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Phytoplankton strategies to exploit nutrients in coastal lagoons with different eutrophication status during re-oligotrophication

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ABSTRACT: We studied a mesotrophic and a hypertrophic Mediterranean coastal lagoon, both of which had been simultaneously subjected to a nutrient input reduction for 9 yr. We compared these 2 lagoons to an oligotrophic coastal lagoon. Using bioassays comprising 24 h incubations with added phosphorus and/or ammonium, we investigated the response of the phytoplankton communities to nutrient enrichment during summer in terms of biomass, size class structure, abundance and growth. For nitrogen and phosphorus, we identified which nutrient limited phytoplankton growth, and what strategies of nutrient exploitation the communities adopted to cope with these limitations. Ultraphytoplankton dominated the 3 communities, but it differed in composition among the lagoons. Green algae dominated in the hypertrophic lagoon, whereas the mesotrophic lagoon presented a higher diversity of phytoplankton groups. Picocyanobacteria and small diatoms were the most abundant groups in the oligotrophic lagoon, although they accounted for less biomass than green algae. The communities of the mesotrophic and the hypertrophic lagoons strongly responded to the nutrient pulse, showing that the re-oligotrophication trajectories of these lagoons were still very vulnerable to occasional eutrophication events. On the other hand, the oligotrophic lagoon marginally responded to the enrichment, indicating its adaptation to nutrient-depleted conditions. We observed a shift along the eutrophication gradient, from a co-limitation by N and P in the oligotrophic and the mesotrophic lagoons to a single and strong N limitation in the hypertrophic lagoon. Each community demonstrated specific use of internal, external or recycled nutrient pools under experimentally induced limitation.

KEY WORDS: Growth rate · Nutrient limitation · Dilution experiment · HPLC

1. INTRODUCTION

Within coastal waters, lagoons are particularly vulnerable to eutrophication processes because of their restricted exchanges with the sea and their long water residence times (Pereira Coutinho et al. 2012). Eutrophication is often caused by nutrient over-enrichment, which stimulates primary producers and strongly impacts the composition of the autotrophic compartment (De Jonge & Elliott 2001). It notably modifies the competition between functional groups depending on their resource acquisition strategies and their growth abilities (Paerl et al. 2003). Hence, availability of phosphorus and nitrogen constitutes the main abiotic factor controlling phytoplankton growth, biomass and community composition in shallow coastal lagoons (Collos et al. 2004) and thus represents an important aspect for eutrophication management (Domingues et al. 2011, Gallegos 2014).

The loss of ecosystem services due to the eutrophication of aquatic ecosystems has been a strong impetus for setting a target of reversing eutrophication,

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which is also in agreement with the objectives of the Water Framework Directive (WFD, 2000/60/EC) (Cartaxana et al. 2009). To improve the ecological status of eutrophic aquatic systems and initiate their ecological restoration, nutrient inputs must be reduced. Some freshwater systems have been submitted to re-oligotrophication processes since the 1970s (Jeppesen et al. 2005, 2007, Van Donk et al. 2008). However, ecological restoration is a very complex and variable process, which often requires a long time to be successful (de Jonge & de Jong 2002). Indeed, several lakes showed hysteresis during their re-oligotrophication, related to persistent nutrients loads. These nutrients originate from the remobilization of organic material in the sediment (Jeppesen et al. 2005, Phillips et al. 2005), or from outside due to an insufficient reduction of external loads (Van Donk et al. 2008). Thus, to be effective, nutrient management of eutrophic waterbodies requires understanding the relationships between primary production and nutrient loads, especially from external and recycled sources (Domingues et al. 2011, Wood & Bukaveckas 2014). For several coastal waters such as lagoons, the responses to ecological restoration through their re-oligotrophication have been assessed (Collos et al. 2009, Leruste et al. 2016). Re-oligotrophication first impacts phytoplankton communities, inducing some functional responses. It results in an increase in nutrient limitation (Nixon 2009), which is one of the keystone drivers of phytoplankton growth and composition (Cloern 2001, Crossetti & Bicudo 2005). Similar to eutrophication, re-oligotrophication particularly affects the size structure of phytoplankton communities (Ruggiu et al. 1998, Collos et al. 2009, Kamenir & Morabito 2009). However, studies of re-oligotrophication are more scarce than those of eutrophication, and their results are quite complex to interpret due to the intrinsic complexity of these ecosystems. There is thus a strong need for studies of re-oligotrophication (Domingues et al. 2011), since this process is still poorly documented and understood (Phillips et al. 2005).

In the coastal lagoons along the French Mediterranean coastline, phytoplankton nutrient limitation is variable, with consequences for their ecosystem functioning. Particularly, along the eutrophication gradient, a shift from phosphorus to nitrogen limitation with increasing eutrophication has been described (Souchu et al. 2010). Among the French Mediterranean coastal lagoons, a complex of 8 lagoons was submitted to an intense eutrophication process since the 1960s, mainly due to the indirect discharge of effluents from the Montpellier city district wastewater treatment plant. Related to the distance from this major point source, a eutrophication gradient formed along the lagoon complex, from mesotrophic in the southwest to hypertrophic in the northeast. Picophytoplankton, which is highly competitive to nutrient pulses, dominated the autotrophic communities of these lagoons (Bec et al. 2011). Since 2005, the lagoon complex has been submitted to a drastic reduction of anthropogenic P and N inputs, leading to the beginning of a re-oligotrophication process. This new dynamic has driven strong responses of phytoplankton communities, reflected by a sharp decrease in phytoplankton biomass and picoeukaryote abundance. It has also triggered drastic changes in community composition related to the functional responses of the different species. The outcome was variable among lagoons, and was dependent on their eutrophication status before the start of the nutrient input reduction (Leruste et al. 2016). Hence, studying stations in different lagoons in this complex offered the unique advantage that, while the period of their re-oligotrophication process was exactly the same, they differed in eutrophic status before the start of this process in 2005. The impact of the previous trophic state on phytoplankton to nutrient conditions during the re-oligotrophication process could thus be assessed.

It is important to understand how phytoplankton functionally respond to nutrient availability, particularly in terms of growth and community composition. This knowledge will in turn help us to understand the result of eutrophication management, and to predict time scales of re-oligotrophication processes (Domingues et al. 2015). Therefore, we formulated 3 main questions: (1) How vulnerable are these lagoons during the re-oligotrophication process to a nutrient pulse? More specifically, we asked: Which organisms within phytoplankton assemblages respond in the short term to a nutrient pulse, depending on the nutrient, and how do they respond? (2) Which nutrients limit the growth of phytoplankton assemblages during re-oligotrophication processes in lagoons? (3) What strategies of resource use can phytoplankton assemblages employ to cope with an experimentally induced nutrient limitation? Specifically: Which nutrient sources are preferentially used, considering internal, external and recycled pools?

To examine these questions, we conducted a study on 2 lagoons from the complex that strongly differed in their eutrophication status before the start of the re-oligotrophication process and presently still show differences. For comparison, we also selected an oligotrophic lagoon which has remained in a good ecological state over the past 2 decades. During the summer of 2014, we experimentally incubated phyto-

plankton communities from these 3 lagoons with an added nutrient enrichment, under *in situ* light and temperature conditions. The full nutrient enrichments allowed us to assess the reactivity of the phytoplankton assemblages to a nutrient pulse. Moreover, we also explored the vulnerability of the re-oligotrophication process to a nutrient pulse. Using the 'All minus one' technique, we induced nitrogen and phosphorus limitations to understand phytoplankton strategies to offset depleted conditions (Andersen et al. 1991).

2. MATERIALS AND METHODS

2.1. Study sites and sampling procedures

Along the French Mediterranean coastline, the mesotrophic north Ingril (IN) and hypertrophic west Méjean (MW) lagoons are located at the southwest and the northeast of the Palavas lagoon complex. They represent in this complex the lesser and the mostly eutrophied lagoons, respectively (Fig. 1). Both lagoons are separated from the sea by the Rhône-to-Sète canal, and hydraulic exchanges with the sea are therefore indirect via this canal only. Both lagoons were exposed to nutrient over-enrichment from the 1960s to 2005 and have followed a re-oligotrophication trajectory since December 2005 (Leruste et al. 2016). However, their benthic nutrient stocks still represent a significant internal source of nutrients (Souchu et al. 2010). The phytoplankton communities of these 2 lagoons were compared to those of a lagoon that has virtually not been impacted by eutrophication: the Ayrolle lagoon (AYR) (Fig. 1). This oligotrophic lagoon has a small watershed (104 km^2) without urbanization. AYR is connected to the sea by a natural inlet in the southeast, while the 2 selected lagoons from the Palavas complex are not directly connected to the sea, but communicate indirectly through the Rhône-to-Sète canal and adjacent lagoons. Since the start of the monitoring program in 1998, the AYR lagoon has been characterized by a good ecological state, low nutrient concentrations and low phytoplankton biomass (Souchu et al. 2010).

Experiments were carried out from 25 August to 4 September 2014. In each lagoon, 70 l of water prefiltered through 1000 µm mesh to remove larger debris without removing zooplankton or larger phytoplankton cells (Collos et al. 2005) were sampled in sub-surface (20 cm depth) between 08:30 and 11:00 h and kept in the dark. At the sampling stations, salinity and temperature were measured with a conductivity meter (Cond 3110 Set 2) at 20 cm depth.

Upon return to the laboratory, water samples were homogenized by gentle shaking and were then aliquoted. Two volumes of 80 ml of water were sampled in 100 ml polypropylene bottles, prewashed with 1 mol l^{-1} HCl and rinsed 3 times with milli-Q water, to measure the concentrations of NH_4^+, PO_4^{3-}, NO_3^-, NO_2^-, total nitrogen (TN) and total phosphorus (TP) (µM). Concentrations of dissolved inorganic nutrients (PO_4^{3-}, NO_3^-, NO_2^-) were obtained by segmented flow automatic colorimetry (Raimbault et al. 1990, Aminot & Kérouel 2004, 2007), and ammonium concentrations were obtained using fluorescence (Holmes et al. 1999).

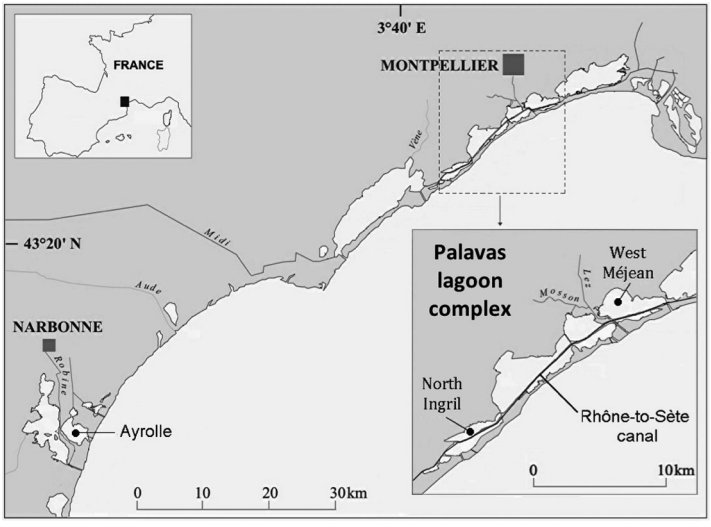

Fig. 1. Sampling stations in Ayrolle (AYR, oligotrophic), north Ingril (IN, mesotrophic), west Méjean (MW, hypertrophic) lagoons in southern France

2.2. Size class structure and phytoplankton community composition

Chlorophyll *a* (chl *a*) concentrations were used as a proxy for phytoplankton biomass. Phytoplankton pigment analysis and biomass measurement were performed on size-fractioned water to assess the contribution of 3 different size classes, i.e. ultraphytoplankton (<5 µm), nanophytoplankton (5–20 µm) and microphytoplankton (>20 µm) to the total phytoplankton biomass, and phytoplankton community compositions for each size class. To obtain size-fractioned water, triplicates of water samples were filtered on nylon filtration tissue with 20 µm and 5 µm meshes to de-

termine the contribution of nanophytoplankton and ultraphytoplankton to total phytoplankton biomass. Known volumes of water samples and size-fractioned water aliquots were filtered on Whatman GF/F membranes (25 mm diameter and 0.7 µm porosity) and stored at −20°C. The volumes chosen for the filtrations depended on phytoplankton biomasses and were maximally 100 ml. The filters were ground in acetone (90%) and extracted during 24 h in the dark at 4°C. Pigments were measured by spectrofluorometry (Neveux & Lantoine 1993). Concentrations are expressed in µg l^{-1}.

The biomass of microphytoplankton was obtained by subtracting chl *a* concentration of size-fractioned water filtered on 20 µm from the total chl *a* concentration. The biomass of nanophytoplankton was obtained by subtracting the chl *a* concentration of the size-fractioned water filtered on 5 µm from that of the water filtered on 20 µm, and the biomass of ultraphytoplankton was obtained from the chl *a* concentration of the size-fractioned water filtered on 5 µm.

Chemotaxonomic analysis using HPLC estimated phytoplankton taxonomic diversity in each size class (Leruste et al. 2015). Triplicates of size-fractioned water samples (150–2000 ml, depending on biomass densities) were filtered on Whatman GF/F membranes (47 mm diameter), and stored at −80°C prior to analysis. Pigments were extracted during 1 h with 5 ml of a mix acetone/methanol/water. Pigment analysis allowed the estimation of some major algal group biomasses using concentrations of pigment markers: heterokonts such as fucoxanthin-rich diatoms using fucoxanthin, dinophytes using peridinin, prasinophytes using prasinoxanthin, cryptophytes using alloxanthin, haptophytes using 19'but-fucoxanthin and 19'hex-fucoxanthin, green algae using chl *b*, lutein, violaxanthin, neoxanthin and zeaxanthin. Zeaxanthin pigment can also indicate cyanobacteria occurrence, although diatoxanthine can indicate the presence of euglenophytes, and violaxanthin can indicate the presence of chrysophytes (Roy et al. 2011).

Flow cytometry and optical microscopy complemented the description of community composition inferred from the HPLC (Sherrard et al. 2006, Paerl et al. 2007), particularly to resolve the zeaxanthin signal that corresponds both to green algae and cyanobacteria. Flow cytometry refined the HPLC results for ultraphytoplankton and especially cyanobacteria, by measuring abundances of cells <5 µm, (FACSCalibur, Becton Dickinson) (Bec et al. 2011). Cyanobacteria and eukaryotic phytoplankton cells (Peuk: picoeukaryotes <3 µm; and large ultra: ultraphytoplankton cells between 3 and 5 µm) were distinguished on the basis of light diffraction (forward scatter, related to cell size) and red fluorescence emissions (chl *a*, wavelength >650 nm), using beads for size calibration. Populations of coccoid cyanobacteria were identified by their orange fluorescence emission, and phycoerythrin-rich picocyanobacteria (PE-cyan) were distinguished from phycocyanin-rich picocyanobacteria (PC-cyan) (Bec et al. 2011). Optical microscopy observations complemented HPLC results for nano- and microphytoplankton, by measuring abundances and taxonomic diversity of cells >5 µm. Triplicates of 1 l samples fixed with formaldehyde (5% final concentration) were stored in the dark prior to analysis. Because of weak phytoplankton biomasses in the oligotrophic lagoon, a modified Utermöhl protocol was used (Leruste et al. 2018). Taxonomic resolution was realized at the species level whenever possible, and verified according to the World Register of Marine Species (www.marinespecies.org/) and the scientific literature.

2.3. Maximal growth and mortality rates

Phytoplankton maximal growth rates were measured by dilution experiments according to Landry & Hassett (1982). Five dilutions containing 9, 17, 43, 74 and 100% of sample in filtered lagoon water (0.2 µm Suporcap cartridges, Pall-Gelman, previously rinsed with 1 l of deionized water and sample water) were carried out in duplicate. The 5 duplicates were incubated in 1 l polycarbonate bottles previously washed with 10% HCl and rinsed 3 times with milli-Q water (Leruste et al. 2019). Before incubation, the bottles containing the different dilutions of sample received an enrichment based on f culture medium (Guillard & Ryther 1962), containing vitamins, silica, trace metals, nitrogen and phosphorus (NaH_2PO_4, final concentration 0.8 µM). The nitrogen was provided as ammonium (20 µM final concentration), assuming that this is the predominant nitrogen form in these lagoons during summer, mostly derived from remineralization processes in the sediment (Collos et al. 2003, Serpa et al. 2007) and in view of previous observations on the lagoons from the Palavas complex and its surroundings (Ifremer 2009). This quantity of nitrogen was chosen to avoid phytoplankton growth limitation, previously observed at 10 µM in another lagoon from the same region (Bec et al. 2005). Two bottles of sample without dilution and enrichment were incubated as controls. Bottles were incubated for 24 h in Thau lagoon water under *in situ* temperature and light conditions at 60 cm depth.

After 24 h incubation, chl *a* concentrations of total, micro-, nano- and ultraphytoplankton were measured in each bottle. The temporal changes of chl *a* concentration of each fraction after $t = 24$ h were used to estimate their apparent growth rate $k(x)$ for each dilution (x) ($x = 1$ for undiluted sample, and $x = 0$ for infinite dilution). For the enrichments, the results were fitted to the linear Eq. (1) (Landry & Hassett 1982):

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

where x is the dilution factor, $k(x)$ is the apparent specific growth rate, μ_{max} is the maximum growth rate, and g is the specific mortality rate assumed to correspond to grazing (all rates expressed as d^{-1}). Hence, linear regression of the apparent growth rate (k) versus dilution factor (x) was used to obtain the maximum growth rate (μ_{max}) as the y-axis intercept, and the grazing rate (g) as the slope of the linear regression. Phytoplankton growth rate in incubations without added nutrient (μ_0) was subsequently extrapolated by adding g (estimated for nutrient-enriched series, see above) to the apparent growth rate (k_0) estimated for undiluted unenriched treatments, assuming that grazing was not impacted by the enrichment. The μ_0:μ_{max} ratio assessed the impact of inorganic nutrient enrichment on growth and estimated the nutrient sufficiency for phytoplankton growth (Landry & Hassett 1982, Landry et al. 1998).

2.4. Nutrient limitation and resource use

To assess the physiological nutrient limitation of phytoplankton, an 'All minus one' experiment was combined with the dilution technique (Landry & Hassett 1982). It consisted of selectively enriching 2 dilution series leaving out either nitrogen or phosphorus (Andersen et al. 1991). We assumed that specific grazing rates (g) were not affected by leaving out one of the nutrients, and can be estimated using the complete enrichment series. All bottles, i.e. both full and partial enrichments, were incubated together for 24 hours.

To identify which nutrient sources were used for phytoplankton growth during experimentally induced nutrient limitation, and to estimate their relative contributions, we considered 3 potential sources: (1) an external source comprising the dissolved nutrients in the water at the beginning of incubation; (2) internal nutrient pools present in the cells at the start of incubation; and (3) nutrients supplied by recycling through grazing, considering excretion, egestion and 'sloppy feeding' (release of organic matter during physical phytoplankton cell breakage, followed by microbial degradation). Hence, the potential biomass production can be divided into $C_E(t)$ from the external nutrient pool, $C_I(t)$ from internal nutrient reserves and $C_R(t)$ from the recycled nutrients. The external nutrient source is not affected by the dilution, while internal reserves are proportionally reduced. Grazing is reduced by a factor x, reducing recycled nutrient supply by the same proportion. Assuming that the amount of recycled nutrient is proportional to the amount of consumed food, the potential growth yield can be expressed as a function of the dilution factor:

$$\Delta C(x,t) = \Delta C_R(t)x^2 + \Delta C_I(t)x + \Delta C_E(t) \quad (2)$$

Accepting Eq. (2), and assuming that initial biomass in each dilution experiment was identical to biomass of the undiluted sample, reduced by the dilution factor (x), i.e. $C(x,0) = x.C(1,0)$, the apparent growth rate $k(x)$ can be expressed by:

$$k(x) = \frac{1}{t}\ln\left(1 + \frac{\Delta C_R(t)x^2 + \Delta C_I(t)x + \Delta C_E(t)}{xC(1,0)}\right) \quad (3)$$

Introducing the potential production coefficient due to each nutrient source (Z) by $K_Z = \frac{\Delta C_Z(x,t)}{C(1,0)}$, Eq. (3) can be re-expressed with production coefficients:

$$k(x) = \frac{1}{t}\ln(1 + K_R x + K_I + K_E x^{-1}) \quad (4)$$

where $k(x)$ is the apparent phytoplankton growth rate at dilution x, and K_E, K_I and K_R are the potential production coefficients of the 3 different nutrient pools. These coefficients represent the relative yields of external, internal and remineralized nutrients, respectively. The values of K_E, K_I and K_R can be estimated by fitting Eq. (4) to estimate $k(x)$ by a non-linear regression method. To estimate parameters K_R, K_I and K_E by multiple linear regressions, Eq. (4) can be antiloged on each side, giving the following expression:

$$\exp(k(x)t) - 1 = K_R x + K_I + K_E x^{-1} \quad (5)$$

with x and x^{-1} as independent variables and exp ($k(x)$ t) – 1 as the dependent variable.

According to these equations, Andersen et al. (1991) showed how mean growth rate expressed the contribution of each nutrient source. To estimate the N and/or P limitation controlling the growth, the Q ratio between maximal growth rate in non-limiting nutrient conditions (μ_{max}) and growth rate under N or P limitation (μ_{-N} or μ_{-P}, respectively) was calculated and is given as Q_N or Q_P.

2.5. Statistical analysis

Data analysis was performed with R (R Core Team 2013). To estimate the maximal growth rate and the grazing rate for the total phytoplankton and the 3 size classes, mixed-effect multiple regression analyses were performed with apparent growth rate for each dilution as the dependent variable. Several equations resulting in the combination of explanatory variables of Eq. (1) were fitted with the 'lmer' function from the 'lme4' library (version 1.1-10, Bates et al. 2015). All combinations were considered using the 'dredge' function of the 'MuMIn' package (Bartón 2013). Model selection was based on parsimony using the small-sample corrected Akaike's information criterion (AIC_c). However, the linear model was selected as it allows calculating μ_{max} and g according to Eq. (1) (Landry & Hassett 1982). To check whether this selection was justified, we calculated the difference between the AIC_c of this model and the one having the lowest AIC_c to obtain a AIC_c value. The linear model was accepted only for $AIC_c < 2$, and we concluded that it does not statistically make sense to calculate μ_{max} and g for $AIC_c > 2$ (Burnham & Anderson 2004).

To estimate the contribution of the 3 different nutrient sources to growth during experimentally induced nutrient limitation in the 'All minus one' incubations, we estimated the K_I, K_E and K_R coefficients reflecting internal, external or recycled sources, respectively. Therefore, we used mixed-effect multiple linear regressions according to Eq. (5). These calculations were performed for total phytoplankton and separately for the 3 size classes. Based on Eq. (5), several models using combinations of the explanatory variables were fitted with the 'lmer' function. To identify the best model (best combinations of the 3 resource coefficients), AIC_c was used to select the most parsimonious model. We then estimated the proportion of biomass produced from these 3 sources, by multiplying initial biomasses with the 3 coefficients (Andersen et al. 1991).

3. RESULTS

3.1. Environmental parameters reflecting the eutrophication status of the lagoons

Environmental parameters and chl *a* concentrations for the 3 lagoons during sampling are presented in Table 1. Salinity was higher than the seawater salinity in the mesotrophic IN lagoon (41), close to that of the seawater in the hypertrophic MW lagoon (37), and lower (31) in the oligotrophic AYR lagoon. Temperatures were comparable among lagoons (21–23°C). Dissolved inorganic nitrogen concentrations were lower than 1.5 µM in the 3 lagoons, and mainly comprised ammonium. Chl *a*, TP and phosphate concentrations reflected the eutrophication gradient displayed by the lagoons. A remarkably high phosphate concentration (2.5 µM) was observed in the hypertrophic MW lagoon. TN concentrations were comparable for the oligotrophic AYR and the mesotrophic IN lagoons (around 30 µM), and 4-fold higher (123 µM) in the hypertrophic MW lagoon. This resulted in a strong decrease of the TN:TP ratio along the eutrophication gradient, from 72 (AYR) to 14 (MW).

3.2. Composition of phytoplankton communities

Phytoplankton communities in the 3 lagoons were dominated by ultraphytoplankton <5 µm. This represented 87, 63 and 84% of the total chl *a* concentrations in the oligo-, meso- and hypertrophic lagoons, respectively (Fig. 2). However, the composition of this major size class changed among lagoons. Green algae dominated the biomass of the ultraphytoplankton of the oligo- and the hypertrophic lagoons, representing 44.5 and 55.3% of the pool of pigments (Fig. 3). Among green algae, *Chlorella*-like cells dominated in the hypertrophic MW lagoon. Green algae and cryptophytes dominated the ultraphytoplankton of the mesotrophic IN lagoon, reaching 38.2 and 37.7% of the pigment concentrations. Cryptophytes were also

Table 1. Environmental parameters and phytoplankton biomasses expressed as chlorophyll *a* (chl *a*) concentration in the 3 lagoons during sampling in summer 2014. Chl *a* concentrations are expressed as mean and standard deviation of triplicates. Lagoons (see Fig. 1) are AYR: Ayrolle (oligotrophic), IN: north Ingril (mesotrophic), MW: west Méjean (hypertrophic). TN: total nitrogen, DIN: dissolved inorganic nitrogen, TP: total phosphorus. Dates are given as d/mo/yr

Lagoon	Date	Temp (°C)	Salinity	Nutrient (µM)							Total chl *a* (µg l^{-1})	TN:TP
				TN	NH_4	NO_3	NO_2	DIN	TP	PO_4		
AYR	03/09/14	21.0	30.9	32.5	0.62	0.00	0.07	0.69	0.45	0.12	0.23 ± 0.01	72.2
IN	25/08/14	20.8	41.5	28.2	0.99	0.15	0.07	1.21	0.89	0.21	0.98 ± 0.04	31.7
MW	27/08/14	23.2	36.6	123	0.68	0.00	0.37	1.05	8.72	2.48	36.1 ± 0.68	14.1

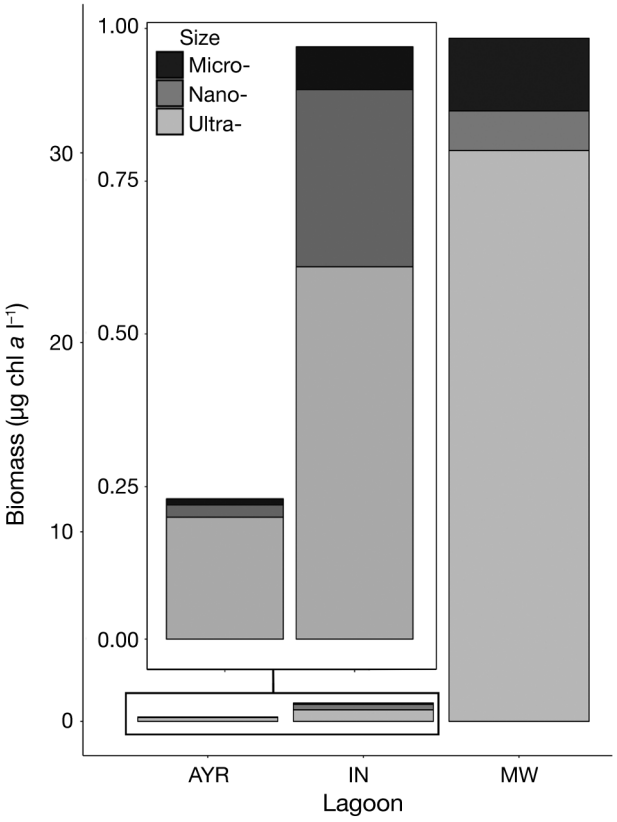

Fig. 2. Contribution of ultraphytoplankton (<5 µm, light grey), nanophytoplankton (5–20 µm, grey) and microphytoplankton (>20 µm, dark grey) to the total biomass, in the oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1) in summer 2014

well represented in the ultraphytoplankton of the oligotrophic AYR lagoon, with alloxanthin representing 19.4% of the pigments of this fraction. Small-size fucoxanthin-rich diatoms also composed the ultraphytoplankton of the 3 lagoons, with fucoxanthin representing 19.7, 12.1 and 16.6% of the pool of pigments in the oligo-, meso- and hypertrophic lagoons, respectively. Ultraphytoplankton also comprised picocyanobacteria, their biomass proportion reaching 7.6% in the mesotrophic, 12.6% in the oligotrophic and 19.7% in the hypertrophic lagoons. In terms of abundance, PC-cyan numerically dominated the ultraphytoplankton of the oligotrophic lagoon (Peuk: 11.9%, PC-cyan: 76.5%), while Peuk dominated those of the Palavas lagoons (IN and MW) (Fig. 4). The numerical dominance of Peuk was particularly marked in the hypertrophic MW lagoon, representing 68% of total cell counts. Moreover, PC-cyan were also abundant in the hypertrophic lagoon, representing 30.6% of the cell counts. In contrast, PE-cyan were only detected in the mesotrophic lagoon, reaching 2.3% of the ultraphytoplankton abundance (Fig. 4).

Nano- and microphytoplankton compositions also changed among lagoons. The hypertrophic MW lagoon still displayed the highest proportion of green algae, representing 64.7 and 61.6% of the pigment pools for nano- and microphytoplankton. Although peridinin was never detected in the hypertrophic lagoon, dinophyte abundances, proportions and richness were high according to microscopic observations. Moreover, the observed taxa, including Dinophysiales (*Oxyphysis oxitoxoides* Kofoid [1926]), Gonyaulacales (*Alexandrium* sp., *Gonyaulax spinifera* [Claparède & Lachmann] Diesing [1866]), Gymnodiniales (*Gymnodinium sanguineum* Hirasaka [1922]), Peridiniales (*Heterocapsa minima* Pomroy [1989], *H. niei* [Loeblich III] Morrill & Loeblich III [1981], *Peridinium quinquecorne* Abé [1927], *Kryptoperidinium foliaceum* Lindemann [1924]) and Prorocentrales (*Prorocentrum micans* Ehrenberg [1834]), were most likely mostly heterotrophic (lacking chl *a*).

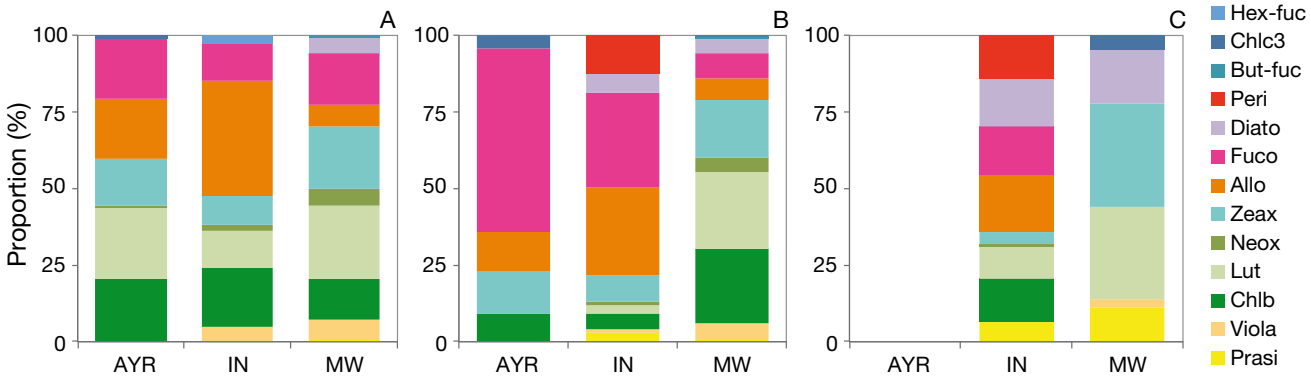

Fig. 3. Relative proportions of pigment biomarkers of phytoplankton communities in oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1), detailed for (A) ultra-, (B) nano- and (C) microphytoplankton. Pigment concentration of the microphytoplankton of AYR was below the detection limits. Hex-fuc: 19′hex-fucoxanthin, Chlc3: chlorophyll *c*3, But-fuc: 19′but-fucoxanthin, Peri: peridinin, Diato: diatoxanthine, Fuco: fucoxanthin, Allo: alloxanthin, Zeax: zeaxanthin, Neox: neoxanthin, Lut: lutein, Chlb: chlorophyll *b*, Viola: violaxanthin, Prasi: prasinoxanthin

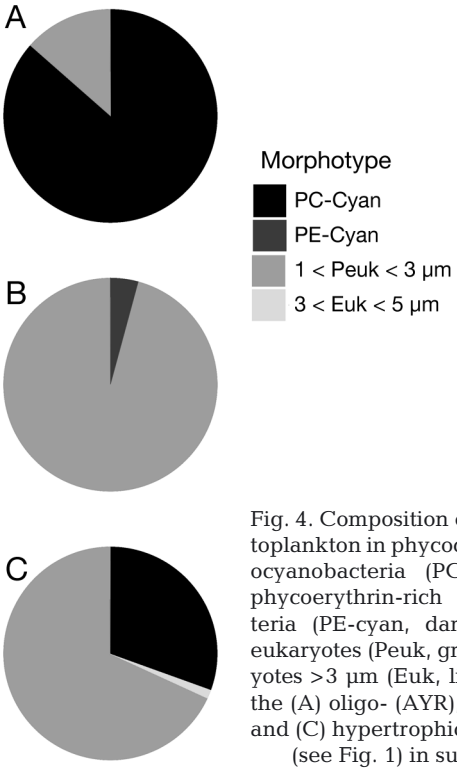

Fig. 4. Composition of the ultraphytoplankton in phycocyanin-rich picocyanobacteria (PC-cyan, black), phycoerythrin-rich picocyanobacteria (PE-cyan, dark grey), picoeukaryotes (Peuk, grey), and eukaryotes >3 µm (Euk, light grey) from the (A) oligo- (AYR), (B) meso- (IN) and (C) hypertrophic (MW) lagoons (see Fig. 1) in summer 2014

3.3. Growth and mortality rate without nutrient enrichment

Growth and mortality rate estimates based on chl *a* concentrations are indicated in Table 2. Without enrichment, the total phytoplankton of the oligotrophic lagoon exhibited a growth rate (μ_0) of 1.20 d^{-1}. In the mesotrophic lagoon, the total biomass strongly decreased during the incubation without enrichment, which impeded the calculation of a positive μ_0. In the hypertrophic lagoon, no meaningful rates could be estimated for the total phytoplankton using the linear model, the trend of the relationship between apparent growth rate and dilution factor being non-linear (see Fig. S1C in the Supplement at www.int-res.com/articles/suppl/a083p131_supp.pdf). However, by dividing the community into size classes, we were able to calculate specific growth and grazing rates for the microphytoplankton and ultraphytoplankton in all 3 lagoons (Table 2).

The microphytoplankton of the oligotrophic lagoon, mainly represented by diatom species, exhibited the highest μ_0, reaching 3.95 d^{-1}. Conversely, the ultraphytoplankton, which dominated the community and

The mesotrophic lagoon exhibited the highest pigment diversity both for nano- and microphytoplankton (Fig. 3). These fractions also displayed nearly even proportions of different taxonomic groups among dinophytes (peridinin), green algae, fucoxanthin-rich diatoms and cryptophytes (alloxanthin). This was the only lagoon to exhibit peridinin, representing 12.4% of the pigment pool for nanophytoplankton and 14.2% for microphytoplankton. In the oligotrophic AYR lagoon, diatoms (2.3×10^4 cells l^{-1}) represented by several *Chaetoceros* species and benthic species such as *Licmophora* sp. dominated the nano- and microphytoplankton. This dominance was illustrated by 60.0% of fucoxanthin in the pigment pool of the nanophytoplankton, and 56% of the total abundance was represented by this class. Fractioning did not allow us to assess the chemotaxonomic diversity of microphytoplankton in the oligotrophic lagoon, probably due to an insufficient pigment concentration.

Table 2. Specific growth rates (μ_0) and grazing rate (g) measured in incubations without enrichment and maximum specific growth rates (μ_{max}) subsequently calculated using complete enrichment in the oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1) and for total phytoplankton and micro-, nano- and ultraphytoplankton fractions. Values were estimated according the linear Landry & Hassett (1982) equation. This model was compared to alternative models by mixed-effect multiple linear regressions of apparent growth rates, based on chl *a* concentration from dilution experiments. The parsimony of the different models was checked by the corrected Akaike's information criterion (AIC_c). The linear model was only accepted when it presented a $\Delta AIC_c < 2$ with respect to the most parsimonious model (see Section 2). AIC_c-w describes the strength of explanation of the model. –: no coefficient

Lagoon	Fraction	Rates (d^{-1})			n	AIC_c	ΔAIC_c	AIC_c-w
		μ_0	g	μ_{max}				
AYR	Total	1.20	0.00	1.29	8	4.2	0.0	0.68
	Micro	3.95	1.38	4.41	7	15.2	0.0	0.65
	Nano	–	–	–	8	15.0	5.6	0.05
	Ultra	1.14	0.46	1.28	10	5.9	0.0	0.43
IN	Total	–0.18[a]	0.22	0.43	10	–8.6	0.0	0.41
	Micro	1.22	0.48	1.83	8	7.4	0.3	0.34
	Nano	–	–	–	10	25.2	4.3	0.09
	Ultra	–0.02[a]	0.35	0.61	9	1.8	0.1	0.34
MW	Total	–	–	–	10	0.3	6.3	0.04
	Micro	2.16	2.10	1.34	10	29.2	0.0	0.71
	Nano	–	–	–	10	27.6	3.0	0.16
	Ultra	0.91	1.17	1.14	10	0.0	0.0	0.86

[a]A strong decrease of the total phytoplankton biomass, estimated with the chl *a* concentration, after the 24 h incubation without enrichment impeded the calculation of a positive μ_0

was mainly composed of PC-cyan (Fig. 4), exhibited a lower μ_0 (1.14 d^{-1}), close to that of its total phytoplankton community (Table 2). In the mesotrophic lagoon, the highly diverse microphytoplankton community displayed a μ_0 of 1.22 d^{-1}, while the ultraphytoplankton, which dominated the community and was mainly composed of Peuk, exhibited a μ_0 close to 0, indicating a growth limitation. In the hypertrophic lagoon, the microphytoplankton, which was mostly composed of green algae and dinophytes, showed a high growth rate (μ_0 = 2.16 d^{-1}), while the ultraphytoplankton, which dominated the community and was mainly composed of *Chlorella*-like cells, exhibited a μ_0 <1 d^{-1}.

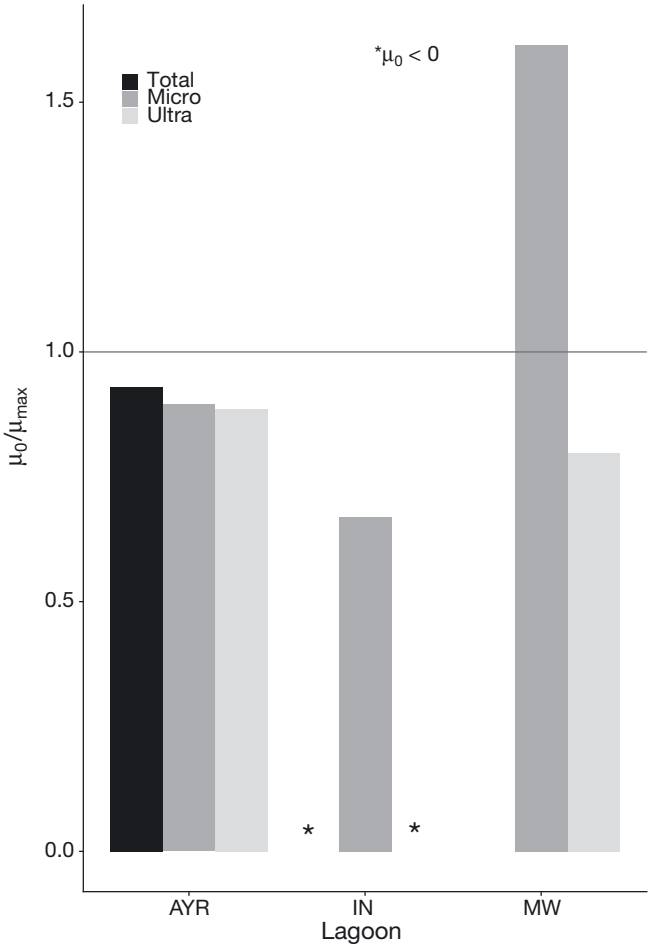

Fig. 5. Ratio between growth without enrichment and maximal growth rate with total enrichment (μ_0:μ_{max}) of total phytoplankton (black), micro- (grey) and ultraphytoplankton (light grey), in the oligo- (AYR), meso- (IN) and hypertrophic (MW), lagoons (see Fig. 1), based on growth rates calculated from chl *a* concentrations in summer 2014. Horizontal line highlights μ_0:μ_{max} ratio = 1, meaning there was no change in growth rate with the full enrichment. Asterisks indicate when $\mu_0 < 0$, leading to a negative μ_0:μ_{max} ratio

3.4. Impact of the nutrient enrichment on growth rate and limitation

The addition of complete enrichment before the incubation resulted in an increased growth rate, except for the microphytoplankton of the hypertrophic lagoon, which decreased by 36% (Table 2). Nevertheless, in the hypertrophic lagoon, the ultraphytoplankton growth rate increased by 25.3%. In the mesotrophic lagoon, the enrichment released the growth limitation, allowing a positive μ_{max}. The microphytoplankton growth rate increased by 50% with the addition of the enrichment. In the oligotrophic lagoon, the enrichment allowed an increase of 7.5% of the growth rate for the total phytoplankton, 11.6% for the microphytoplankton and 12.2% for the ultraphytoplankton.

The ratio of μ_0:μ_{max} can be an index of the severity of nutrient limitation under natural conditions (Fig. 5). Hence, the total community and the ultraphytoplankton of the mesotrophic lagoon were strongly limited, as reflected by μ_0:μ_{max} ratios <0. A moderate limitation was observed for the microphytoplankton of the mesotrophic lagoon ($0.5 < \mu_0$:$\mu_{max} = 0.67 < 0.75$). A minor degree of nutrient limitation was detected for the total, the micro- and the ultraphytoplankton of the oligotrophic lagoon and for the ultraphytoplankton of the hypertrophic lagoon (μ_0:$\mu_{max} > 0.75$). The total phytoplankton of the hypertrophic lagoon was also limited, as suggested by the non-linear trend of the relationship between the apparent growth rate and the dilution factor (Fig. S1C).

3.5. N and P limitation and resource exploitation during experimentally induced N and P limitation

The growth rates of the total phytoplankton and the size classes under experimentally induced N and P limitations are presented in Table 3, as well as the Q_N and the Q_P ratios illustrating the severity of these limitations.

The total phytoplankton, the micro- and the ultraphytoplankton of the 3 lagoons most often exhibited nitrogen limitation, expressed by μ_{-N} lower than μ_{max}, leading to Q_N values <1 (Table 3, Fig. 6A). More particularly, in the oligotrophic lagoon, after 24 h incubation with the enrichment minus N, we were not able to detect microphytoplankton biomass and were thus unable to calculate a μ_{-N} value, suggesting a sharp N limitation for this fraction. The ultraphytoplankton in the hypertrophic lagoon showed a strong N limitation (Q_N = 0, Fig. 6A).

Table 3. Maximum growth rates with complete enrichment (μ_{max}), with enrichment but without N (μ_{-N}) or without P (μ_{-P}), and Q_N and Q_P values (see Section 2.4) based on μ_{max} and μ_{-N} or μ_{-P} ratios respectively, for the oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1) and for total phytoplankton, micro-, nano- and ultraphytoplankton. –: data missing or not measurable

Lagoon	Fraction	Rates (d^{-1})			Q_N	Q_P
		μ_{max}	μ_{-N}	μ_{-P}		
AYR	Total	1.29	–1.06	0.76	–	0.53
	Micro	4.41	–	1.54	–	0.38
	Nano	–	1.48	1.18	–	–
	Ultra	1.28	0.91	1.13	0.70	0.88
IN	Total	0.43	0.21	0.26	0.48	0.59
	Micro	1.83	0.66	1.52	0.36	0.83
	Nano	–	0.20	–0.08	–	–
	Ultra	0.61	0.16	0.13	0.27	0.21
MW	Total	–	–0.01	1.25	–	–
	Micro	1.34	–0.21	2.10	–	1.57
	Nano	–	0.85	1.35	–	–
	Ultra	1.14	0.00	1.13	0.00	0.99

The total phytoplankton of the oligo- and the mesotrophic lagoons also exhibited a P limitation, illustrated by Q_P values <1. The P limitation increased with decreasing eutrophication for the micro- and the dominant ultraphytoplankton in the 3 lagoons (Fig. 6B). The micro- and ultraphytoplankton of the hypertrophic lagoon were not P-limited (Q_p > 1). In summary, along the studied eutrophication gradient, limitations varied from a co-limitation by N and P in the oligotrophic and the mesotrophic lagoons to a single and strong N limitation in the hypertrophic lagoon (Fig. 6B).

To cope with the experimentally induced nitrogen limitation, the total phytoplankton of the 3 lagoons differently used the 3 considered N pools (Fig. 7A,C, Table S1). In the hypertrophic lagoon, phytoplankton mainly used recycled N, while in the mesotrophic lagoon phytoplankton mainly used the internal pool and 18% of external N (Fig. 7A). The size class decomposition specified different trends across the communities (Fig. 7C). In the hypertrophic lagoon, the dominant ultraphytoplankton exhibited an exclusive use of the recycled pool, while the 2 other size classes only used the internal N pool (Fig. 7C). In the mesotrophic lagoon, the N use of the 2 main size classes echoed the total phytoplankton: ultraphytoplankton used internal resources and nanophytoplankton used the external one. The microphytoplankton used 84% of recycled N and 16% of internal N pools. In the oligotrophic lagoon, the ultra- and nanophytoplankton used internal N resources.

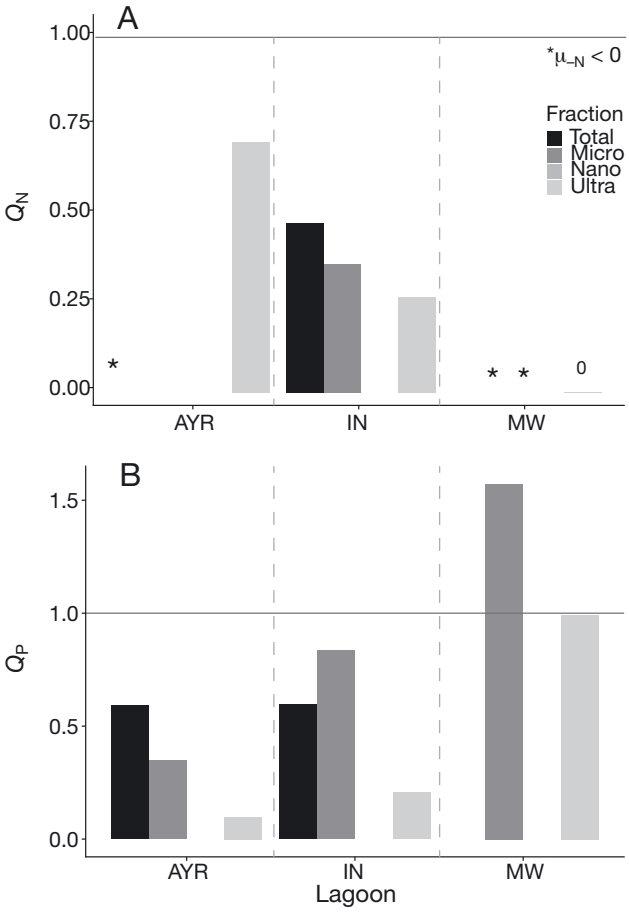

Fig. 6. Ratio between maximal growth rates with total enrichment and enrichment (A) without N (Q_N) and (B) without P (Q_P) for total phytoplankton (black) and micro- (dark grey) and ultraphytoplankton (light grey), in the oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1), based on growth rates calculated from chl *a* concentrations in summer 2014. Lagoons separated by dashed vertical lines. The horizontal line highlights ratios equal to 1, meaning there was no change in growth rate with the enrichments minus N or minus P. Asterisks indicate when μ_{-N} were <0,leading to negative Q_N or Q_P

To cope with the experimentally induced phosphorus limitation, the total phytoplankton in the 3 lagoons used the 3 different P pools (Fig. 7B,D, Table S1). Phytoplankton mainly used 67% of internal and 33% of recycled P loads in the oligotrophic lagoon (Fig. 7B); 91% of recycled P and 9% of external P loads in the mesotrophic lagoon; and 85% of internal P and 15% of external P loads in the hypertrophic lagoon. The decomposition in size classes highlights several strategies of P use (Fig. 7D). In the oligotrophic lagoon, the microphytoplankton only used recycled P, while the nano- and ultraphytoplankton only used internal P loads. In the mesotrophic lagoon, the microphytoplankton used 90% of recycled P and 5% of external and internal loads,

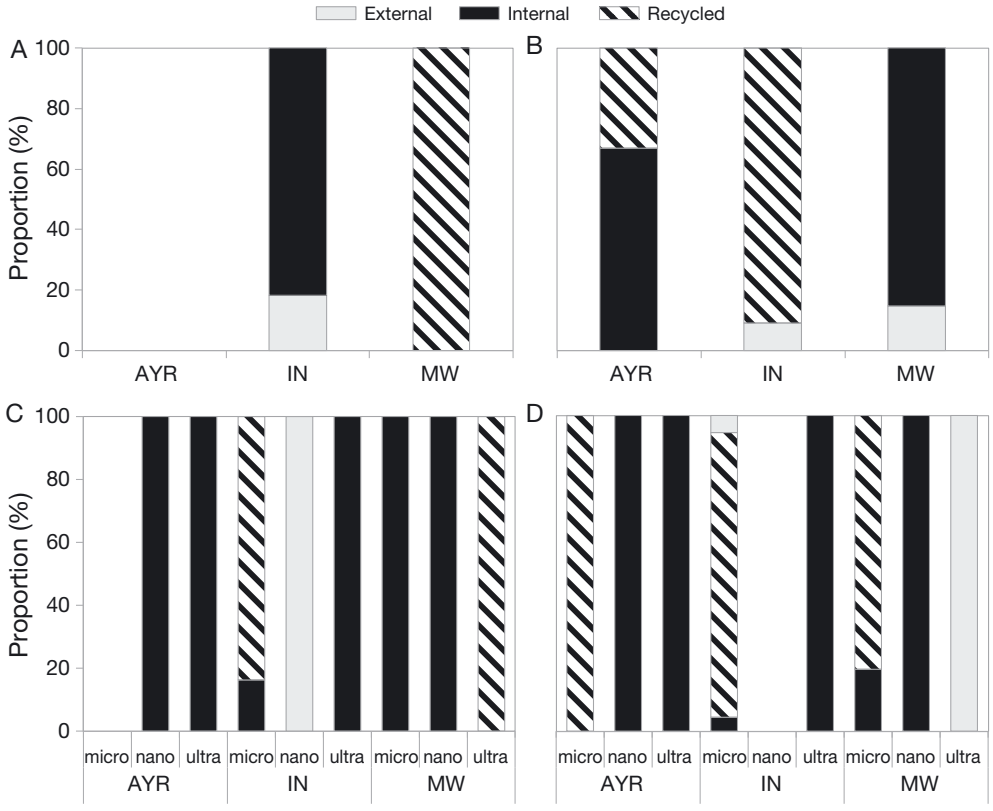

Fig. 7. Contribution of internal (black), external (light gray) and recycled (hatched) (A,C) nitrogen and (B,D) phosphorus resources used for growth under limited condition, for total phytoplankton (A,B), and decomposed for micro-, nano- and ultraphytoplankton (C,D) in oligo- (AYR), meso- (IN) and hypertrophic (MW) lagoons (see Fig. 1) during summer 2014. Negative values of *K* coefficients have not been taken into account

while the ultraphytoplankton only used internal P loads. In the hypertrophic lagoon, external P loads were exclusively used by the ultraphytoplankton. The microphytoplankton used 80% of recycled and 20% of internal P, and the nanophytoplankton exclusively used internal P loads.

4. DISCUSSION

4.1. Phytoplankton community composition related to different eutrophication status

In this paper, we describe the phytoplankton communities of 3 contrasting lagoons sampled in summer 2014, and discuss how their adaptive strategies of nutrient use can be linked with the characteristics of the lagoons, especially their eutrophication status. We particularly pay attention to how the re-oligotrophication processes initiated in 2005 in the hypertrophic and mesotrophic lagoons impact these strategies.

The summer phytoplankton communities were dominated by ultraphytoplankton in all 3 lagoons. This is a characteristic feature of most Mediterranean coastal lagoons (Bec et al. 2011). Small autotrophic cells are particularly competitive in taking up nutrients due to their high nutrient affinities (Chisholm 1992, Raven 1998). Indeed, DIN concentrations were below 1.5 µM in the 3 lagoons, showing the ability of these small cells to take up DIN. Ammonium dominated the DIN during summer, as it results from internal recycling and benthic fluxes (Collos et al. 2003, Ouisse et al. 2013). Both cell counts and biomasses showed that Peuk mainly comprising *Chlorella*-like cells dominated the ultraphytoplankton of the hypertrophic lagoon. Among the green algae, prasinophytes and chlorophytes observed in this lagoon both possess a good tolerance to high NH_4 concentrations, as well as a high affinity for uptake (Bec et al. 2011). In addition, these organisms are also well adapted to turbulence, turbidity and variable salinity (Paerl et al. 2007), as observed in the hypertrophic lagoon (Leruste et al. 2018). Moreover, their high photosynthetic efficiency also confers a competitive advantage (Raven 1998, Litchman et al. 2010), which may explain their success in this turbid system (Bec et al. 2005, 2008, 2011).

The abundance of heterotrophic dinophytes in the hypertrophic lagoon can be linked to their competitive advantage in acquiring nutrients in systems displaying important stocks of organic matter and low dissolved inorganic nutrient concentrations. Indeed, when dissolved inorganic nutrients are depleted or when there is a strong competition for nutrients, mixotrophic or strictly heterotrophic species can use alternative nutrient resources and have high growth efficiency (Litchman et al. 2006). Moreover, compared to strict autotrophs and heterotrophs, mixotrophs are more successful under N limitation, because of their higher growth efficiency when consuming N-starved prey (Fischer et al. 2017). For example, in the nearby Thau lagoon, dinophytes, comprising mixotrophic and heterotrophic species blooming under N limitation during dry periods, and some mixotrophic species consumed dissolved organic nitrogen (Collos et al. 2014). Moreover, several dinophyte species, such as *Gyrodinium* sp. and *Protoperidinium* sp. observed in the hypertrophic and the mesotrophic lagoons, have demonstrated abilities to graze on diatoms or ciliates (Legrand et al. 1998). These abilities could explain their persistence in these systems dominated by Peuk and despite the nutrient depletion (Zhang et al. 2005). Dissolved and particulate organic nutrient concentrations in the lagoon water column and sediments, and the remineralization of these nutrient stocks, were not estimated in this study, and would be very interesting to explore. Such studies would reveal more about the real availability of nutrients for the potentially mixotrophic and heterotrophic species we observed in this study.

In the oligotrophic lagoon, cell counts showed that picocyanobacteria were more abundant than Peuk, while Peuk biomass dominated over picocyanobacteria. This apparent contradiction can be explained by the larger size of Peuk (1–3 µm) than picocyanobacteria (≤1 µm). Hence, picocyanobacteria represented a lower biomass, but were still able to outnumber Peuk in this oligotrophic system because of their small cell size. Picoyanobacteria in both oligotrophic and hypertrophic lagoons were dominated by PC-cyan, which contrasted with the mesotrophic lagoon where picocyanobacteria were dominated by PE-cyan. PC-cyan efficiently use red light, which dominates the spectrum in turbid waters with high plankton densities or high sediment re-suspension. Hence, their presence in the oligotrophic lagoon appears to be linked to turbidity due to recurrent sedimentary suspension affecting the water column light quality (Stomp et al. 2007). Conversely, the presence of PE-cyan in the mesotrophic lagoon reflected an adaptation to low turbidity (Vörös et al. 1998).

Without enrichment, the highest calculated specific growth rates were 3.95 and 2.16 d^{-1} for the microphytoplankton in the oligo- and hypertrophic lagoons, respectively. In the oligotrophic lagoon, this size fraction was mainly composed of diatoms, which have already shown high growth rates in Mediterranean lagoons (Bec et al. 2005). The microphytoplankton of the hypertrophic lagoon in this sampling was mainly composed of mixo- or heterotrophic dinophytes that displayed competitive advantages in coping with the nutrient limitation.

4.2. Phytoplankton nutrient limitation

During the summer season, nutrient pulses in the lagoons are scarce, and nutrients can be rapidly depleted, depending on the sediment stocks. Nutrient limitation impacts phytoplankton biomass, community composition in terms of functional groups, and growth (Domingues et al. 2011). The TN:TP ratio, which is a proxy for elemental ratios in phytoplankton, provided a first insight into the nutrients limiting their growth (Ptacnik et al. 2010, Souchu et al. 2010). This ratio was higher than the Redfield ratio for the oligo- and mesotrophic lagoons, and slightly lower for the hypertrophic lagoon. This suggests that phosphorus was the limiting factor in the oligo- and mesotrophic lagoons, while nitrogen was the limiting factor in the hypertrophic lagoon, which is in agreement with earlier observations of a shift from phosphorus to nitrogen limitation with increasing eutrophication (Souchu et al. 2010). The high dissolved inorganic phosphorus concentration in the hypertrophic lagoon further supports the notion that phosphate was not limiting.

The 'All minus one' experiments give further indications about *in situ* growth-limiting factors. Indeed, nitrogen limitation in the hypertrophic lagoon was reflected by Q_N and Q_P values (0 and 1). In contrast, in the oligotrophic and mesotrophic lagoons, Q_N and Q_P values consistently below 1 indicate the ease of inducing N and P limitation in experimental incubations. This raises the question of whether phytoplankton in these lagoons was co-limited by N and P simultaneously. Although this is in principle in contradiction with Liebig's 'law of the minimum' (von Liebig 1840), there is increasing evidence of N and P co-limitation in freshwater, marine and coastal ecosystems (Sakka Hlaili et al. 2006, Elser et al. 2007, Harpole et al. 2011). Such co-limitation is consistent

with both low DIN and DIP concentrations in the oligotrophic and mesotrophic lagoons. Moreover, a more gradual shift from P limitation via N and P co-limitation to an exclusive N limitation between lagoons with increasing eutrophication has already been demonstrated (Souchu et al. 2010). Among the co-limitation, different size classes expressed some specificities according to their composition, their affinity with nutrients determining their uptake efficiency and the amount of available nutrients. For example, in the oligotrophic lagoon, PC-cyan were more N- than P-limited, probably because of their high affinity for P due to specific mechanisms of acquiring this nutrient (Donald et al. 1997). To sustain their growth under limitation, phytoplankton used other resources in addition to those externally supplied. For example, in the hypertrophic lagoon, the dominant ultraphytoplankton was the most competitive because it used recycled N and external P. In this lagoon, the nanophytoplankton used internal N and P pools, while the microphytoplankton predominantly used recycled P.

4.3. Reactivity of phytoplankton to nutrient pulses

To better understand phytoplankton adaptations to environmental changes during the re-oligotrophication process, it is necessary to assess the sensibility and the responsiveness of these communities to a nutrient pulse (Duarte et al. 2000, Wood & Bukaveckas 2014). We assume that re-oligotrophication, which reduces nutrient availability, results in an increase in the nutrient limitation for phytoplankton communities. Moreover, the nutrient requirement is expected to increase with increasing eutrophication (Domingues et al. 2015). Eutrophication has the greatest impact on ecosystem functioning in Mediterranean lagoons during summer, when temperature and irradiance are optimal (1) for the growth of autotrophic organisms, and (2) for the recycling of nutrients from the sediment (Collos et al. 2003). In most cases where we were able to calculate specific growth and grazing rates, nutrient enrichment enhanced growth rates, reflecting the nutrient limitation occurring in summer. The only exception was for the microphytoplankton of the hypertrophic lagoon, for which we observed an unlikely ratio of μ_0:μ_{max}= 1.6, which was probably due to an underestimation of μ_{max}. Hence, despite the high concentration of added nutrients (20 µM final concentration of nitrogen), the enrichment was insufficient to allow a linear trend of the total phytoplankton apparent growth rate as a function of the dilution factor (Fig. S1C) (Landry & Hassett 1982). Most likely, the very high phytoplankton biomass resulted in a nutrient depletion for the less diluted samples and consequently in an underestimation of their specific growth rates. This was supported by the very low apparent growth rate in the bottles containing 100 and 75% of unfiltered water.

In the oligotrophic lagoon, the nutrient pulse only marginally stimulated the phytoplankton growth rate (less than 12%) compared to the response of the communities of both lagoons (IN and MW) from the Palavas complex. This may reflect a luxury consumption of N and P, which is a common strategy used by phytoplankton to deal with variable nutrient variability. Cells use nutrient enrichment to build up an intracellular storage that can later be used for growth after the depletion of the external nutrient supply (Domingues et al. 2015). Several phytoplanktonic groups commonly use these strategies. For example, many diatoms use storage (Litchman et al. 2007, Domingues et al. 2011), and several picophytoplanktonic species use luxury consumption in variable nutrient regimes (Glover et al. 2007). The phytoplankton of the mesotrophic lagoon responded most to the enrichment, showing the highest increase (50%) in growth rates of microphytoplankton. This result highlights that the re-oligotrophication trajectory of this lagoon is still very vulnerable to occasional eutrophication events. The ultraphytoplankton of the hypertrophic lagoon also responded to the enrichment by an increase in its growth rate. However, this increase was lower than that of the phytoplankton of the mesotrophic lagoon. Considering the high phytoplankton biomass and abundances in the hypertrophic lagoon, the enrichment was insufficient to satisfy their nutrient requirement and allow the estimation of their maximal growth rate.

4.4. Vulnerability of lagoons to nutrient pulses during re-oligotrophication

Our experiments may provide some indications whether an incidental nutrient pulse impacts phytoplankton communities, suggesting a vulnerability of the lagoon to eutrophication, even during a re-oligotrophication process. In the mesotrophic lagoon, the micro- and ultraphytoplankton size classes quickly reacted to the enrichment, showing that they are highly sensitive to a nutrient pulse. Among the phytoplankton and the macrophytic communities in this lagoon, Peuk always seem to be the most reac-

tive to a nutrient pulse. This highlights the fragility of the re-oligotrophication process during the summer period, even in a relatively less degraded lagoon compared to other eutrophicated systems.

Despite the 9 yr of re-oligotrophication, the hypertrophic lagoon still presented eutrophic characteristics, and was still dominated by picophytoplankton (Bec et al. 2011), which was the main group reacting to the nutrient pulse; within this group, *Chlorella*-like cells were particularly reactive. These cells are highly competitive in turbulent systems, during summer, and when DIN supply is dominated by ammonium (Margalef 1978, Reynolds & Lund 1988, Litchman et al. 2007). However, the enhancement of the ultraphytoplankton growth by the enrichment was attenuated by the particularly high phytoplankton biomasses observed in August 2014. Indeed, the chl *a* concentration mainly stemming from Peuk reached 36 µg l^{-1}. This high biomass was much higher than that observed during the preceding summer periods (median chl *a* concentrations of 3.68 µg l^{-1} between June and August from 2007 to 2013). This was the first return to levels (mean ± SD) observed at the beginning of the re-oligotrophication process in summer 2006 (79.9 ± 40.7 µg chl *a* l^{-1}, Leruste et al. 2016). Such density may reflect the lagoon's vulnerability and potential inertia to the re-oligotrophication, due to occasional nutrient inputs and recurrent internal loads sustained by benthic fluxes (Phillips et al. 2005). These fluxes of ammonium and phosphate are particularly important during summer (Collos et al. 2003, Ouisse et al. 2013), and were partly reflected by the high phosphate concentration (2.48 µM). This release impacts benthic and pelagic communities, particularly in shallow systems with less than 5 m depth, such as most coastal lagoons (Cowan et al. 1996). Hence, this may have enhanced Peuk and PC-cyan growth (Velasco et al. 2006), leading to their bloom (Carstensen et al. 2007).

In contrast, in the oligotrophic lagoon, the very low biomass and the numerical dominance of PC-cyan reflect an adaptation to the oligotrophic conditions (Caroppo 2000). Compared to those of the other lagoons, the community of Ayrolle was slightly enhanced by the nutrient enrichment. In this respect, the μ_0 values were surprisingly high both for the micro- and the ultraphytoplankton. Moreover, these were only partly compensated by the measured grazing rates. In this shallow oligotrophic lagoon, the ecosystem functioning is largely impacted by the benthos. An important cover of seagrasses (*Zostera noltei*, >80% of coverage) predominates the primary production and inorganic nutrient uptake (De Wit et al. 2017). In addition, the benthos is home to suspension feeders that exert grazing pressure on phytoplankton, maintaining low population densities despite high μ_0 values. Thus, benthic and macrophytic communities mainly benefit from nutrient input (Bricker et al. 2008). The phytoplankton may have adapted by using other nutrient sources, such as internal pools, to cope with this low access to nutrient loads (Glover et al. 2007). We expect that the short-term vulnerability of the currently meso- and hypertrophic lagoons will only decrease after prolonged re-oligotrophication, and particularly upon return of the benthic communities that are characteristic for the oligotrophic conditions, such as the marine Magnoliophyta.

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