higher than in half diluted, but the opposite occurred in number of trichomes,
451 possibly due an increase in trichome and cell volumes (Fig. S9). In Heath and
452 Cooke (1975), higher cell volume was coincident with increased APA, contrasting
453 with our results. Alterations in cells ultrastructure following changes in P status
454 are expected (SOLOVCHENKO; GORELOVA and KARPOVA, 2020), but it is not
455 possible to conclude which mechanisms drove the changes observed in trichome
456 and cell volumes from our data.
457 In relation to our second prediction (i.e. absence of species exclusion in
458 at least one of the treatments), we confirmed the no complete exclusion and

459 effects of P pulse. At a first glance, one could argue that the persistence of both
460 species until the end of the experiment was due insufficient time to complete the
461 exclusion (i.e. a non-equilibrium situation, HUTCHINSON, 1953). Although we
462 cannot ensure *Dolichospermum* sp. would not be excluded from *A. gracile*
463 dominated cultures if the experiment lasted longer, probably time duration of the
464 experiment was not the main reason avoiding species exclusion because: i) for
465 instance, previous works in chemostats using other cyanobacteria species found
466 population density reduction (<20 % of total cells density) and complete exclusion
467 in mixed cultures before 14 days of experiment time-course (DE NOBEL et al.,
468 1998; STOMP et al., 2004; respectively); ii) by attending the invasibility criterion
469 (i.e. capacity of recovering from low density), we enhanced the probability of
470 exclusion by the species in initial reduced abundance and, iii) in laboratory
471 experiments, the strength of selection in competition experiments are stronger
472 than in natural environments (FOX; NELSON and MCCAULEY, 2010). Thus, our
473 results provide evidences of the role of pattern of P availability on species
474 coexistence.

475 Nevertheless, clearly there was dissimilar performances in recovering
476 from low abundance between species. The small size of *Dolichospermum*'s
477 populations at the end of mixed cultures experiment contrasts with previous
478 studies showing *Aphanizomenon* exclusion by *Dolichospermum* (formerly
479 *Anabaena*; DE NOBEL et al., 1997, 1998). One possible explanation might be
480 the intrageneric and intraspecific variation in Cyanobacteria (WILLIS et al., 2016;
481 GUEDES et al., 2019; KELLY; RYAN and WOOD, 2019; XIAO et al., 2020). But,
482 despite the contrasting results and the distinct experimental conditions employed,
483 some general and coincident results were found. De Nobel et al. (1997) reported
484 less dependence on residual P and absence of adjustments in N fixing activity
485 according the growth rate in *Aphanizomenon* cultures when comparing to
486 *Dolichospermum*. Analogously, we found independence between APA and SRP,
487 and invariant TN according P pulses in cultures dominated by *A. gracile*. In a
488 posterior investigation, De Nobel et al. (1998) also found that N fixing in
489 *Aphanizomenon* was less sensible to light levels than *Dolichospermum*. Taking
490 together, the evidences raise the question of possible compensatory
491 mechanisms being used by *Aphanizomenon* to maintain a certain N_2 fixing rate.
492 In fact, acquisition of N and P are correlated, and the alleviation in the limitation

493 by one nutrient can impact the acquisition of the other (WANG et al., 2018;
494 AUBRIOT, 2019). It seems reasonable to hypothesize that higher APA in *A.*
495 *gracile* is a compensatory mechanism of P scavenge in order to sustain N_2 fixing.
496 Bradburn, Lewis and McCutchan (2012) found higher N fixing efficiency
497 in natural populations of *Aphanizomenon* when compared to *Dolichospermum*,
498 and even in dark conditions the former was able to fix N. Even though we did not
499 assess the N fixation, we found a temporal increase in APA for the same range
500 of TN, and usually higher TN in cultures dominated by *A gracile*. Taking together,
501 the set of evidences points out to the use of organic P to attend high P demand
502 for N fixation, but not in a direct response to SRP levels. It is interesting that in *A.*
503 *gracile* cultures the SRP decrease to similar levels as in *Dolichospermum* sp, and
504 this indicates that the compensatory APA is not reflecting, *a priori*, any
505 disadvantage in the P uptake system.
506 Another interesting feature is the long-term effect of P pulses. It was
507 already described the heritage of adapted P uptake systems by descendent cells
508 (FALKNER; PRIEWASSER and FALKNER, 2006; AMARAL; BONILLA and
509 AUBRIOT 2014), but it is still surprising that the difference between uptake
510 systems apparently lasts for more than five days, as indicated by the SRP and
511 APA levels. In both cultures, the SRP tended to be lower in 10P than 1P and this
512 suggests that longer exposition to higher P concentration occurred in 1P, and not
513 10P. Indeed, higher exposition time in 1P rather than 10P was reported in a
514 previous study with *Dolichospermum* (FALKNER; PRIEWASSER and FALKNER,
515 2006), while higher exposure time in 10P occurred in natural phytoplankton
516 communities dominated by *Planktothrix* and *Raphidiopsis* (AUBRIOT; BONILLA
517 and FALKNER, 2011; AUBRIOT; BONILLA, 2012). Taking together, these
518 opposite results show that the exposure time will depend on the uptake activity
519 of populations and P pulse concentration, since small pulses can be taken up
520 very fast or can overlap one after the other, resulting in short or long exposure
521 times to P, respectively. In addition, the variation in the combination of high and
522 low affinity proteins between species probably reflects these dissimilar adapted
523 states, considering even in the same species there is variation in number of
524 copies (WILLIS et al., 2018) and differential expression of genes involved in P
525 acquisition (WILLIS et al., 2019). And, these different adjustments might or not
526 be reflected in other populational parameters, as in the case of *Dolichospermum*

sp. dominated cultures, where the total final biovolume in 1P was higher than 10P regardless the growth rate was the same.

The dependence of *A. gracile* on organic P was suggested to function as a mechanism to promote coexistence, and it was reported a temporal coincidence in *Aphanizomenon* bloom and TP concentration in Baltic Sea, and no response on growth after dissolved inorganic P addition (SCHOFFELEN et al., 2018). In our experiment, higher APA and evenness in species relative biovolume were associated to 10P in *Dolichospermum* sp. dominated cultures. However, our results do not allow to disentangle the roles of pulses and APA in coexistence probability, and at what extent their roles can change along the time. For instance, the enhanced APA in *A. gracile* dominated cultures did not guarantee a better performance of *Dolichospermum* sp. invasion. Therefore, we greatly recommend the study of the interplay between adjustments to fluctuating P concentrations and the use of organic P.

1.6 CONCLUSIONS

Here, we evaluated and compared the effects of biomass density and P availability (i.e. concentration and pattern of pulses) on monocultures and mixed cultures of two potentially competing cyanobacteria isolated from the same lake sample. We found differences in growth rate according the P concentration in monocultures, and an effect of P pulses resulting in distinct outcomes of mixed cultures. The effects of P pulses lasted for mostly the duration of the experiment, as demonstrated by SRP and APA. Likely, in 1P was achieved a longer exposition to enhanced P concentration and then, leading to a more energetically efficient P uptake. The higher APA in *A. gracile* is probably associated to increased P demand to N_2 fixing metabolism in comparison to *Dolichospermum* sp. Taken together, our results reinforce the importance of environmental fluctuations and species ecophysiology on modulating coexistence. Finally, more studies are necessary to investigate under what circumstances the increased phosphatase production by *A. gracile* can alleviate the P limitation to the whole community and to what extent it might be important in promoting coexistence.

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734 1.8 SUPPLEMENTARY MATERIAL

735 Can resource pulses shape freshwater Cyanobacteria communities?

736 Elena Fukasawa Galvanese, André Andrian Padial, Luis Aubriot

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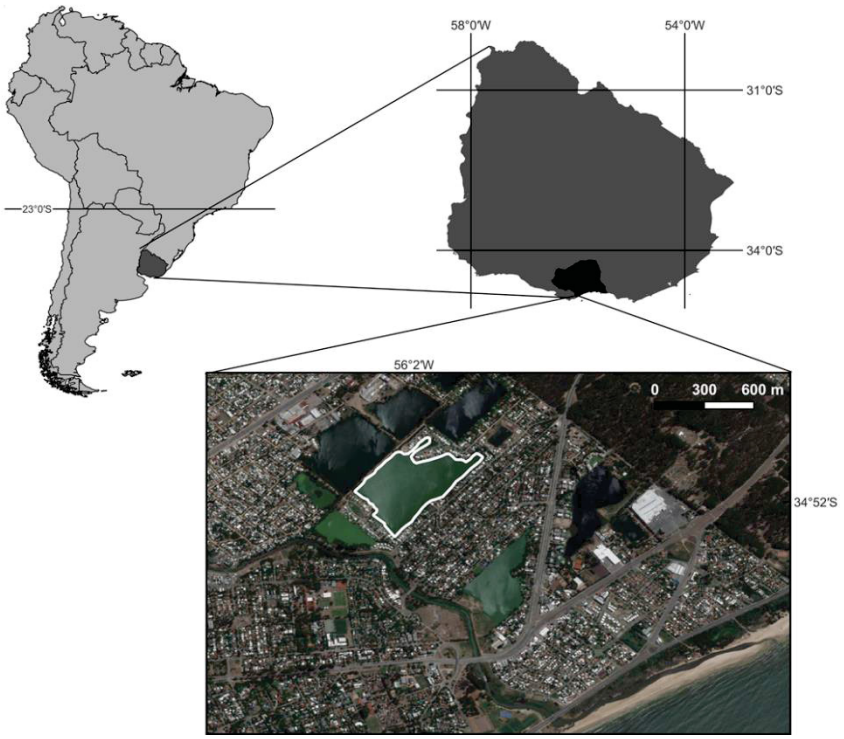

Figure S1. Localization of Lago Javier. From the same samples the two species were isolated.

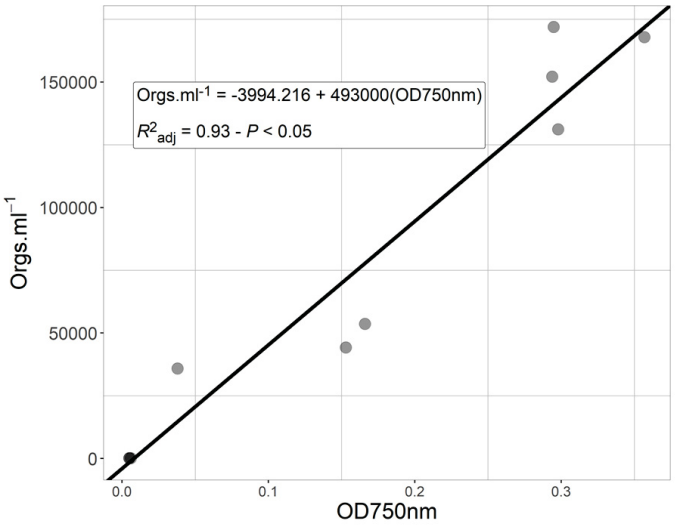

Figure S2. Scatter plot of optical density at 750 nm and number of organisms .ml^{-1} in *Dolichospermum*. The data includes 4 data from a different strain (n=11). The line is the resulted regression.

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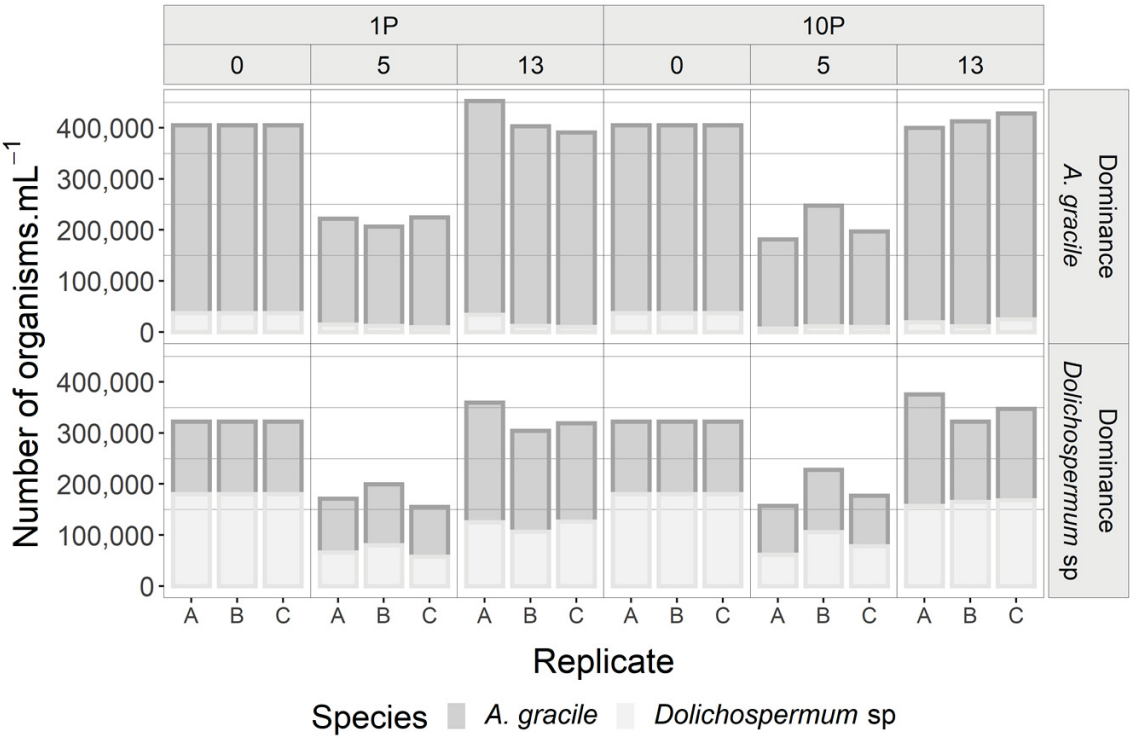

Figure S3. Number of organisms and the relative contribution of each species in mixed cultures experiment, in all replicates.

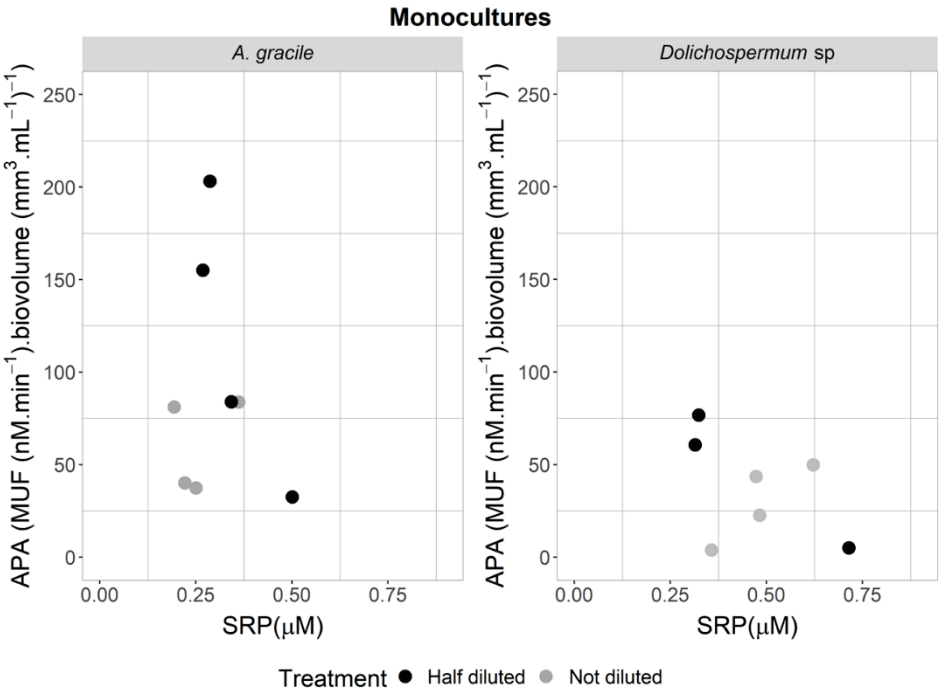

Figure S4 Scatter plots of SRP and APA in monocultures (n=4 in *A. gracile* and n=3 in *Dolichospermum* sp in each treatment).

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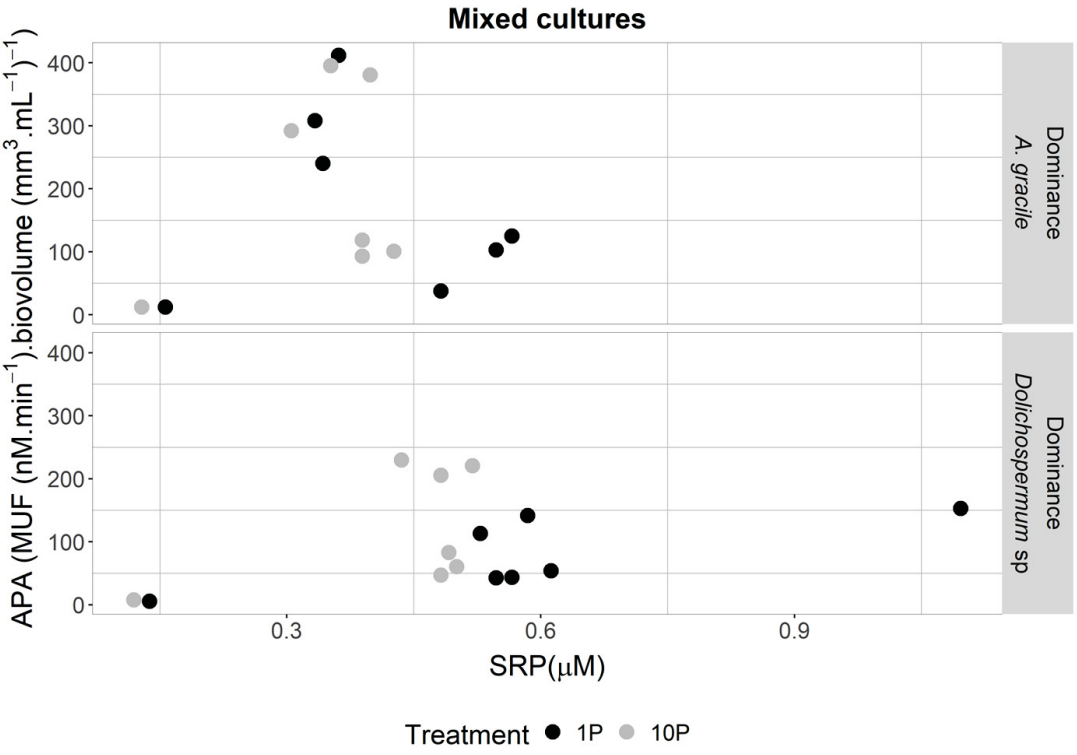

Figure S5 Scatter plots of SRP and APA in mixed cultures experiment (n=7 in 1P and 10P).

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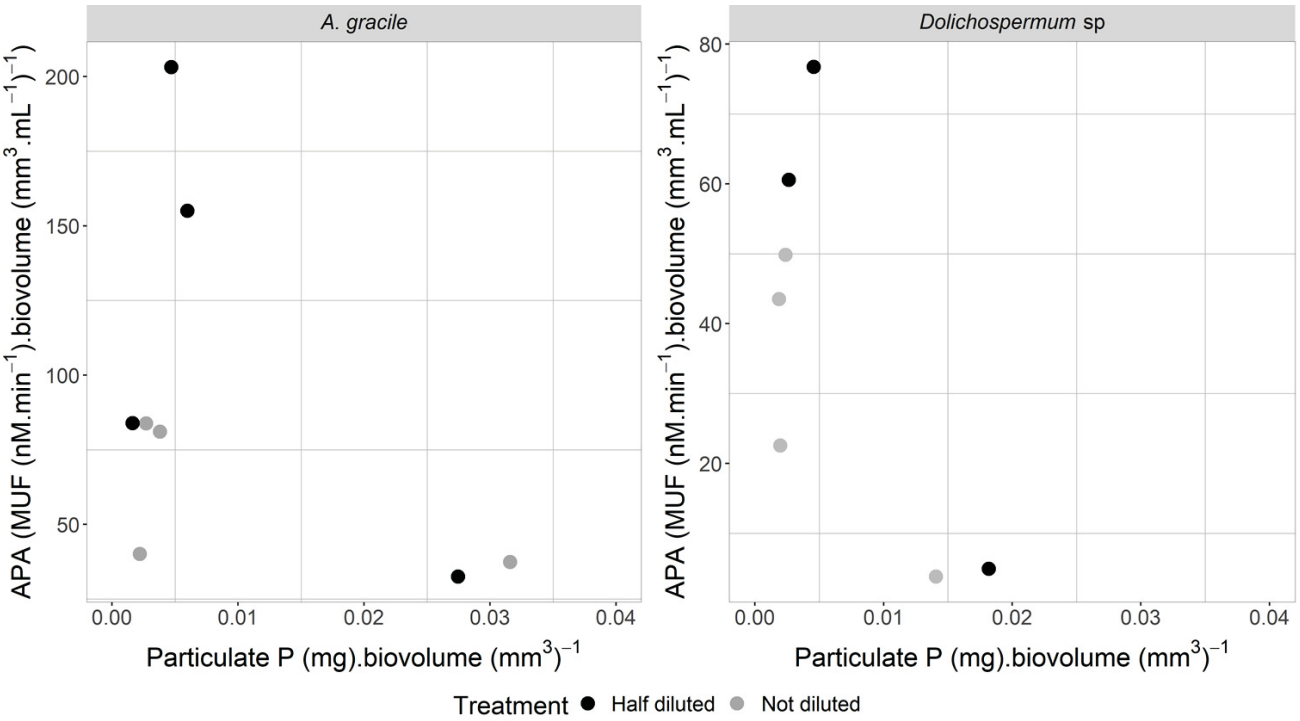

Figure S6 Scatter plots of PP and APA in monocultures experiment (n=4 in each treatment).

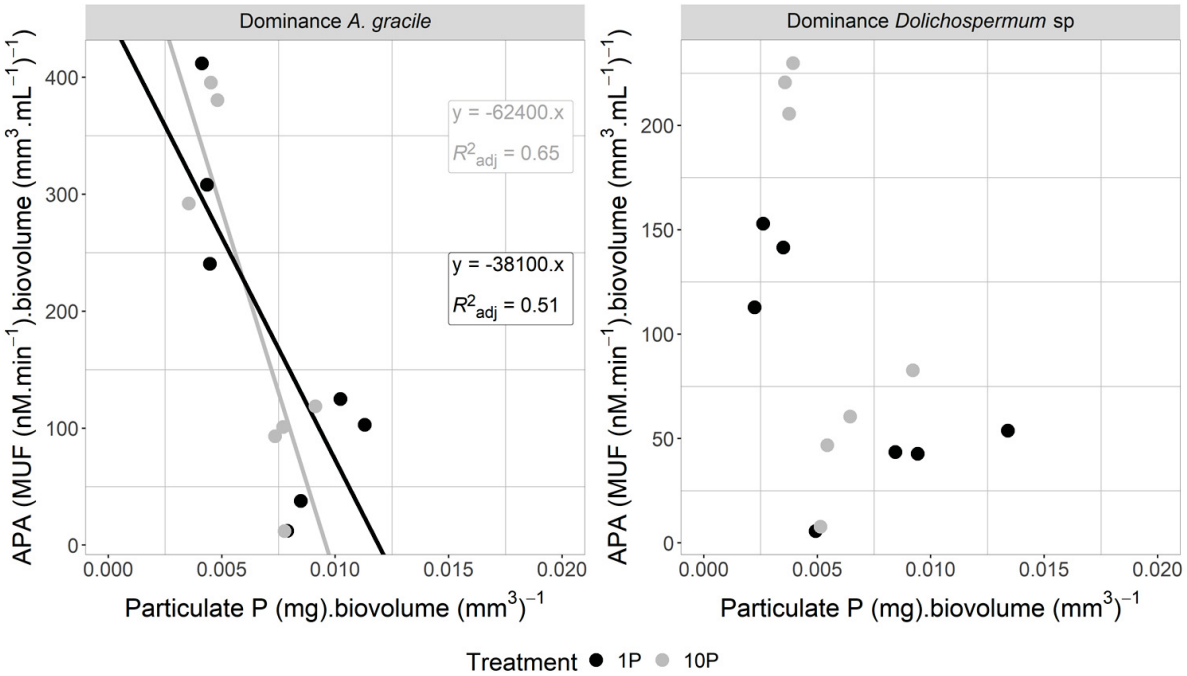

Figure S7 Scatter plots of PP and APA in monocultures experiment (n=4 in each treatment). Lines are significant linear regressions with permutations.

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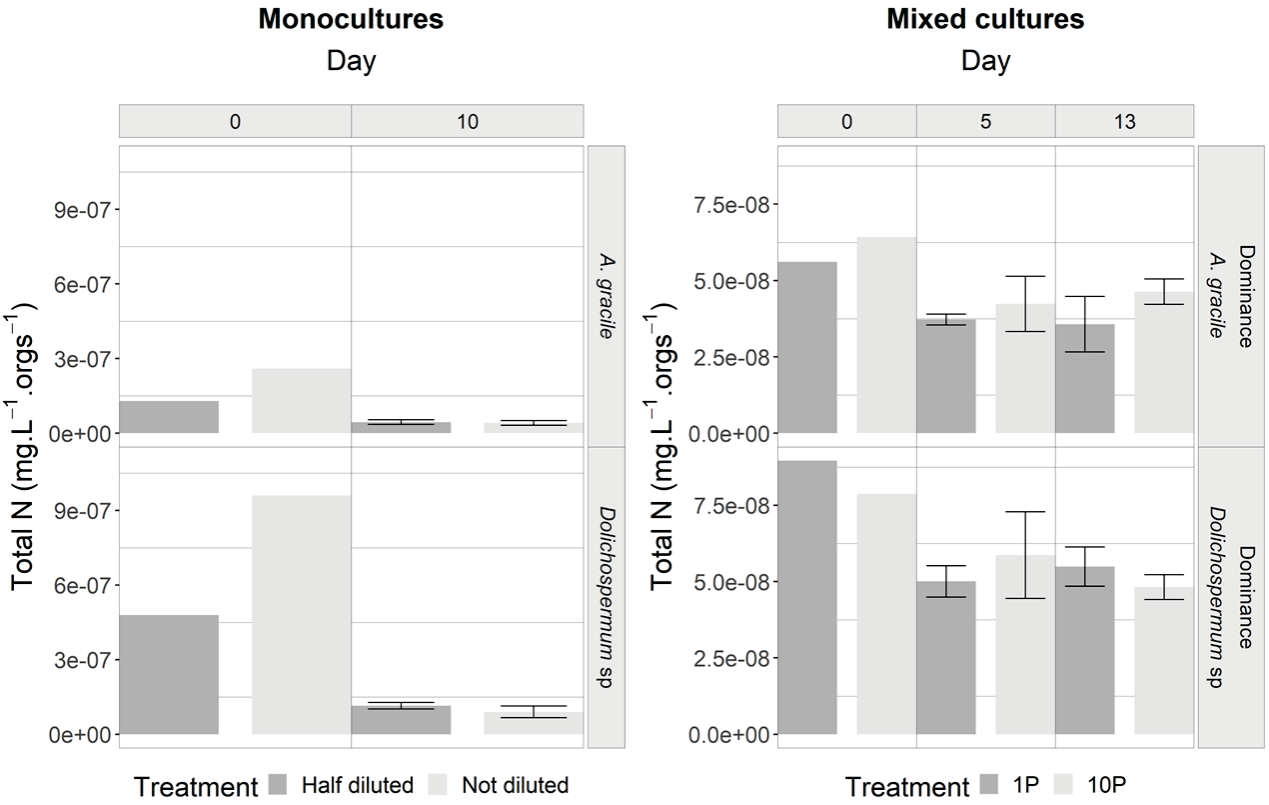

Figure S8 Total nitrogen in initial and final days, in monocultures and mixed cultures experiments. Bars are averages and bars standard deviation.

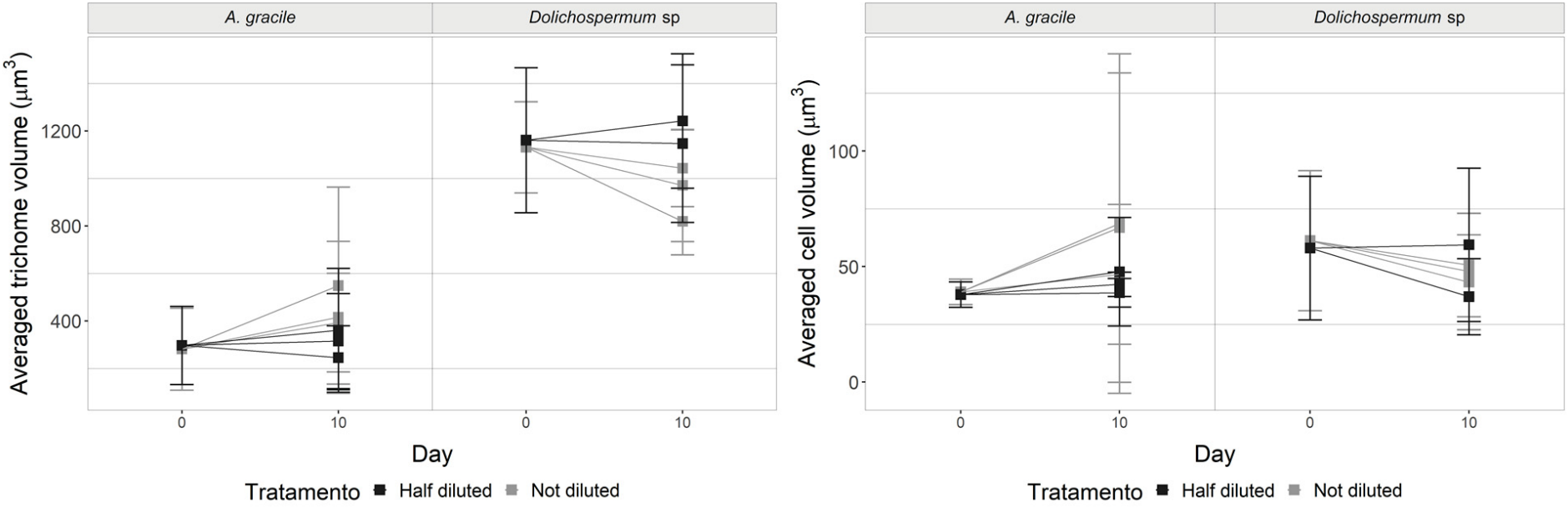

Figure S9 Averaged trichome and cell volumes obtained in monocultures experiment at initial and final days. Bars are standard deviation.

CAPÍTULO 2

PHOSPHORUS PULSES MODULATING CYANOBACTERIA INTERACTIONS UNDER SATURATED AND NON-SATURATED CONDITIONS

Phosphorus pulses modulating cyanobacteria interactions under saturated and non-saturated conditions

Elena Fukasawa Galvanese, Ann Chuang, Luis Aubriot, André Padial, Michele Burford

2.1 ABSTRACT

Natural communities are constantly subjected to fluctuations in environmental conditions. In this sense, how organisms respond to changes in key environmental drivers, during favorable and unfavorable conditions, is helpful in the understanding of species coexistence and diversity. Intending to evaluate the impacts of distinct patterns of phosphorus (P) supply on freshwater cyanobacterial communities, we performed experiments with mono and mixed cultures. The experiments encompassed submitting deficient cultures to saturating and non-saturating P additions. Patterns of P supply had the same total P concentration, but the addition was either in one or divided into ten pulses (hereafter 1P and 10P, respectively). We measured the P uptake, the growth by using optical density at 750nm, and assessed organic P use by measuring alkaline phosphatase activity (APA) in monocultures. The P pulses affected the growth of *Aphanizomenon gracile*, and APA in *Raphidiopsis raciborskii* under saturated conditions. *Aphanizomenon gracile* had the higher APA. In mixed cultures, 1P favored *A. gracile* and 10P *R. raciborskii*. Cultures had subtle differences in remaining external P and P uptake curve, despite the P saturated conditions. In limited conditions, species varied in responses after pulses, in spite of the similar kinetics parameters before the pulses. The uptake ceased at different thresholds according the pulses, and growth was favored in 1P in all three species, contrasting to previous studies with *R. raciborskii*. Taken together the sensitivity to P fluctuations, even when P concentration is high, and the suggested convergence of uptake kinetics under P limitation, our results provide evidence for a constant variation in intra and interspecific competition, probably increasing the scenarios for coexistence.

Keywords: *A. gracile*, *D. circinale*, flow-force model, P uptake, *R. raciborskii*

2.2 INTRODUCTION

One of the expressions of biological diversity is the multitude of life forms and organisms' interactions. The environment is not static, implying that organisms also have the capacity of adjusting to environmental conditions as an inherent character (FORSMAN, 2015). The capability of organisms to adjust to different environmental conditions has two important outcomes: firstly on the environment itself, because the organisms are changing the environment and, in some way, constructing the environment they will experience in future (FALKNER; FALKNER, 2008); and secondly organisms affect other organisms, due to direct interaction or mediated by environmental changes as stated before. Thus, biological diversity is a complex integration of many chain reactions, and it is hard to disentangle the order of stimulus and responses. Conceptually, biological diversity could also be defined as the temporal and spatial dependence of diversity (USINOWICZ et al., 2017).

The spatio-temporal approach of studying processes associated to community dynamics has been useful in explaining the diversity at different scales (e.g. DE LEÓN et al., 2014; WOJCIECHOWSKI et al., 2017). Also, through the temporal sequencing of biological processes, it becomes possible to make predictions of future scenarios and to test them (e.g. STORK, 2010). One of the most common predictions we usually use is the population growth using the logistic curve (VERHULST, 1838), and the combination of two or more logistic curves used to predict competitive outcomes (ROSS, 1908; DUCOBU et al., 1998). The growth of a certain population is the ultimate result of the organisms' adjustments to match its requirements in a changing environment. Even so, growth itself (i.e. the observable pattern) does not provide complete information about the kind of adjustment made, because there are many possible avenues (i.e. physiological and/or behavioral responses) to overcome a given stress or condition (FORSMAN, 2015). In this sense, subsequent environmental changes will result in sequential responses by the organisms, in such a way that an observed response to a specific environmental condition relies on previous environmental stimulus (FALKNER; FALKNER, 2000; WOJCIECHOWSKI; PADIAL, 2015). The better the capacity to establish temporal correlation between

events, the better the prediction of future conditions by the organisms (BERNHARDT et al., 2020).

Aquatic systems, mainly the phytoplankton community, are particularly interesting habitats to study how environmental changes affect organisms' responses. This is because in the same lake or reservoir, there are distinct micro-habitats changing in space and time, in combination with short generation times of microorganisms (hours to days). For example, there might be differences in nutrient and light availability within few meters (e.g. TONETTA et al., 2016), besides daily and seasonal variation (e.g. FONTES et al., 2013). And, more importantly, as the phytoplankton community might be subject to drastic, rapid or slow changes, it is not surprising that these organisms possess a high level of physiological flexibility (e.g. BOLIUS; WIEDNER and WEITHOFF, 2017).

Regarding the acquisition of the resources phosphorus (P), nitrogen (N) and light, phytoplankton community exhibit a wide range of strategies. For instance: some species can fix atmospheric N to achieve their requirements, regardless the high energetic cost of this process (YEMA; LITCHMAN and DE TEZANOS PINTO, 2016); different pigment composition between and within species to optimize light absorption (STOMP et al., 2008) and; the use of organic P (both, direct uptake organic forms, such as phosphonates (HEATH, 2005); and after release of phosphate from organic compounds (CHU, 1946; PRENTICE et al., 2019)), besides the high P affinity proteins, allowing to reach external P concentration at nanomolar level (RIGLER, 1956; FALKNER; WAGNER and FALKNER, 1996).

Phytoplankton, and particularly cyanobacteria, show high flexibility in the modulation of P uptake, adjusting the uptake system towards an efficient energy balance (FALKNER; WAGNER and FALKNER, 1996). Falkner, Falkner and Schwab (1989) proposed a flow-force model to mathematically evaluate this adaptive behavior. The model considers that, as the availability of P decreases, more energy is required to transport P from outside to inside the cell (FALKNER; HORNER and SIMONIS, 1980). And this extra energy, which allows the cells to scavenge P at nanomolar levels (FALKNER; PRIEWASSER and FALKNER, 2006), means that more ATP must be provided concomitantly with the increase in the H^+ gradient across the thylakoid membrane (FALKNER; HORNER and SIMONIS, 1980). The ATP is produced by photophosphorylation in different

systems than those directly related to the transport of P. The functioning of the P transport and the systems related to energy production can be more or less coupled, and the higher the level of coupling, the lower the P external concentration at which the cell can uptake P (PLAETZER et al., 2005). Therefore, the external P concentration at which the uptake ceases, represents an energetic equilibrium, the so-called threshold value (FALKNER; STRASSER and GRAFFIUS, 1984). Moreover, the systems will work near the maximum efficiency because, in one hand, an excess of P entering the cells cannot be accommodated in infinitely increasing polyphosphate granules within the cells; and in the other, insufficient amount of P entering the cells will compromise the growth, the cell activity, and finally will lead to the death or dormant cell (i.e. akinetes, PADISÁK; ISTVÁNOVICS, 1997). Such consumption and production of energy is anticipatory of the subsequent growth, as under limited conditions P uptake and growth are decoupled, a situation that can be observed in batch cultures (WAGNER; FALKNER, 2001). A change in this equilibrium can be induced and observed when P limited cells are subjected to an external P concentration that surpass the threshold value (AUBRIOT; BONILLA and FALKNER 2011).

Considering that the threshold value is expressing a given energetic state (e.g. low threshold values indicating high activity and affinity of uptake systems and strong P deficiency), the temporal pattern of P availability plays an important role in changing P cell status and changing the energetic equilibrium (i.e. new threshold value). For example, providing the phosphate at one single time or dividing the same phosphate concentration in multiple pulses, or inverting their order might result in different thresholds (FALKNER; PRIEWASSER and FALKNER, 2006; AUBRIOT; BONILLA and FALKNER, 2011). Moreover, differences in P cell metabolisms induced by the patterns of P availability can also impact the growth (AMARAL; BONILLA and AUBRIOT, 2014) and CO_2 fixation (WAGNER; SAHAN and FALKNER, 2000).

Usually, the equations describing growth and nutrient concentration do not account for the flexibility in nutrient acquisition, considering it as dependent only on nutrient concentration and enzyme's affinity (WAGNER; FALKNER, 2001). Accordingly, the parameters are kept fixed in equations (e.g. maximum growth rate). This lack of flexibility can constrain a wider understanding of what may be happening in natural communities, and using the flexibility in acquisition

and use of nutrients in a wide range of P availability to assess species' interactions outcomes, might help us to address important ecological questions, mainly those related to species coexistence (e.g. Paradox of Plankton; HUTCHINSON, 1961) and management of aquatic system under anthropic pressures (BERTHOLD; CAMPBELL, 2021).

In order to provide information about how communities of freshwater cyanobacteria respond to variations in P concentration, we aimed to compare the responses of different commonly co-occurring and bloom-forming species of cyanobacteria to distinct patterns of P availability in mono and mixed cultures. We submitted the cultures to saturated and non-saturated P additions, and the P additions consisted of the same total amount of P provided in one single or divided in ten additions. We expected different responses according the P pulses in no saturated conditions, and also no species exclusion in at least one P pulse treatment, assuming that in at least one treatment the same equilibrium in P uptake would be achieved by all species.

2.3 MATERIALS AND METHODS

2.3.1 Culture conditions and species

Non axenic cultures of *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek 2009, *Aphanizomenon gracile* (Lemmermann) Lemmermann 1910 and *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno 2018 previously isolated from Australian reservoirs were cultivated in BG 11 medium and kept in 12/12 light-dark cycle, under approximately 80 µmol photons m^{-2} s^{-1} and 27-28 °C, in culture flasks of 600 mL. Through consecutive dilutions with BG 11 without N (-N) and P (-P) along three months and approximate 10 days frequency, the dissolved inorganic N and total P levels were reduced. The total P was reduced to 0.2 mgP L^{-1} (6.45 µM). The N limiting condition was confirmed by presence of heterocysts. Cultures under these conditions were used for three types of experiments (see below). In the experiments, radioactive ^{33}P was used to analyze P uptake kinetics by the cells and consequently evaluate the possible changes in P uptake system (see Fig. 1).

2.3.2 Experiment 1

In this experiment, the goal was to assess the effects of 1P and 10P pulse treatments on monocultures of each species, under P saturated conditions. Previous to the experiment, 1.4 L of culture which had been grown under batch conditions for 17 days since the last subculture was centrifuged (2 min at 2000 m s^{-2}, Stratos, Sorvall Centrifuge) and resuspended in BG11 -N -P. This procedure was repeated three times before the final resuspension in 200 mL of BG 11 -N -P, four days before the start of the experiment. The concentrated cultures were diluted to achieve a volume of 2 L and the optical density at 750nm (hereafter OD750nm) standardized across the cultures. Then, 450 mL of each culture was distributed in four bottles: two to receive the pulses with ^{33}P and the remaining only receiving ^{31}P solution.

The phosphate pulses applied to the three cultures totaled 7 µM P (^{33}P and ^{31}P to each set of bottles), which corresponds to 15 times the total P in the diluted cultures. Half of this concentration (i.e. 3.5 µM P) was added in single or ten pulses (100 µL every 6 minutes). After 2 hours of the first pulse, another 3.5 µM P was added and 15 mL of each bottle was collected. In cultures which received pulses containing the tracer ^{33}P, the remaining external P concentration (i.e. threshold) and the uptake were measured. The uptake curve was obtained by spiking 1.07 µM P final concentration and proceeding to the steps described in section 2.3.8 Analysis of P uptake (Post-pulse, Fig. 1). The cultures were distributed in three culture flasks (triplicates). The culture conditions were 12/12 light-dark cycle (80 µmol photons m^{-2} s^{-1}) and 27-28 °C. Every two days, samples from the cultures that receive only ^{31}P were collected for OD750nm measurement and APA analysis. Every day the flasks were gently shaken and placed back randomly.

2.3.3 Experiment 2

The aim of this experiment was again to compare the effect of 1P and 10P pulse pattern under saturating conditions, but applied to both mono and mixed cultures of the three species. Each culture in BG 11 -N and with 6.45 µM total P was maintained 23 days since the last subculturing. Then, 600ml of culture was centrifuged (2 min at 2000 m s^{-2}, Stratos, Sorvall Centrifuge) three days before

198 the experiment. After centrifuging, the three cultures were diluted until 600 mL
199 using BG 11 -N -P. One day before the experiment, *R. raciborskii* culture was
200 diluted until 1.6 L with BG11 -N -P and the obtained OD750nm value was used
201 as reference to dilute the other two cultures. On the day of the experiment, mixed
202 cultures were prepared combining 225 mL of two of the cultures and 50 mL of the
203 third (e.g. 225 mL *D. circinale* + 225 mL *A. gracile* + 50 mL *R. raciborskii*, named
204 'Low *R. raciborskii*'). These different combinations were made to meet the
205 invasibility criterion of the current theory of coexistence, which states that stable
206 coexistence pre-supposes that all species, in a certain community, can recovery
207 from small abundance in a system under equilibrium (CHESSON, 2000). To
208 establish the equilibrium between two species before mixing the third would be
209 complex, mainly under P limitation. Therefore, to avoid adding an unaccountable
210 effect to the experiment, the three cultures were mixed just before the P pulses.
211 The P concentration (sterile ^{31}P + ^{33}P solution) applied in either 1P and
212 10P was 1.5 µM P, that was 1.6 times the total P of the diluted cultures. Also, to
213 check changes in the uptake system, 200 nM P final concentration was spiked to
214 the cultures before and after the pulse patterns (Pre- and Post-pulse,
215 respectively; Fig. 1). After the pulses, the procedure was done 1h20' after the last
216 pulse, collecting 11 mL of culture.
217 Finally, the cultures were redistributed in three culture flasks (triplicates)
218 and incubated under 12/12 light-dark cycle (80 µmol photons m^{-2} s^{-1}) and 27-28
219 °C. In day 3 of the experiment, samples were collected for OD750nm
220 measurements. Then, until day 11, samples were collected every two days. The
221 end of the experiment was at day 16. Samples were collected at days 0 and 16
222 for counts, and fixed with Lugol solution. Every day the cultures were gently
223 shaken and randomly repositioned in the experimental trays.
224
225 2.3.4 Experiment 3

226 Considering the proportion between total P and the P concentration added in the
227 pulse treatments, the two first experiments were performed under high P levels
228 (saturation experiments, see comments above). In the third experiment, we
229 assessed a non-saturating P pulse in monocultures. Cultures maintained during
230 53 days since the last subculturing were used for the experiment 3. The viability

of the cultures was evaluated before the starting of the experiment by checking the uptake activity of each culture and the general culture conditions under the microscope. The P uptake activity was assessed two times before the experiment, repeating the procedure of spiking 400nM P final concentration and measuring the remaining external P during the first minutes (see 2.3.8 Analysis of P uptake section). The linear slope of the P influx obtained was used to estimate the necessary time to deplete the P in the medium; which was then used to estimate the time span to repeat the P spike after the pulses.

Differently from the other two previous experiments, we did not centrifuge the cultures. In order to even the cultures biomasses, 200 mL of BG 11 -N -P was added to *R. raciborskii* culture and the estimated number of trichomes (based on OD750nm and counts relationship) guided the dilution of the other two cultures. The estimated number of trichomes and the OD750nm before the start of the experiment were: *D. circinale* - 18706.59 orgs mL^{-1}, 0.024; *A. gracile* - 10032.8 orgs mL^{-1}, 0.020; and *R. raciborskii* - 19071.72 orgs mL^{-1}, 0.032. The cultures were grown under the same conditions described for the other experiments.

The P concentration (sterile ^{31}P + ^{33}P solution) in the 1P and 10P was 2µM (61.94 µgP L^{-1}), approximately 2.2 times lower than the total P in the diluted cultures. As performed in the experiment 2, the uptake was measured before and after the pulses (Pre- and Post-pulses, Fig. 1), through spiking 200nM P final concentration and filtering the cultures at pre-determined time intervals. Lastly, the cultures were redistributed in three culture flasks (triplicates) and incubated under the same conditions described above. Samples for OD750nm measurements were done at days 0, 2 and 12.

2.3.5 Growth rate

The OD750nm data were used to calculate the growth rate according to the equation (WOOD; EVERROAD and WINGARD, 2005):

$$\mu^{-d} = \frac{\ln(\text{OD750nm final}) - \ln(\text{OD750nm initial})}{\text{T final} - \text{T initial}}$$

where initial and final correspond to the first and last days of exponential growth (i.e. linear increasing in OD750nm data when in log scale) in experiment 1, and to initial and final days in experiment 2 and experiment 3.

2.3.6 Trichome counts

In experiment 1, the number of trichomes was estimated using equations from linear regressions between number of trichomes and OD750nm, using data from the same strains (Fig. S1), and these estimates were then used to standardize APA. In experiment 2, the number of trichomes was counted in Lugol's fixed samples from days 0 and 16. The counts were made in an optical microscope (Opton), and at least 100 trichomes were counted in a Sedgwick-Rafter chamber. In cases which the abundance of trichomes was very low, the whole chamber was counted.

2.3.7 Alkaline phosphatase activity (APA)

In experiment 1, we assessed alkaline phosphatase activity (APA). Alkaline phosphatase activity was measured using the fluorescence method described in Ammerman (1993). The method consists of adding the MUF-P (4-Methylumbelliferyl Phosphate, Sigma Aldrich M8168) to the sample and compute the increase in fluorescence along the time as a result of the activity of alkaline phosphatases. The MUF-P is the substrate for alkaline phosphatases, and here we added 300 µL of MUF-P at 1mM to 2.7 mL of sample (final MUF-P concentration 0.1 mM). The fluorescence is dependent on high pH >10, so 1 mL of boric buffer was added to the solution. The excitation and emission wavelengths were 360nm and 440nm, respectively. The reads were performed in a fluorescence spectrophotometer (Cary Eclipse, Varian Inc.), every minute for ten minutes. The APA was calculated by first estimating the final MUF using the standard curve with MUF (4-methylumberlliferon sodium salt, Sigma Aldrich M1508), then dividing this estimate by the minutes passed (i.e. MUF nM min^{-1}). This rate was standardized by number of trichomes. Samples were collected from all triplicates at day 2, then every two days until day 10, and at day 16.

2.3.8 Analysis of P uptake

To estimate the amount of P uptake in the cultures we used a ^{33}P phosphate solution (American Radiolabeled Chemicals, Inc). Both the remaining external P in the cultures after the pulses and P uptake curves before and after the pulses were obtained following the steps: 1) spiking ^{31}P + ^{33}P solution and filtering the sample (2 mL in experiment 1, 1 mL in experiments 2 and 3) using polycarbonate filters; 2) collecting the filtrates (1 mL in experiment 1; and 500 µL in experiments 2 and 3) in scintillation vials; 3) adding 4 mL of previously diluted Ultima Gold™ cocktail (Perkin Elmer); 4) homogenizing the mixture using a vortex and reading in liquid scintillation analyzer (Tri-Carb® 2910TR, Perkin Elmer). ^{31}P at 0.2mgP L^{-1} (6.45 µM) was used to stop the ^{33}P uptake in two different ways in experiments 1 and 3. In experiment 1, 1 mL the ^{31}P was added before the filtration stops. In experiment 3, 1 mL of ^{31}P solution was applied just after the filtration finishes. In the case of uptake curves, the P spiked and time intervals of filtration for each experiment were: experiment 1 – spike of 1.07 µM, filtration every 15”, 30”, 60”, 1’30”, 2’ and 3’; experiment 2 – spike of 200 nM, filtration every 15”, 30”, 45”, 60”, 1’30” and 2’; experiment 3 – spike of 200 nM, filtration every 30”, 1’30”, 2’30”, 15’ and 45’.

2.3.9 Data analysis

Permutational ANOVA (function “lmp” from package “lmPerm”, WHEELER; TORCHIANO, 2016) was used to compare growth rates, total number of trichomes (in experiment 2) and APA between P pulse treatments. Linear regressions with permutations were also performed between number of trichomes and OD750nm. All these analysis and graphics were done with software R version 4.0.5 (R Core Team, 2021).

For the analysis of uptake rate in experiment 3, the data were adjusted using the flow-force model proposed by Falkner, Falkner and Schwab (1989), in software MLAB (Mathematical Modeling System, Civilized Software, Inc.). The model is based on the electrokinetic approach proposed by Thellier (1970), and describe the adaptive behavior of P uptake system (see 2.2 INTRODUCTION). Considering the energy provided by the flow of H^+ from the thylakoid to the cytoplasm, and the rate of formation of inert chain of phosphates (i.e.

polyphosphates) as P enters the cell, the final linear equation describing the P uptake rate (J_P) is (see FALKNER; FALKNER, 2011):

$$J_P = \frac{dP_e}{dt} = -L_P(\log[P_e] - \log[P_e]_A)$$

where L_P is the coefficient expressing the conductivity of membranes, $[P_e]$ the external P concentration, and $[P_e]_A$ the external P concentration at which the uptake ceases (i.e. threshold value).

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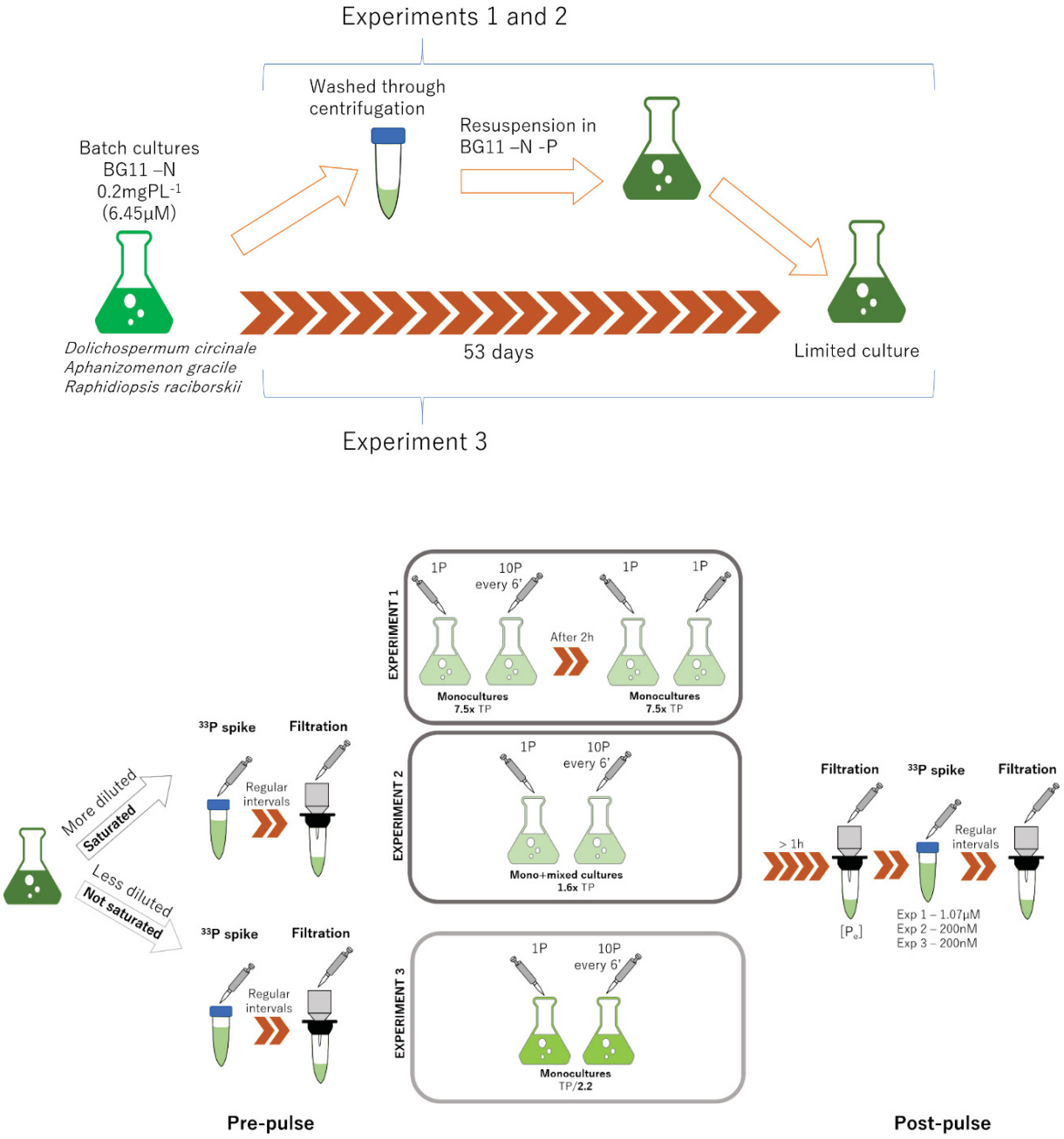

Figure 1 Scheme of procedures adopted in each experiment. Top: preparation of cultures for the experiments. Bottom: pulse treatments and measurement of P uptake in each experiment.

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2.4 RESULTS

2.4.1 Growth curves

Given the low initial cell concentration, the cultures had an extended lag phase before the start of exponential phase (Fig. 2). Given *D. circinale* have not started the exponential growth, the growth rate was not calculated. The calculated growth rates for the other two species were: 1P - *A. gracile* – 0.253±0.006 d^{-1} (days 10-18) and *R. raciborskii* 0.130±7.740215e-05 d^{-1} (days 18-29); 10P - *A. gracile* – 0.277±0.012 d^{-1} (days 12-18) and *R. raciborskii* 0.127±0.003 d^{-1} (days 18-29). The growth rate in 10P treatment was higher in *A. gracile* ($F_{1,4}$= 9.75, P = 0.0354).

In experiment 2, the OD750nm data had a great variation, and as in experiment 1, *D. circinale* did not grow (Fig. 3). The specific growth rate between the initial and final days of the experiment are shown in Table 1. The growth rates in 10P were higher than 1P in Low *D. circinale* and Low *A. gracile*. In Low *R. raciborskii* cultures, the 1P growth rate was higher than 10P. Unfortunately, the high dilution guided by the OD750nm favored *R. raciborskii* initial number of trichomes, which is coherent with its dominance in all mixed cultures. However, despite this advantage *A. gracile* achieved similar number of trichomes in Low *R. raciborskii* 1P (Fig. 4).

In experiment 3 (Fig. 5), there were not found significative differences between 1P and 10P (Table 1), although clearly there was trend of higher growth rates in 1P in *A. gracile* and *R. raciborskii*.

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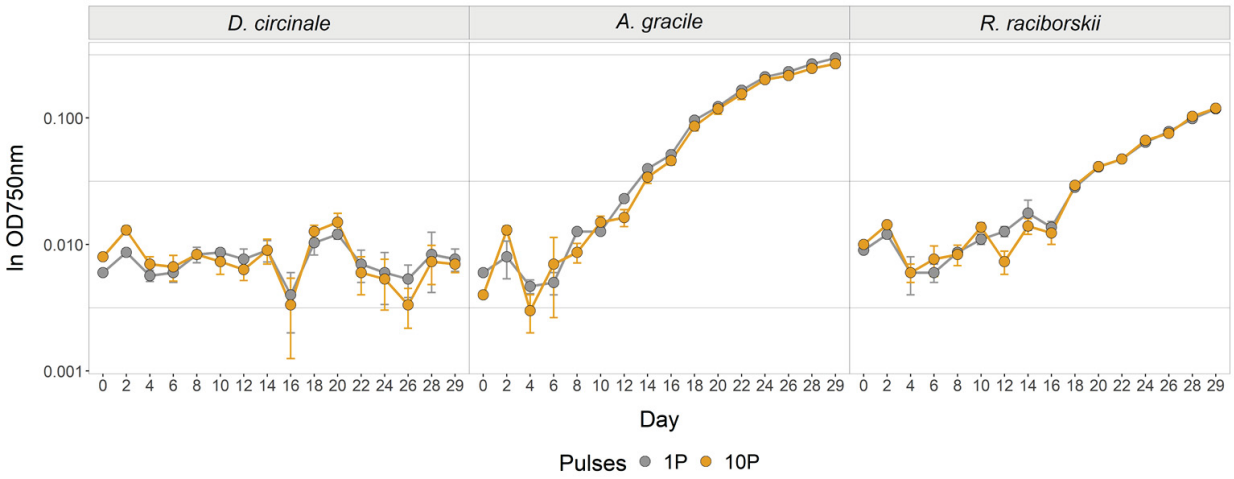

Figure 2 Growth curves obtained from averaged OD750nm data (n=3) in the experiment 1. The bars represent standard deviation.

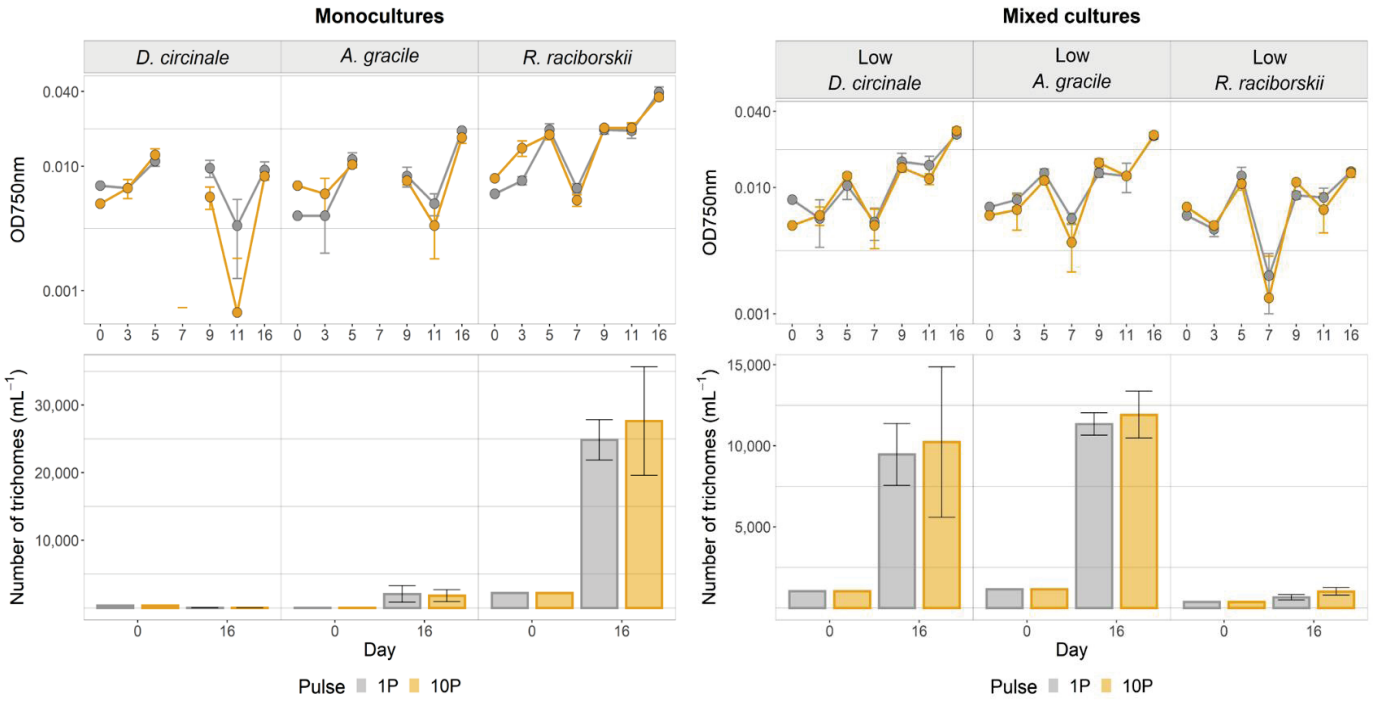

Figure 3 Growth curves obtained from averaged OD750nm data (n=3) and number of trichomes in the experiment 2. Left: monocultures, right: mixed cultures. Bars represent standard deviation. Note the different scales in number of trichomes. Missing values in OD750nm data are due negative values.

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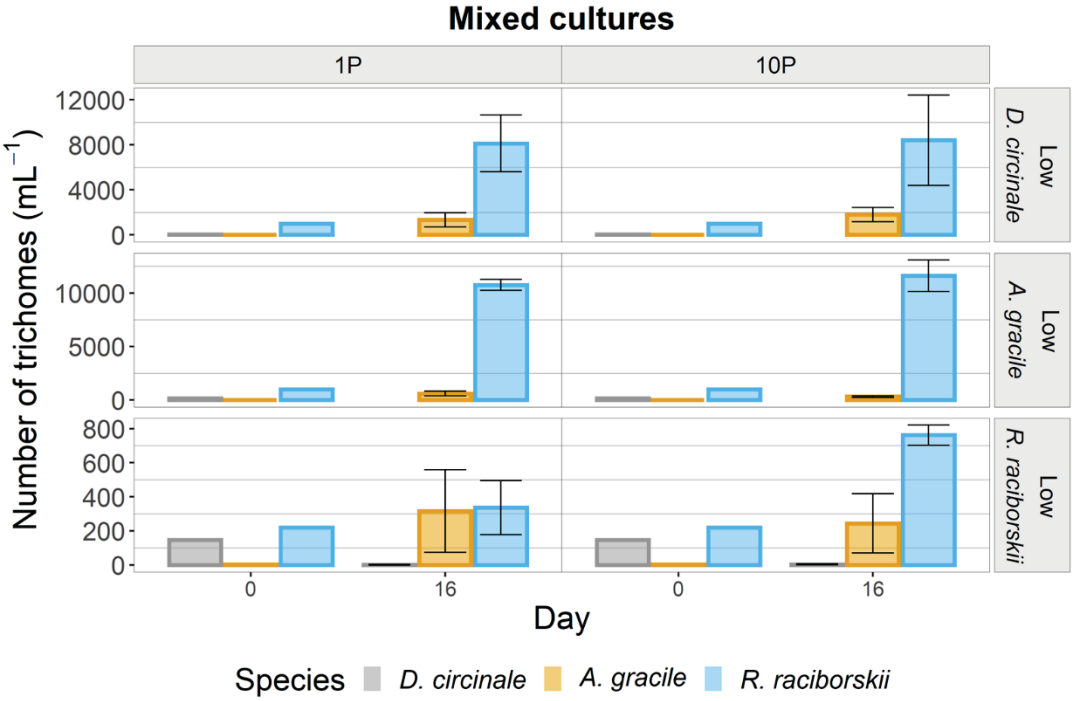

Figure 4 Average of number of trichomes from each species in mixed cultures of experiment 2 (n=3). Standard deviation is also shown. Note the different scales.

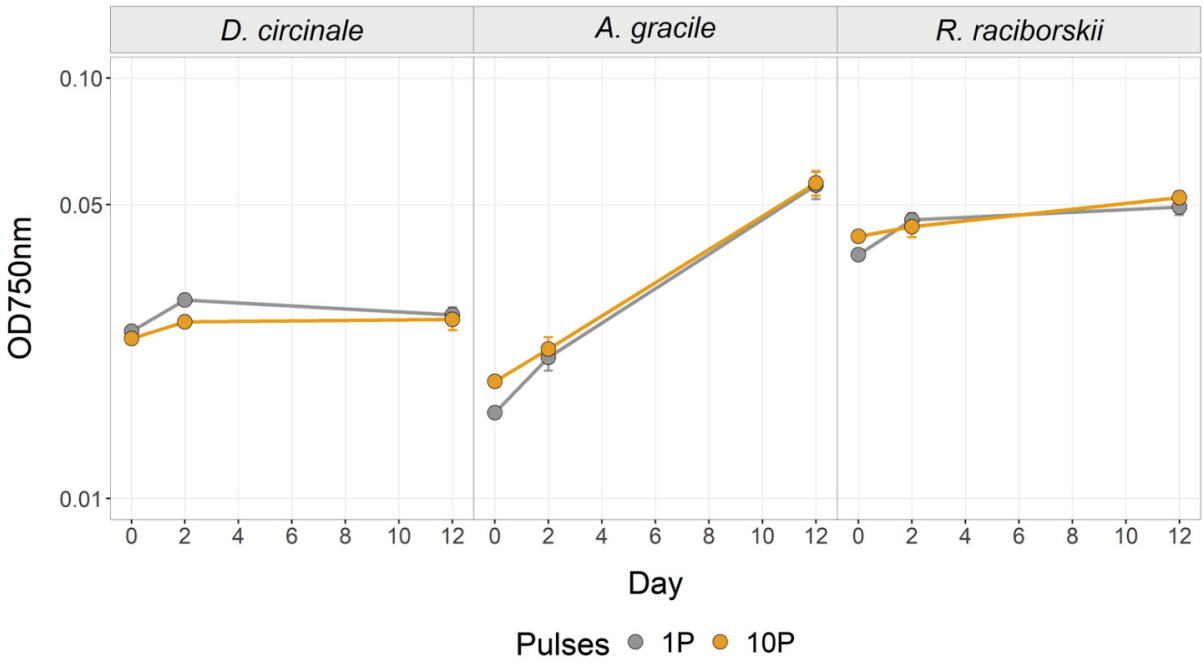

Figure 5 Growth curves of experiment 3, from averaged OD750nm data (n=3). Bars are the standard deviation.

361 Table 1 Average specific growth rates (i.e. final-initial) calculated in experiments 2 and
362 3. No growth rate was calculated for *D. circinale* in experiment 2 because the absence of growth. Results of ANOVA with permutations comparing 1P and 10P are also shown.
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			1P	10P	1P *vs* 10P
EXPERIMENT 2	Monocultures	*A. gracile*	0.098±0.002 d^{-1}	0.078±0.002 d^{-1}	$P > 0.05$
		R. raciborskii	0.110±0.007 d^{-}	0.099±0.007 d^{-1}	$P > 0.05$
	Mixed cultures	Low *D. circinale*	0.074±0.002 d^{-1}	0.107±0.004 d^{-1}	$F_{1,4}=118.9$, $P=0.0004$
		Low *A. gracile*	0.081±0.004 d^{-1}	0.092±0 d^{-1}	$F_{1,4}=23.48$, $P=0.0083$
		Low *R. raciborskii*	0.05±0.003 d^{-1}	0.038±0.005 d^{-1}	$F_{1,4}=12.62$, $P=0.0237$
EXPERIMENT 3		*D. circinale*	0.007±0.003 d^{-1}	0.009±0.005 d^{-1}	$P > 0.05$
		A. gracile	0.104±0.006 d^{-1}	0.091±0.006 d^{-1}	$P > 0.05$
		R. raciborskii	0.022±0.003 d^{-1}	0.017±0.003 d^{-1}	$P > 0.05$

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2.4.2 APA

The APA measured in experiment 1 is shown in Fig. 6. Data for *D. circinale* cultures are omitted because the absence of growth, and the consequent negative values in APA. Despite the very low initial inoculum in *A gracile*, the APA was usually in high levels than in *R. raciborskii*. No difference between 1P and 10P was found in *A. gracile*. In *R. raciborskii*, 1P differed from 10P in days 2 ($F_{1,4}$=22.11, *P*=0.00929) and 10 ($F_{1,4}$=15.98, *P*=0.016). In both cases, higher APA was associated to 10P. Interestingly, APA increased in the first days and then reduced, coinciding to the start of exponential growth.

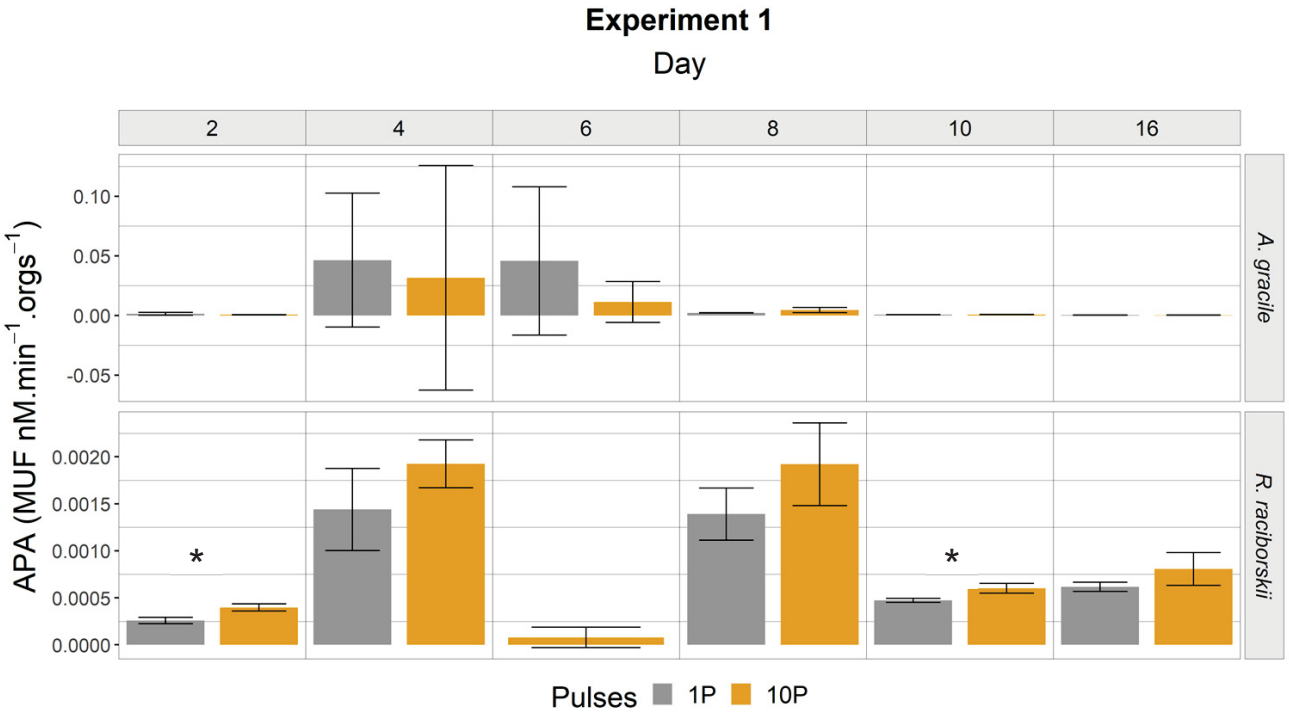

Figure 6 Alkaline phosphatase activity (APA) standardized by number of trichomes in experiment 1. The bars are averages (n=3), and standard deviation is also indicated. Note the different scales. Asterisks represent significative difference between 1P and 10P.

2.4.3 P uptake

The uptake data obtained from experiment 1 confirmed the saturation condition provoked by the pulses (Fig. 7). Despite that, a small difference can be observed in the remaining P after 2h of the first pulse. On day 10, the external P reduced

383 in comparison to day 0. The P levels were above 5000nM, and as also indicated
384 by the growth curve, at this point there was no P limitation to growth.
385 In experiment 2, we also confirmed the P saturation, and pre pulse curves
386 show negligible uptake (Fig. 8). As in experiment 1, although the saturation
387 condition, a slightly higher amount of P remained in treatment 1P, both
388 monocultures and mixed cultures. On day 16, except for *D. circinale*, the
389 dissolved P in the cultures was below 30nM, what is coherent with the suggested
390 start of the exponential growth from OD750nm. Moreover, at day 16 the cultures
391 showed a subtle active uptake, which suggests that the P spiked was higher than
392 the threshold established in the cultures at this point.
393 In the third experiment, before the pulses the three species confirmed the
394 highly active uptake (Fig. 9), therefore the P uptake kinetics could be evaluated
395 with flow-force equation. Different kinetic parameters were attained after the
396 pulses (L_P and $[P_e]_A$; Table 2), but in both *A. gracile* and *R. raciborskii* cultures
397 the threshold values attained in 10P were lower than in 1P. After 1h30 of the last
398 pulse, the remaining external P was higher in 10P in *R. raciborskii* culture; and
399 the opposite in *A. gracile* (for *D. circinale* the sample for remaining P after pulses
400 was lost). Interestingly, in *D. circinale* the uptake changed greatly, and it did not
401 conform to the flow-force model, instead the data fitted a linear regression (1P –
402 $F_{1,3}$=60.16; P=0.0044; 10P – $F_{1,3}$=474.5; P=0.0002).

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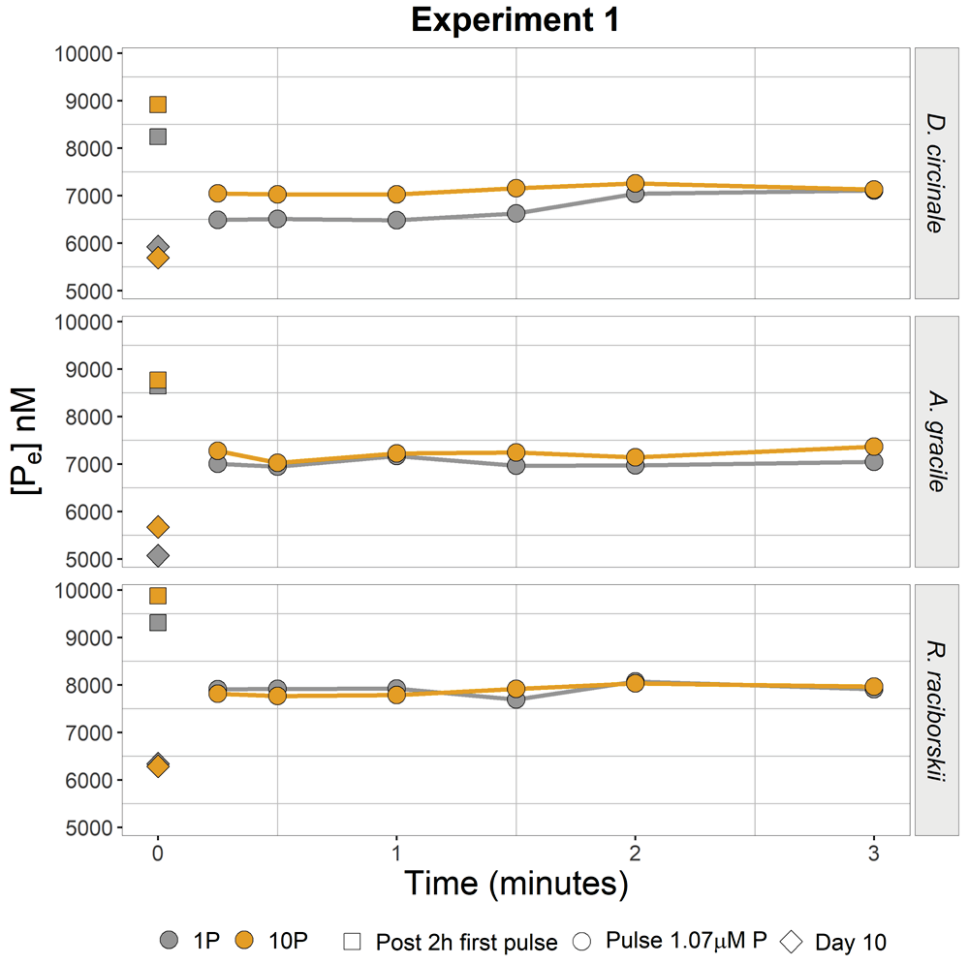

Figure 7 Remaining external phosphorus in experiment 1 in three distinct situations. After the pulses, during the second addition of P and following the external P for 3 minutes, and the remaining external P at day 10.

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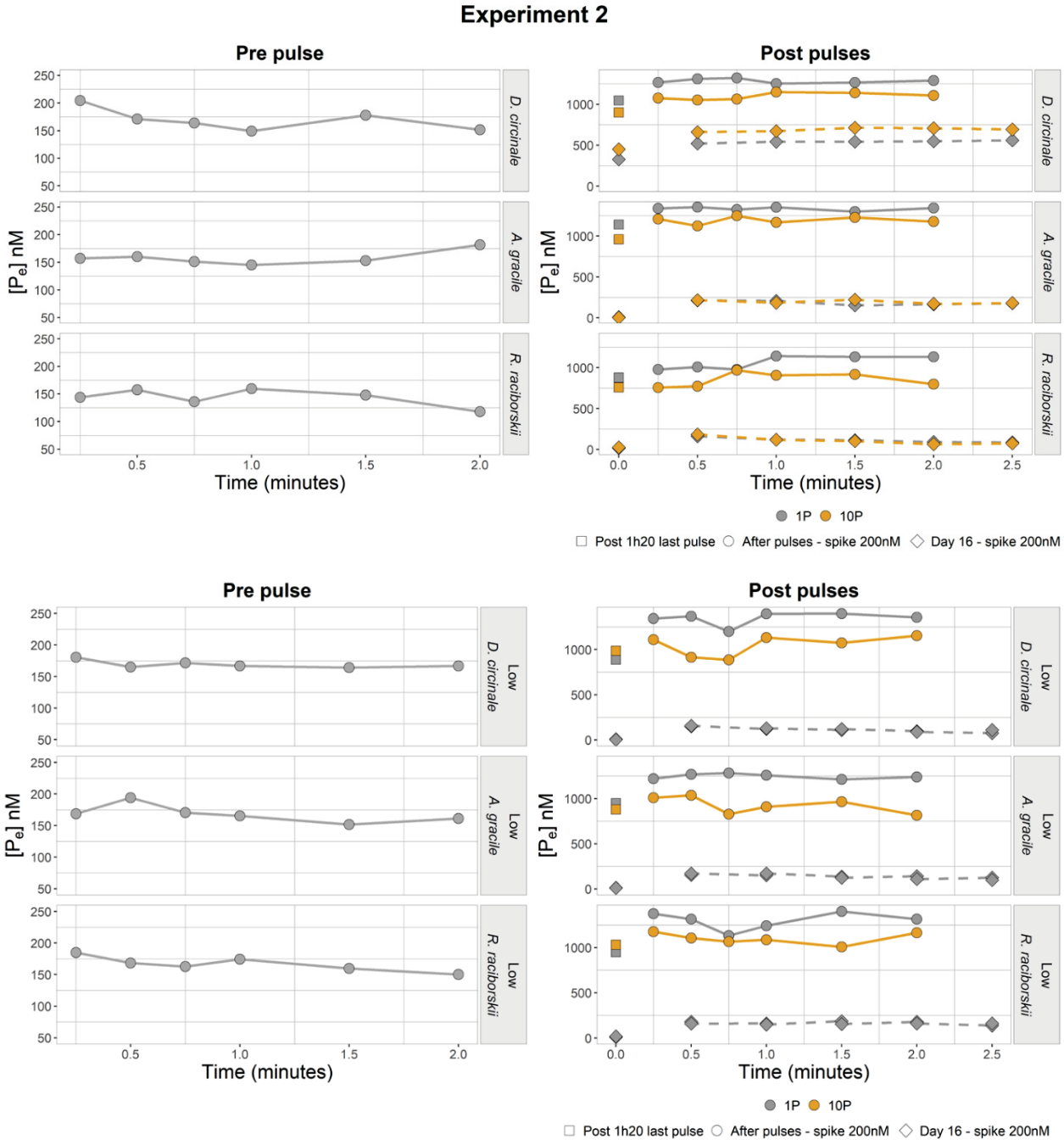

Figure 8 Remaining external P in experiment 2, in monocultures (top) and mixed cultures (bottom), before (left) and after (right) the pulses. After the pulses, the data shown correspond remaining P after the pulses and after spiking 200nM in day 0; and the remaining external P and repeating the 200nM spike at day 16.

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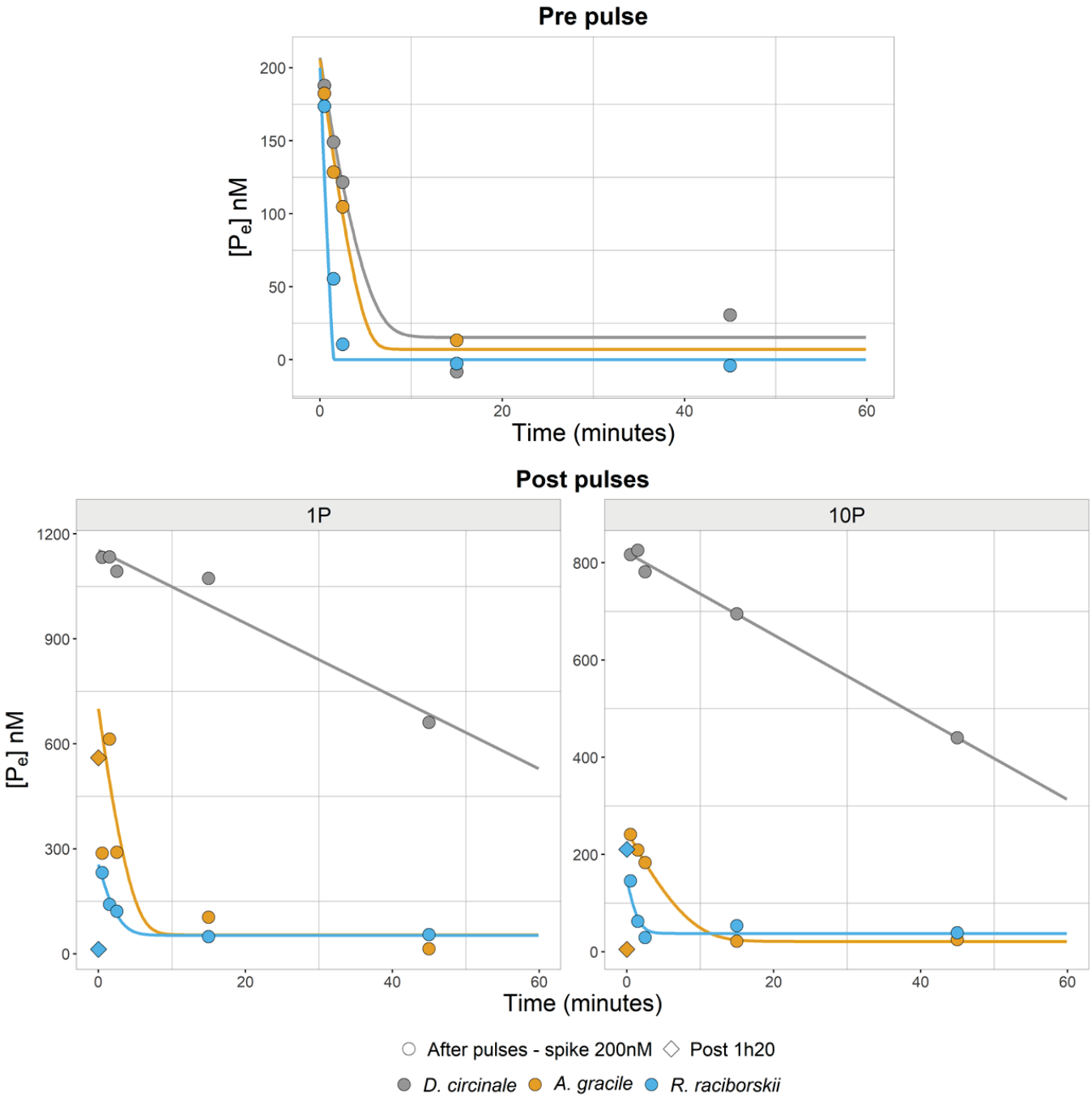

Figure 9 Uptake curves as the reduction in external P concentration in experiment 3, before (top) and after the pulses. The lines are the flow-force model adjusted to the data. Also are indicated the remaining external P after pulses and before spiking 200nM. Note the different scales. For each curve, R^2 values were: Pre pulse – *D. circinale* R^2 = 0.98; *A. gracile* R^2 = 0.99; *R. raciborskii* R^2 = 0.97; Post 1P: *D. circinale* R^2 = 0.94; *A. gracile* R^2 = 0.87; *R. raciborskii* R^2 = 0.97; Post 10P: *D. circinale* R^2 = 0.99; *A. gracie* R^2 = 0.99; *R. raciborskii* R^2 = 0.82.

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Table 2 Parameters estimated by the flow-force model adjusted to data from experiment 3, before and after the pulses (2 µM). In both cases, 200nM was spiked and the reduction in external P followed. Units: L_P – nM.min^{-1}, $[P_e]_A$ - nM.

	Pre pulse		**Post 1P**		**Post 10P**	
	L_P	**$[P_e]_A$**	**L_P**	**$[P_e]_A$**	**L_P**	**$[P_e]_A$**
D. circinale	14.86	15.33	-	-	-	-
A. gracile	14.13	7.16	57.80	54.04	11.56	21.18
R. raciborskii	20.57	<1nM	48.81	52.71	53.36	37.65

2.5 DISCUSSION

In this study, we aimed to investigate the role of saturated and non-saturated P fluctuations on mixed and monocultures of three species of freshwater Cyanobacteria. Our findings reinforce the diversity of processes that might be involved in population growth, responding to P pulses even when there is no P limitation. Thus, in real ecosystems, the outcomes of species interactions might also be reflecting periods of no P limitation, thus extending the temporal range of coexistence mechanisms. The species were affected differently regarding the pulses, *A. gracile* was usually favored in 1P, and *R. raciborskii* in 10P, considering the mixed cultures in experiment 2. In addition, under saturated conditions, we also found differences between 1P and 10P in growth rates and APA, and subtle difference in the uptake system. Under limited conditions, the species were similar in kinetic parameters before the pulses, but diverged after the pulses. This probably reflects distinct groups of proteins with dissimilar kinetics, present in each species, and conforming different growth responses after the disturbance caused by increase in external P concentration.

Different growth rates between pulses in experiments 1 and 2 are unexpected considering the non-limiting P status. In discontinuous cultures, P acquisition is temporally separated from growth. This leads to a link between the growth rate and the previous context when P was acquired. Therefore, the changes in P uptake promoted by distinct patterns of P availability have the potential to induce different growth rates (AMARAL; BONILLA and AUBRIOT, 2014). In this context, the saturated pulses are not expected to induce different P adjustments because both 1P and 10P will expose the cells to high P concentration for enough time to lead to similar adaptive responses (FALKNER; PRIEWASSER and FALKNER, 2006; AUBRIOT; BONILLA, 2012), and subsequent similar growth rates. However, our results suggest that some initial responses to 1P and 10P, not usually considered, are different enough to result in different growth rates. Also, responses are probably dependent on the presence of other species, as evidenced by the differences between monocultures and mixed cultures in experiment 2 ; which raises questions about the direct use of parameters from monocultures to predict multiple species scenarios. These growth responses contrast with the very similar levels of

444 remaining P and uptake curves between 1P and 10P, repeated in the middle of
445 experiment 1 and end of experiment 2. Our results extend the range of the effect
446 of P patterns and flexible P uptake adjustments, not only under P deficient
447 condition but also to ambient high P levels.

448 Alkaline phosphatase activity is expected to increase as a response to P
449 limitation (LI; DITTRICH, 2019). Since the cultures were saturated in either 1P
450 and 10P in experiment1, it is again surprising the difference in APA found
451 between pulses. Other studies reported constant expression of some alkaline
452 phosphatase genes irrespective of P load and the decoupling of enzymatic
453 activity from gene expression for these enzymes (LIU; WU, 2012); others
454 reported deviations from the SRP-APA negative relationship (HEATH; COOKE,
455 1975). The distinct APA found regarding the pulses, highlight the intricated
456 functioning of these enzymes and the possible roles they play beyond P supply
457 (e.g. regulation of organic compounds which serve as messengers; HEATH,
458 1986; supply of carbon in heterotrophic aquatic bacteria; SIUDA; CHRÓST,
459 2001). Furthermore, APA might be dissimilar between species under same
460 conditions (MATEO et al., 2006). In our case, APA was consistently higher in *A.*
461 *gracile* than in *R. raciborskii*. This is in accordance with previous works describing
462 concomitant dominance of *Aphanizomenon* and high APA (HEATH; COOKE,
463 1975; SCHOFFELEN et al., 2018). The reduced N_2-fixing efficiency in
464 comparison to other species (DE NOBEL et al., 1997) could be pointed out as
465 the reason for increased APA, but considering the absence of P limiting condition,
466 our results reinforce the above-mentioned alternative roles of alkaline
467 phosphatases, not directly linked to inorganic P levels.

468 In the third experiment, the condition of no saturation allowed the
469 observation of the changes in the parameters P uptake system, both between
470 treatments and species. Each species had different responses regarding the
471 uptake rates and the P threshold values. *Dolichospermum circinale* had the
472 greatest change in P uptake after the pulses, and the flow-force model could not
473 be adjusted to the data. Thus, for this species was not possible to estimate the
474 threshold value. Even though, considering the general trend of the data, we can
475 suppose the remaining P was higher in 1P. This agrees with a previous study
476 using a species from the same genus (FALKNER; PRIEWASSER and FALKNER,
477 2006). In the case of *A. gracile*, the phylogenetic proximity to *D. circinale*

(GUGGER et al., 2002) reinforce the expectation of similar responses to P pulse, and it was the case. The growth of *A. gracile* in 1P in Low *R. raciborskii* in experiment 2, and the trend of higher growth rates in 1P in experiment 3, suggest that under 1P this species is more prone to growth and use P in an efficient way. In *R. raciborskii*, the very low threshold and minimum time to achieve it before the pulses, support previous reports of high P affinity in this species (ISVÁNOVICS et al., 2000). Similar to *A. gracile*, in *R. raciborskii* the threshold increased in 1P and the growth rate tended to be higher than in 10P. The difference in threshold between 1P and 10P was smaller than in *A. gracile*, which could suggest a longer reaction time than *A. gracile* (see AUBRIOT; BONILLA, 2012). However, the similar increase in growth rate in both species suggest that *R. raciborskii* probably has not a longer reaction time than *A. gracile*. The increase in thresholds after the pulses suggest that in our experiment the higher exposure time, which could not be directly evaluated, was achieved under 1P in accordance with results of Falkner, Priewasser and Falkner (2006) and in experiments with initial high P pulses in Aubriot, Bonilla and Falkner (2011). In regard to another study reporting enhanced growth rate in *R. raciborskii* cultures subjected to 10P in comparison to 1P (AMARAL; BONILLA and AUBRIOT, 2014), our results provide evidence that it is not the number of pulses *per se* but the initial concentration and the P uptake rate that determine the exposure time to ambient P. In addition, contrasting results can also emerge from intraspecific variation (XIAO et al., 2020) and experimental conditions (e.g. degree of P limitation, order of pulses, concentration of pulses).

Regarding the effects of pulses in natural communities, changes towards enhanced threshold were associated to both single (AUBRIOT; BONILLA and FALKNER, 2011) and multiple pulses (AUBRIOT; BONILLA, 2012) in samples from the same lake but collected in different dates. The dissimilar responses to one or multiple of pulses might be attributed to changes in community composition and/or in the total P concentration in relation to P uptake rate, making direct comparisons tricky. Despite the highly variable responses of the populations and communities, it is interesting the convergence of the curves we found before the pulses. This suggests shared ‘attractors’ of the uptake systems under P starved conditions, in analogy to the ‘attractors’ which would be driving the compartments of uptake system towards stability, as proposed by Falkner,

Wagner and Falkner (1996). A future insightful research topic would be the role of organic P forms and the stability of a certain adapted P uptake.

Recently, Kamennaya et al. (2020) demonstrated the accumulation of P in the periplasm of marine cyanobacteria through electrochemical gradient generated by monovalent protons. The complex formed by the proton and phosphate would prevent the leakage back to environment, while still allowing the interaction with enzymes in cytoplasmic membrane to transport the P to inside the cell. Thus, the marine cyanobacteria would have a mechanism to concentrate P in the periplasmic membrane. In the context of P uptake adjustment, this P concentration in the periplasm could work as another mechanism to maintain the stability of a certain uptake adapted state, beyond the presence of membrane proteins with distinct kinetics. The validity of this hypothesis, and the interaction with the use of organic P, needs further investigation.

2.6 CONCLUSIONS

Here, we evaluated the P uptake, growth and species interactions under different pulse patterns, both in saturated and limiting conditions. Studying the processes under contexts in which the responses are less evident might improve our understanding of the dynamics of natural communities (SIUDA; CHRÓST, 2001). We found effects of P pulses on growth rates, APA and relative abundances of mixed cultures even under saturated conditions, and it might be important in eutrophic lakes or situations when high inflow of nutrients enter aquatic bodies. Usually, 1P favored *A. gracile* and 10P favored *R. raciborskii* in mixed cultures, but both seemed favored in 1P in monocultures in limited condition. Our results emphasize the dependency of species responses on experimental conditions and highlight the uncertainties associated to predictions involving species interactions but based on monospecific experiments. Different exposition times can be achieved by choosing distinct P pulses and submitting the organisms to distinct levels of P limitation. Interestingly, before the pulses the kinetics parameters were similar among the species, which might suggest convergence of physiological processes under limited conditions and divergence as it is alleviated. This reinforces the existence of energetic constraints shared by species under the same environmental conditions. And in this sense, we could expect convergence

545 of P uptake machinery in organisms from the same environment, possibly
546 favoring the persistence of species under high resource limitation. Finally, the
547 complexity and flexibility of species responses to P fluctuations found here
548 expands the scenarios where species coexistence is possible.

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718 2.8 SUPPLEMENTARY MATERIAL

719 Phosphorus pulses modulating cyanobacteria interactions under saturated and
720 non-saturated conditions

721 Elena Fukasawa Galvanese, Ann Chuang, Luis Aubriot, André Padial, Michele
722 Burford

723

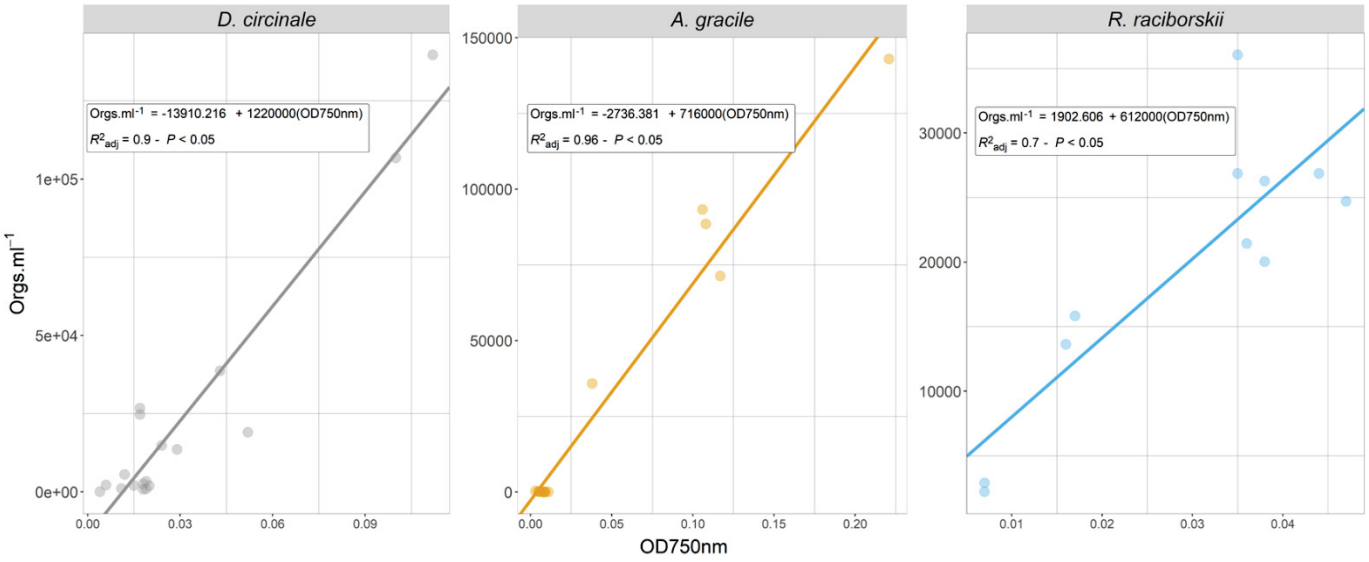

Fig. S1 Linear regression of optical density at 750nm and number of trichomes used to dilute cultures in experiment 3.

CAPÍTULO 3

WHAT IF THE PHOSPHORUS FORM MATTERS? TOWARDS BETTER UNDERSTANDING OF HOW PHOSPHORUS CAN SHAPE POPULATIONS AND COMMUNITIES

What if the phosphorus form matters? Towards better understanding of how phosphorus can shape populations and communities

Elena Fukasawa Galvanese, André Padial, Luis Aubriot

3.1 ABSTRACT

Resource competition is expected to be the major driver of community structure. Once established, ultimately is the capability of a species to maintain its population in face of limited resources and competitors that will determine its permanence in a given location. Many relationships between resource consumption and community diversity have been proposed along the time, but it remains surprising the persistence of multiple species under known resource limited systems. That is the case of microorganisms in the aquatic environment, usually limited by the same nutrients at least part of the time. Altogether, the high flexibility in phosphorus (P) acquisition, the capability of change the uptake kinetics and, the diverse use of organic P make traditional models describing growth and P availability inadequate. Here, we suggest the understanding of P limitation, and the role of its availability on populations and communities, would be benefited by approaching it like the light harvesting systems. The presence of different photosynthetic pigments, which allow the cells to capture light from different wavelengths is paralleled to the use of inorganic and organic P forms. To that, we briefly reviewed the main concepts, and discussed theoretically the relationship between the two forms of P as a single nutrient or as a ratio between organic and inorganic forms.

Keywords: cyanobacteria, competition, physiological flexibility, species diversity, P uptake

3.2 INTRODUCTION

Organisms are expected to born, grow, reproduce and die. Although obvious, the phenomenon of life cycle is not completely understood in both micro and macro scales. On micro scales, the regulation of many genes and the interactions between the environment and the DNA expression are still unknown (GIBSON, 2008). On macro scales, the drivers of diversification and the patterns of species' distributions are not fully understood (SCHLUTER; PENNELL, 2017) though it is well established the use of large scale data to predict and explain species distributions by niche modelling (SOBERÓN; 2007). Despite the great advances provided by the theory of natural selection developed by Darwin and Wallace, recent studies have challenged the basic definitions of biological entities such as 'species' (e.g. FIGUEIRÓ et al., 2017), and even the evolutionary mechanisms have been reevaluated in a new framework (i.e. the Extended Evolutionary Synthesis, LALAND et al., 2015), emphasizing the need to constantly improve the relationship between empirical findings and theoretical frameworks.

The evolutionary history of organisms might seem to one harder than the study of their current distributions. Nonetheless, it can be surprisingly hard to investigate species distributions and go beyond the description of occurrences, in a way that provide testable mechanistic explanations for the observed patterns. In fact, Ecology as a discipline is under great debate about how generalizable the observed patterns are (LINQUIST et al., 2016).

The first and basic approach to begin to explain why a species is where it is found is surveying its abiotic and biotic constraints. The abiotic constraints comprise the chemical and physical conditions which the organisms cannot overcome to remain in certain area considering its physiological tolerance. The biotic constraints refer to the interactions between organisms, and in general those interactions are classified as competition, predation, parasitism or symbiosis. Together, the abiotic and biotic constraints constitute the niche of a species (GRINNELL, 1917; HUTCHINSON, 1957). And, although the biological interactions are considered strong enough to restrict species distributions more than the physiological tolerances, we are still stunned by the high biological diversity and trying to decode the enigma of what mechanisms allow the coexistence of multiple species.

Here, we do not intend to solve entirely the enigma. Rather, we revisited some basic concepts and present a more integrated and dynamic scenario of resources and species interactions using cyanobacteria and phosphorus. Our aim is to propose a different theoretical perspective on the relationship between resource usage and population growth that might help deeper the understanding of which drivers and how they might be modulating species coexistence and consequently biodiversity patterns.

3.3 FIRST THINGS FIRST: HISTORICAL CONTEXT AND FUNDAMENTAL CONCEPTS

3.3.1 Theoretical and mathematical development

Mathematically, the definite growth of a population in a limiting resource scenario was expressed before Darwin, by the Belgian mathematician Pierre-François Verhulst (1838). Based on Malthus idea of geometric growth and using human population data, Verhulst proposed what would be latter known as the logistic curve of growth. The asymptote of the logistic curve represents the carrying capacity (K), i.e. the maximum number a population can reach under certain conditions. The constant challenges imposed by the factors limiting population growth was called “struggle for existence” by Darwin and Wallace (DARWIN; WALLACE, 1858).

The “struggle for existence” is the core of Darwin’s and Wallace’s ideas, and it depends on: i) the resources limiting the population, i.e. intraspecific competition, ii) the interspecific competition and the iii) top down control. The first attempt to describe and understand the dynamic of two populations interacting using the “struggle for existence” framework was made by Ronald Ross (1908). Ross studied the malaria disease and solved the mystery of parasites transmission, which is through the mosquitoes. He also proposed the calculus of the rate of infection in a time period based on populational data from human and mosquitoes, and probabilities of interactions between them, which finally resulted in a reduced equation showing the conditions of increase and decrease of the rate of infections.

The second great step made in community dynamics were the formulations describing a system composed by two logistic curves interacting,

developed by Alfred J. Lotka in 1925 and Vito Volterra in 1926 (SCUDO; ZIEGLER, 1978). The same mathematical framework can be used to competitive and predatory interactions (e.g. MACARTHUR, 1972) and, basically, the equations express the effects of one individual from a species on the growth of an individual from the other species. Even though using the basic description of population growth, the system of equations has limitations. For example, Kostitzin (1940), one of Volterra's collaborator, highlighted temporal and spatial variability of some coefficients, e.g. natality, mortality and competition; and the growth curve itself. And indeed, Lotka and Volterra proposed ways to make the models more biologically realistic. For instance, Volterra (1927) included environmental variations; and Lotka (1932) permitted to vary some coefficients assumed constant by Volterra, making possible the coexistence of species.

It is interesting that until this point, the theoretical development was made through propositions of 'supposed' mechanistic models. The models do not explicitly account for the biological variations in response to environmental changes (FLYNN et al., 2015), and therefore treat the organisms as completely predictable units. For example, though some variability can be modelled by the equations, phenotypic plasticity is not explicitly assumed.

3.3.2 Experimental and empirical development

Following the great theoretical and mathematical advance described above, probably the major experimental advance on species interaction was made by Georgii Frantsevich Gause. Gause (1934) performed many experiments using yeast, protozoan and bacteria organisms to disclose what makes a species victorious upon other, in a pursuit of understanding, in biological and physiological terms, of the original "struggle of existence" presented in Darwin's book. In his investigations, Gause established logistic curves of monospecific cultures and then estimated the coefficients to be used in the differential equations of mixed cultures. Although not among the most mentioned results, there were two interesting findings in Gause's experiments: i) other factors than resource availability might work as limiting factor, adding more complexity to the system and, ii) changes or differences in mathematical coefficients might also be related to distinct states in the system, which means the context-dependence of

interactions. Despite the concerns expressed by Gause about generalization of the results, Gause is considered the father of the “principle of contention”, defined as “two species with similar ecology cannot live together in the same place, and the bearing of this, if true, on the origin and persistence of species” (HARVEY, 1945).

Corroborating empirically the “principle of contention”, we can mention the study published by Charles S. Elton (1946). Elton reported low frequencies of congeners in the same habitat, both plants and animals. Even though less common, he also found cases of congeners inhabiting the same habitat. Interestingly, Elton pointed out a pivotal issue in Ecology: the scale. Without knowing the environment and how species use it, the definition of a certain habitat is arbitrary.

3.3.3 (Re) defining the conceptual problem

A few years after the definition of the “principle of contention”, George Evelyn Hutchinson (1957) redefined the set of necessary conditions to allow the persistence of a given species. Hutchinson proposed a wider definition of niche, that he called “Volterra-Gause principle”, considering not just a specific limiting resource inhibiting species to coexist, but the group of conditions required for the persistence of species that might overlap among them. In this overlapping region, only the superior competitor should be present. Furthermore, he also stressed the difficulties of directly identifying competition in natural communities and the cases which the “Volterra-Gause principle” should not work. The cases stated by him are concerned about random occupation of habitats by the less superior competitor, allowing its persistence; the intraspecific territorial disputes and, the scenarios where the environmental changes are faster than the competitive outcome, preventing the complete exclusion of the inferior competitor.

Following Hutchinson, in 1960 Garret James Hardin termed the “competitive exclusion principle” and explicitly defined it. The definition gave by Hardin was “complete competitors cannot coexist” or “ecological differentiation is the necessary condition to coexistence” (HARDIN, 1960). Just a year later, Hutchinson published another article, entitled “The Paradox of the Plankton” (HUTCHINSON, 1961). He presented the problem of no empirical confirmation

of the competitive exclusion principle in plankton communities, commenting some possible explanations to the paradox: niche differentiation, stimulatory effect of one species upon other or selective predation. Although they are plausible explanations when considering terrestrial systems, none of them can satisfactorily be applied to plankton communities, given the homogenization of the environment, the higher number of species involved and the non-equilibrium condition. The non-equilibrium condition, according to Hutchinson, is when the time of environmental change and the time necessary to complete exclusion are approximate (HUTCHINSON, 1953). If the environmental changes would be determining the species presence, then it would be also very clear the extinction in sedimentary deposits (in the case of diatoms), which is not the case. Besides, Hutchinson states that the continuous sourcing of species from other regions would not explain the regularity of the records.

It is interesting to note that, the “struggle for existence” driving populations as mentioned previously, changed slightly to the requirement of niche differentiation to allow coexistence of species due the “competitive exclusion principle”. Indeed, Chase and Leibold (2003) and Pocheville (2015) highlighted the close link made along the time between niche and competition theories. And probably, this link helps to understand the great role attributed to competition in structuring communities (TILMAN, 1982).

George David Tilman is probably the most famous researcher who experimentally evaluated species competition, performing experiments with different biological groups. When studying freshwater diatoms, he found that two species might stably coexist when limited by different resources, but not when both are limited by the same resource. In this case, one species will exclude the other and Tilman predicted the prevalent species from monospecific cultures, through estimating the species capable of surviving under the lowest resource level (i.e. R*) at a given mortality rate (TITMAN, 1976).

After Tilman’s investigations, what was looking like a set of evidences, gained a higher status. It was shown not just the relationship between resources and population growth, but also the possibility of predicting the outcome of species interactions based on their requirements. Understanding the important role of competition found experimentally and the explicative power for field data (TILMAN, 1977), it is comprehensible that the modern theory of coexistence

proposed by Peter Chesson (2000) is based on the mechanisms which modulate the competition among species. These mechanisms are classified in two main groups: mechanisms reducing fitness differences are called equalizing; and mechanisms related to increase intraspecific competition in comparison to interspecific competition, are termed stabilizing (see Spaak and De Laender (2020) for new mathematical definitions of niche and fitness differences).

Although the competition is the core of most theories involving biological diversity, it is important to highlight studies conducted mainly by Daniel Simberloff in islands, showing the variable role of species interaction along the time on the number of species during colonization (SIMBERLOFF; WILSON, 1969), observed number of species similar to the expected by a random distribution (CONNOR; SIMBERLOFF, 1979), and the theoretical and methodological problems in studies and investigations assuming the competition as the best explanation to community diversity and structure (SIMBERLOFF, 1983).

3.4 CYANOBACTERIA AND PHOSPHORUS

Microorganisms are great models to study the links between species requirements and tolerances (i.e. fundamental niche, *sensu* HUTCHINSON (1957)), and the possible outcomes, i.e. coexistence or competitive exclusion, when the resources are shared in the presence of other species. Besides the convenience in manipulation, microorganisms such freshwater cyanobacteria impose practical challenges to human society, e.g. deteriorating water quality and toxin production, making fundamental the study of the factors promoting its growth and persistence. Indeed, many authors investigated the cyanobacteria and the phytoplankton community regarding the limiting resources (e.g. AGUILERA et al., 2017; BURSON et al., 2018) and mainly through the comparison of parameters from Monod (e.g. ISTVÁNOVICS et al., 1992; HOFMANN et al., 2021) and Droop equations (e.g. SOMMER, 1991; ISVÁNOVICS et al., 2000).

The macronutrients phosphorus (P) and nitrogen (N) are the most frequent investigated nutrients in aquatic systems, although they are not always the main limiting factors (STERNER, 2008; HOFMANN et al., 2021). Despite the huge effort devoted to study the dynamics of these nutrients and microorganisms'

responses, some limitations of the classical Monod and Droop approaches, together with characteristics of P acquisition systems, might be undermining a comprehensive understanding of community dynamics. Before discussing those limitations, we will start by stressing the basic classification of organic and inorganic P according to their relationships with growth isoclines, following Tilman (1982).

3.5 ORGANIC OR INORGANIC: IS THAT THE QUESTION?

Tilman (1980, 1982) proposed a graphical classification of pairs of resources, based on its relationship with growth isoclines. The growth isoclines represent the conditions in which natality and mortality are equal, therefore when the population growth is zero. From this representation, different species isoclines can be compared in relation to the same pairs of resources. Basically, pairs of resources can be substitutable, essential or hemi-essential (Fig. 1). When two resources are substitutable, it means a population is able to survive when one of the resources is not available. If both are required at certain minimal level, they are said essential. If one resource is nutritionally complete and the other is not, the last being dismissible, they are called hemi-essential. Inside each category, there are also different curves, depending on the way the resources are consumed (e.g. if they can be acquired together or there is negative or positive effects when consumed together). So, back to our focus on P, one interested in disentangling the role of competition for P on phytoplankton or cyanobacteria communities, should start by representing P and the corresponding growth isocline. Here, we face the first difficulty relative to P: the use of inorganic and organic forms of P. Given our intention is not to discuss the biochemistry of P but rather its acquisition by the microorganisms, we use the widely spread terms ‘inorganic’ and ‘organic’ to refer to reactive and non-reactive forms of P, respectively. But it is worth mentioning that other classifications of different P forms exist (QUIQUAMPOIX; MOUSAIN, 2005).

The knowledge of organic P use by phytoplankton is not new (CHU, 1946; COTNER JR; WETZEL, 1992). And, species performance may vary according to the organic compound (BAI et al., 2014). Usually, it is expected the use of organic form under depletion of inorganic form in the environment

(DYHRMAN et al., 2006; LI; DITTRICH, 2019). However, evidences have been reported showing high activity of alkaline phosphatases (AP, group of enzymes capable of breaking organic compounds, mainly phosphatemonoesters, and with optimum pH in alkaline range) even when organisms are not limited by inorganic P (e.g. ISTVÁNOVICS et al., 1992; CAO; SONG and ZHOU, 2010), what might suggest a more common use of organic forms than previously thought (SIUDA; CHRÓST, 2001; LIU; WU, 2012) – not considering the possible direct use of some organic compounds (HEATH, 2005). Though not surprising the distinct strategies to acquire P, it is indeed surprising that few studies investigated the possible role of organic P in community structure. A quick search in all data bases in Web of Science (ran in April 19 2021) using the search string TOPIC=("organic phosphorus" OR phosphatase) AND TOPIC=(phytoplankton AND competition) returned only 56 studies. In comparison, the search string TOPIC=(phosphorus OR phosphate) AND TOPIC=(phytoplankton AND competition) NOT TOPIC =("organic phosphorus" OR phosphatase) accounted for 676 studies. Despite these are raw results without any further selection, they illustrate the reduced attention that has been given on integrating the use of organic P in competitive dynamics in comparison to inorganic P. Probably, the cause is the assumption that the use of organic P is driven by the scarcity of inorganic P, mentioned before. Another possible explanation is the difficulties associated to the measurement and manipulation of organic P in cultures or in micro- or mesocosms (MCKELVIE, 2005).

At a first glance, one could just ignore the two forms of P, arguing there is no evidence that the cell discriminates the P demand according its origin, so it would be just one main resource. However, if one considers the roles each form might play in maintaining populations in the same community (SCHOFFELEN et al., 2018), their different availabilities (HEATH, 1986) and, the different mechanisms to acquire (i.e. production of enzymes to break organic compounds *vs* membrane proteins to transport phosphate) and measure (WORSFOLD; MCKELVIE and MONBET, 2016) them, then organic and inorganic P could be posed in different axes as different resources. For that, it is necessary to establish the relationship between these two resources and the growth isocline. Then, appears the second difficulty: there is no clear understanding about organic P use for the majority of species and environmental conditions.

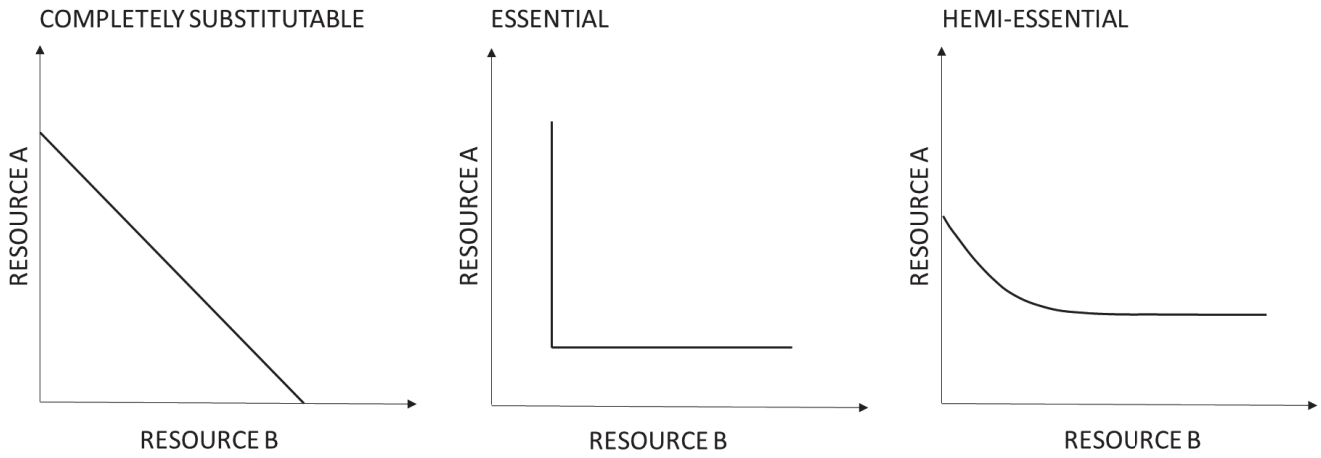

Figure 1 Types of relationships between different resources. See the text for explanations why organic and inorganic P forms do not fit in none of these relationships. Modified from Tilman (1982).

Even when experimentally possible to test exclusive additions of one or the other P form (e.g. BAI et al., 2014), exclusively uptake of only one chemical form is very unlikely to occur in nature. First, because phosphate is one of the products from enzymatic activity or changes in pH, thus the same P uptake structure is necessary for the transport of phosphate released from organic compounds (JANSSON; OLSSON and PETTERSSON, 1988). Second, organisms can take advantage of pulses of phosphate due disturbances in stratified water column and/or release after cell lysis (RIGLER, 1956), in an opportunistic way. Finally, evidences reporting no directly proportional relationship between enzymatic activity and phosphate concentration (see Chapters 1 and 2 of this thesis) also reinforces the inadequacy of classifying the two forms as substitutable resources.

It is also unlikely that the organisms will require a minimum amount of organic and inorganic P to grow, as in the case of essential resources. Firstly, because there is no evidence that cells differentiate between the two forms during the uptake, secondly because the high costs associated to synthesize different proteins (i.e. phosphatases to break organic compounds and high affinity proteins to uptake P), and thirdly because the cells have a limited space to storage P

(SMITH; KALFF, 1982), and it would be a disadvantageous spent of energy to increase the availability of P without uptaking it.

The hemi-essential relationship, which could be considered a type of complementarity between the resources, seems adequate to fit organic and inorganic P relationship. However, it is not adequate conceptually because the hemi-essential relationship assumes one resource is incomplete regarding its nutritional status. Organic and inorganic P are not distinct resources due nutritional differences (although organic compounds might also serve as carbon source for some bacteria; SIUDA; CHRÓST, 2001); thus, it is not their nutritional characteristics that define the dispensability of one or other form.

The points raised above are assuming the main function of organic P is nutritional. However, it is important to highlight the suggestion of Heath (1986) that dissolved organic P might have other functions, for example as chemical messengers. Irrespective of other functions the different organic P-containing compounds might have, the possibility of substantial organic P use adds complexity to the resource-based models. It is not to say the organic P will remove completely the competitive interaction between species, but adds more possible scenarios and may increase the time to competitive exclusion be completed. Besides that, there are still other factors, such as availability of N (KENESI et al., 2009) and temperature (AHLGREN, 1988), that can shape the P demand (GALVANESE; PADIAL and AUBRIOT, 2019) and, thus, shape the consumption curves of inorganic and organic P.

3.6 GROWTH, UPTAKE RATE AND MINIMUM P REQUIREMENT

In general, the core of competition models is the growth rate, the uptake rate and the minimum concentration of the limiting resource being assessed, i.e. R* (TILMAN, 1980). All parameters are considered proxies of success (e.g. STEWART; LEVIN, 1973), in a complementary way. For example, in two species scenario sharing resources and without initial resource limitation (i.e. below the carrying capacity), the species with the higher growth rate is expected to dominate and lead to the exclusion of the other species. If considering the uptake rate, the species with higher uptake rate would be able to consume more rapidly the resource, making it unavailable to the other species and thus excluding it,

irrespective of the growth rate. Finally, when comparing the R* under resource limitation scenario, the species with the lowest resource requirement will exclude the other, and it is independent of initial population size. Given the relatively simplicity of the predictions using these parameters in resource-based models, several studies have used these parameters and models to explain and predict the outcome of co-occurring species (e.g. DUCOBU et al., 1998; LITCHMAN; KLAUSMEIER and BOSSARD, 2004; SUOMINEN et al., 2017). But some limitations, inherent of these models, might prevent a better understand of the dynamics in natural communities.

The flexibility in nutrient acquisition is a premise, given the high variability in environmental conditions (e.g. THRANE; HESSEN and ANDERSEN, 2017). However, not just the demand for P might change according the environmental conditions such as temperature (AHLGREN, 1988) or N concentration (AUBRIOT, 2019), but also the uptake rate and the R* (i.e. threshold value). In a context of P limitation, the uptake rate and R* are dependent on the pattern of P availability (FALKNER et al., 1995). Changes in the P uptake systems are controlled by the energy required by the membrane transporters, in the direction of an equilibrium between the limited space to storage polyphosphate granules and the cell demand for growth (FALKNER; FALKNER, 2011). Given the cells cannot monitor the polyphosphate granules, the changes in the uptake system along the time are also important to guide the adjustment itself (PLAETZER et al., 2005); which imposes a temporal dependence of the P acquisition machinery. In this sense, the same amount of P, but alternating the order of P additions (FALKNER; PRIEWASSER and FALKNER, 2006), or differing between one single addition or divided in ten additions of regular interval (AUBRIOT; BONILLA, 2012), will result in different threshold levels at which the uptake of P ceases. This new equilibrium, expressed in the change of threshold after P addition to a previously limited population, means that more than one R* is possible to the same population and same total P concentration. Beyond that, higher threshold attained means that the population will not deplete completely the P, as expected in Monod equations (i.e. the hyperbola does not cross the axes origin, THELLIER, 1970; WAGNER; FALKNER, 2001). Therefore, models assuming fixed parameters are not able to describe adequately the phenomenon (FALKNER; WAGNER and FALKNER, 1996). Relatedly, the ability to achieve a new

equilibrium in the time interval of hours (WAGNER; FALKNER and FALKNER, 1995) in the range of nanomolar (WAGNER; SAHAN and FALKNER, 2000; AUBRIOT; BONILLA and FALKNER, 2011) is very relevant regarding fluctuations the natural communities experienced (e.g. ISTVÁNOVICS; OSZTOICS and HONTI, 2004; TONETTA et al., 2016).

Changes in uptake rate and threshold also can impact the growth rates. For example, the comparison of one and ten P pulses resulted in distinct growth rates of freshwater cyanobacteria. However, the P treatment resulting in higher growth rate varied in separated studies according the species tested (WAGNER; SAHAN and FALKNER, 2000; AMARAL; BONILLA and AUBRIOT, 2014). Moreover, it is well established the reliance of growth rate on other factors such as light (WOJCIECHOWSKI; FERNANDES and FONSECA 2016), temperature (BRIAND et al., 2004), the interaction among them (BONILLA et al., 2016) and the interaction with nutrients and temperature (THRANE; HESSEN and ANDERSEN, 2017; GALVANESE, PADIAL and AUBRIOT, 2019) or light (AGUILERA et al., 2017). Considering this variation can be strain dependent (GALVANESE, PADIAL and AUBRIOT, 2019; GUEDES et al., 2019), there is more than one possible avenue in the same population and a multitude when investigating the communities. This might be a problem specially in the theoretical assumptions of models that are based on the balance between intra and interspecific competition as a mechanism to prevent species exclusion, because the intraspecific variation can be higher than the interspecific (XIAO; WILLIS and BURFORD, 2017). Finally, as for the uptake rate and R*, fixed maximum growth rates or direct dependency on resource levels will oversimplify the natural dynamics.

3.7 MOVING FORWARD IN SPECIES COMPETING MODELS

From the exposed above, it seems complex to accommodate in models the most conditions and possible outcomes that can be found in nature. Nonetheless, we argue it is possible to improve the models, by using in a different perspective the frameworks and approaches already developed.

The use of multiple sources or forms of the same nutrient is not surprising, as mentioned before. The issue is how to design the growth isoclines

according each P form. Surely, more studies are required to a proper understanding of the role of organic P forms on growth in different species and conditions, but as stated by Thellier (1973) “in such cases where true mechanistic models cannot be deduced, one will have to accept purely phenomenological models, the analytical form of which will sometimes have to be proposed by analogical reasoning with other domains of biology, chemistry or physics where such analytical forms are already known”, and in this sense we can benefit from the understanding of how organisms use another important resource: light. In order to absorb and be protected from the excess light, organisms possess distinct types of pigments (KIRK, 2011). Pigments are classified in chlorophylls (e.g. chlorophyll *a*), carotenoids (e.g. *β* carotene) or biliproteins (e.g. phycocyanin). The first two are widespread among the phototrophic organisms, and biliproteins restricted to Cyanobacteria, red algae and Cryptophytes (KIRK, 2011). Chlorophyll *a* is considered the main pigment responsible for light harvesting, having absorption peaks in the blue and red regions of light spectrum. The light from region which chlorophyll *a* does not absorb, is not wasted because the complementary absorption of biliproteins (GANTT, 1975). The biliproteins are organized in complexes called phycobilisomes, and the energy absorbed is transferred through excitation and emission of the pigments until it reaches the chlorophyll *a* in photosystem II (GANTT, 1975). The presence of accessory pigments (i.e. biliproteins and carotenoids) helps organisms deal with the reduction in intensity and the narrowing of wavelengths available in aquatic environments. Thus, the accessory pigments are an important mechanism to overcome the light limitation. And in fact, the distinct biliprotein pigments were shown to explain the prevalence of distinct strains according the light quality (STOMP et al., 2004, 2007).

Perhaps because of the wave character of the light beam, it is easy to realize the light availability as a gradient, and the compensation made by organisms to overcome its limitation by increasing relatively the amount of accessory pigments in relation to chlorophyll *a* or the number of photosystems (DE NOBEL et al., 1998). We argue we can benefit from using the same approach when assessing the phosphorus usage by organisms. To acquire light and phosphorus, in both cases organisms possess complementary systems for qualitatively and quantitatively distinct forms of the available resources. And, in

both cases, there is a general acquisition system, i.e. the chlorophyll *a* and the inorganic P acquisition machinery, which is complemented by the alternative systems, i.e. accessory pigments and use of organic P, according the species and the interactions with other resources required for cell growth. Furthermore, in both cases is the sum of all available forms considered when defining where the primary producers can potentially growth, i.e. euphotic zone in case of light and, potentially bioavailable P which considers the inorganic and organic portions. Finally, the availability of the forms of each resource is mainly shaped by the environment. For example, the presence of colored dissolved matter will affect the light penetration, as pH will affect the availability of P forms. Thus, a gradient-approach of P acquisition could help understand the spatial distribution of species and also the co-occurrence of distinct populations in the same water layer, as occurs for light gradient (e.g. STOCKENREITER et al., 2021).

In regard the graphical representation of the growth isoclines according the resource availability as developed by Tilman (1980, 1982), firstly one must think if, for the species under consideration, there is evidence of significant use of organic P. Although it is well accepted phosphatases are widespread in all biological groups (MCCOMB; BOWERS and POSEN, 1979), the extent to which the organisms might rely on this source might be variable (SCHOFFELEN et al., 2018; XU et al., 2020). It is important to highlight that the assessment of the organic P use must include some consideration about the direct production of phosphatase by the species under investigation, because correlation between organic P content and growth might be found if a species is benefited from the release of extracellular phosphatase by other species (e.g. BAR-YOSEF et al., 2010). This first picture of P use might be represented as in Fig. 2 (left). The isoclines in the figure represent two distinct situations: A - there is always a minimal use of organic P; B – the use of organic P starts when inorganic P reaches a certain level (e.g. PRENTICE et al., 2019). Probably, species can alternate between the two curves, being context dependent – for example, for example, depending on the growth phase (GIORDANO, 2013).

When assessing the relationship between P and other essential resource, e.g. N, there will be a minimum required of each resource, as in the classical representation (Fig. 2, right). However, as the availability and/or demand for each P form might change, the representation of P should be the ratio

480 organic:inorganic, rather than the mere quantification of inorganic P. Even though
481 the organic:inorganic rate should be expected to vary for different species and
482 scenarios, here we still assume that the use and/or availability of organic P is
483 associated to a certain reduction in availability of inorganic forms. In addition, the
484 R* for P might not change, because it still represents the minimal P demand to
485 sustain a population. One possible mechanism to increase the use of organic P
486 without changing R* could be the accumulation of phosphate salts in the
487 periplasm membrane. Kamennaya et al. (2020) proposed that the
488 electrochemical gradient generated by the concentration of monovalent ions
489 (such as Na^+ and H^+) in the periplasm, would promote the massive movement of
490 external phosphate in the periplasm. In the periplasm, the phosphate would
491 bound to cations and the complex formed would not return to the external
492 environment, but yet would be available to interact with high affinity proteins in
493 the cytoplasmic membrane. In this sense, the release of extracellular
494 phosphatases could provide phosphate to be concentrated in the periplasm
495 without new synthesis of high affinity enzymes. But it is still important to keep in
496 mind that the contribution of each P form may vary, and so the mechanisms for
497 their use, and consequently the energy balance between the two systems.

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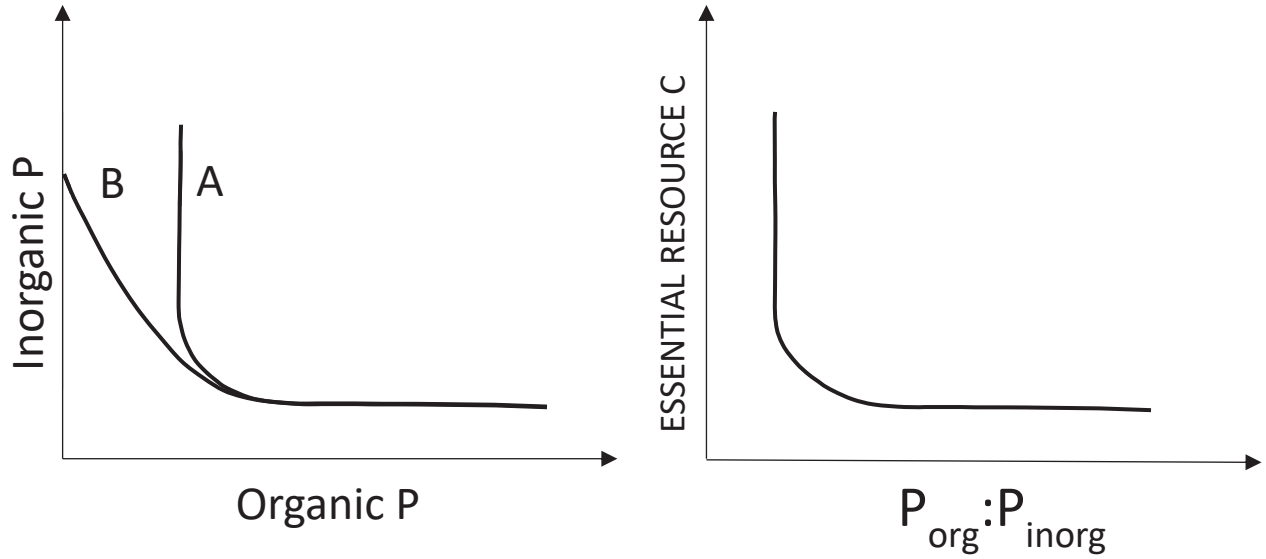

Figure 2 Left: graphic representation of two possible relationships between organic and inorganic P, and the growth isoclines. A – when a minimum of organic P is used; B – when the organic P use is driven by the inorganic P availability. The choice between the curves depends on the actual dependence on each form by the organism under consideration. Right: growth isocline according to two essential nutrients. Instead of using inorganic P as one single nutrient, we advocate use the proportion between organic and inorganic P.

3.8 CONCLUDING REMARKS AND FUTURE DIRECTIONS

Here, we intended to advocate in favor of a less dichotomic view in resource acquisition by organisms. It seems obvious that the resources are consumed continuously, and that many mechanisms were developed along the time to overcome bioenergetic limitation, besides the phenotypic plasticity itself. But the merge of the niche theory with competition (CHASE; LEIBOLD, 2003), together with the methodological limitations, lead to a narrow understanding of how organisms use resources, and consequently the outcomes of populations co-occurring. In cyanobacteria, changes in R* and in the growth rate can be induced by modifying the pattern of availability of P, and this variability makes the classical resource-based models inadequate. Moreover, the possibility of significant supply of organic sources makes a more complex scenario arise when evaluating the relationship between resource consumption and diversity. We argue that the knowledge accumulated regarding the light harvesting systems is a useful analogy in understanding the processes underneath the population's growth, and this will open space for more coexistence outcomes. Thus, we greatly encourage more studies using this theoretical framework to investigate the effects of quantitative and qualitative fluctuations in P forms on populations and communities.

3.9 REFERENCES

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6 CONCLUSÕES GERAIS

Neste trabalho, o principal interesse foi entender como diferentes maneiras de disponibilizar um recurso podem modular a coexistência das espécies. Utilizamos o fósforo (P) porque é um macronutriente muitas vezes limitante em ambientes aquáticos (e.g. PRENTICE et al., 2019), e os microrganismos apresentam diversas estratégias para crescer frente condições desfavoráveis (WAGNER; FALKNER, 2001). Nesse sentido, as cianobactérias são organismos muito interessantes, porque possuem alta variação intra (WILLIS et al., 2019; XIAO et al., 2020) e interespecíficos (BONILLA et al., 2012) de atributos. Frente à flutuação de P num cenário de limitação por esse nutriente, a depender do tempo que as células ficam sujeitas a um aumento da concentração de P, as características do sistema de absorção podem mudar (FALKNER; PRIEWASSER and FALKNER, 2006). Essas mudanças podem potencialmente aumentar as chances de coexistência se elas forem na mesma direção nas diferentes espécies (WAGNER, SAHAN and FALKNER, 2000; AUBRIOT, BONILLA and FALKNER, 2011; NARWANI et al., 2017). Assim, testamos pulsos de P totalizando a mesma concentração final, em monocultivos e cultivos mistos. Para induzir diferentes tempos de exposição, aplicamos um pulso (1P) e dez pulsos (10P) a cultivos limitados, em condições de saturação (i.e. alta concentração de P adicionado em relação ao P total) e não saturação (i.e. baixa concentração de P adicionado em relação ao P total); e manipulamos a relação entre o tamanho da população e o P adicionado. Além de acompanhar o crescimento dos cultivos, avaliamos a atividade da fosfatase alcalina como um indicador de uso de fontes orgânicas de P.

Nossos resultados mostraram que há efeito de diferentes pulsos de P nas taxas de crescimento e na atividade das fosfatases alcalinas em algumas situações, incluindo situações em que as células não estão limitadas por P. Ainda, essa resposta aos pulsos variou entre as espécies. *Aphanizomenon gracile* teve maiores valores de atividade de fosfatases alcalinas, corroborando outros estudos que sugeriram um uso pronunciado de formas orgânicas de P (HEATH; COOKE,1975; SCHOFFELEN et al., 2018). Ao longo do tempo, a atividade das fosfatases aumentou para um mesmo intervalo de nitrogênio total, o que sugere uma maior demanda de energia para fixação de nitrogênio

conforme o tempo foi passando. Esse aumento pode ou não estar relacionado com o aumento de demanda de outros processos dentro das células.

Geralmente, o crescimento de *A. gracile* foi maior em 1P que em 10P. *Raphidiopsis raciborskii*, em monocultivos sob pulsos não saturantes, teve maior taxa de crescimento em 1P, diferentemente do encontrado para uma outra cepa (AMARAL; BONILLA and AUBRIOT, 2014). Quando em cultivos mistos, entretanto, maior aumento da população de *R. raciborskii* foi em 10P. Algo similar aconteceu em cultivos mistos de *A. gracile* e *Dolichospermum* sp., o que sugere que pode não ter sido o efeito da quantidade de pulsos *per se*, e sim um efeito da melhor performance de *A. gracile* em 1P. Ademais, nos dois experimentos mistos realizados, *Dolichospermum* foi a espécie menos dominante. Isso indica uma inferioridade em relação às outras duas espécies nas condições usadas nos experimentos, ainda que tenham sido cepas diferentes usadas nos experimentos; e *Dolichospermum* sp. e *A. gracile* tenham sido isoladas de uma mesma amostra no primeiro experimento.

Em relação às curvas de absorção de P sob pulsos não saturantes, a similaridade entre os parâmetros cinéticos antes dos pulsos entre as três espécies sugere uma convergência dos sistemas de absorção. Essa convergência é alterada pelos pulsos, que aumenta a concentração de P externo. Essa convergência sugere que há atratores energéticos para um equilíbrio, o qual corresponde a um determinado intervalo de concentrações de P (FALKNER; WAGNER and FALKNER, 1996).

Considerando as evidências de maior uso de formas orgânicas de P por *A. gracile*, o provável uso dessas formas mesmo em condições não limitantes de P inorgânico (SIUDA; CHRÓST, 2001), e a variação da disponibilidade dessas formas no ambiente (BOSTRÖM; PERSSON and BROBERG, 1988), propomos uma abordagem mais ampla do P como recurso. Essa nova abordagem veio da analogia com a absorção de luz, em que os organismos possuem pigmentos fotossintéticos que são complementares em seus espectros de absorção, favorecendo a coexistência de espécies (STOMP et al., 2004). Em termos práticos, propomos que seja considerada a proporção de uso de formas orgânicas e inorgânicas, em lugar apenas da concentração de P inorgânico. Em termos teóricos, isso significa mudar a maneira como o nutriente P é classificado segundo as classificações de recursos de Tilman (1980). Uma nova perspectiva

teórica é particularmente importante não só para entender as dinâmicas naturais de uso do P ao longo do tempo, mas principalmente porque a representação gráfica usando isoclinas de crescimento zero como proposto por Tilman (1980) já foi utilizada para definir nicho (CHASE; LEIBOLD, 2003) e coexistência (CHESSON, 2000).

Por fim, nossos resultados confirmam que os padrões de disponibilidade ou flutuações de P podem influenciar as interações entre espécies, e consequentemente nos padrões de coexistência. Porém, a importância dessas flutuações é variável, não sendo sempre determinante, uma vez que outros fatores, como o uso de P orgânico, podem interagir. Nosso trabalho reforça o papel da Ecofisiologia na dinâmica de comunidades, e também a necessidade de estudar as comunidades ao longo do tempo, considerando que os organismos estão constantemente respondendo ao ambiente e modificando-o, e assim diversas respostas fisiológicas são possíveis (BERTHOLD; CAMPBELL, 2021).

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