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PRIMARY RESEARCH PAPER

Complementarity of the multidimensional functional and the taxonomic approaches to study phytoplankton communities in three Mediterranean coastal lagoons of different trophic status

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Abstract We used the individual-based multidimensional functional diversity and the taxonomic approaches in a complementary way to describe phytoplankton communities in three coastal lagoons with different eutrophication status in the South of France. We sampled communities during three seasons, i.e., in autumn, spring, and summer. Using classical taxonomy, 107 taxa/morphotypes were identified in the nine communities. The individual-based functional approach allowed grouping these individuals into 20 functional entities according to their values for 5 traits related to trophic adaptations (cell size, mobility, trophic regime, coloniality, and pelagic/benthic life). Some species (e.g., *Prorocentrum micans*) emerged in multiple functional entities, showing the importance to consider intraspecific variability. The functional description of phytoplankton communities better reflected the hydrological functioning and the different eutrophication status of the lagoons than the taxonomic approach. Specific functional adaptations were identified in the nine communities. For example, phytoplankton organisms with heterotrophic and potentially mixotrophic abilities occurred when the availability of inorganic nutrient decreased, or when organic matter and small preys were potentially the main nutrient resources. The limitation has also favored small cells highly competitive for nutrients. Using functional indices together with taxonomic description has also helped revealing important aspects of community assembly, such as competitive exclusion in summer.

Keywords Classification · Functional entity · Functional traits · Taxonomic diversity

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Introduction

Ecosystem conservation and sustainable management need to understand and predict the effect of environmental changes and disturbances on biodiversity. Indeed, biodiversity loss has significant impacts on ecosystem functioning, affecting their resistance and resilience to disturbances. However, specific diversity cannot completely explain the adaptations of communities to the variability of environmental characteristics. This is because of functional redundancy, i.e., species performing similar functions in the ecosystem (Mouillot et al., 2013), or because of the complex and intrinsic dynamics of communities (Scheffer et al., 2003). Hence, diversity indices based only on taxonomic identity provide an incomplete view of the biodiversity and its relationship with environmental characteristics (Villéger et al., 2010), while the functional trait approach facilitates the synthesis between community ecology and ecosystem ecology (McGill et al., 2006).

Biodiversity of phytoplankton represents a widely used biological indicator of aquatic ecosystem functioning, and has been used to understand the impacts of human activities on these ecosystems. This is thus essential to understand how the variability of environmental conditions acts on the structure and the composition of phytoplankton communities (Litchman et al., 2007, 2010; Litchman & Klausmeier, 2008; Paerl et al., 2010). However, it is sometimes difficult to relate the taxonomic composition of the phytoplankton communities directly to the many environmental constraints and fluctuations that influence these communities. Indeed, in highly variable ecosystems, such as shallow lakes (Tafas & Economou-Amilli, 1997; Naselli-Flores & Barone, 2003), or transitional waters (Cloern & Jassby, 2010), these environmental changes differently impact the pool of species, leading to a high diversity but to real difficulties to link it with ecosystem dynamics.

To overcome this difficulty for linking the taxonomic diversity of communities with their ecosystem functioning, several functional approaches have been developed. To describe phytoplankton and especially its adaptations to environmental conditions and its ecological role, Margalef (1978) first proposed a graphical representation of the niche occupancy by the major phytoplankton taxonomic groups, using a two-dimensional space. Its representation known as phytoplankton mandala (Margalef et al., 1980) was largely based on how the form and structure of phytoplankton taxa are related to their functionality in the environment. Since then, more comprehensive functional classifications based on groups of species sharing the same functional traits have been proposed (Reynolds et al., 2002; Weithoff, 2003; Litchman & Klausmeier, 2008; Salmaso et al., 2015). Functional traits are defined as morpho-physiological or phenological traits which impact fitness via their effects on species growth, reproduction, and survival (Violle et al., 2007). These approaches were based on (1) the known responses of species to environmental conditions, including abiotic, biotic parameters, and disturbances; and (2) their role in ecosystem functioning. More recently, studies have used multivariate statistics to uncover the links between the community composition in terms of functional groups and the environmental conditions (Kruk et al., 2010; Palffy et al., 2013; Roselli & Basset, 2015; Weithoff & Gaedke, 2017). So far, functional approaches in phytoplankton ecology have been mainly based on allocating species into functional groups without observing the traits of individual organisms. Nevertheless, traits may be subjected to a strong intraspecific variability (Naselli-Flores et al., 2007). This can be related to intraspecific genetic variability, as well as to phenotypic plasticity (Salmaso & Padisak, 2007; Salmaso et al., 2015). Indeed, individuals of a single species may affiliate to different functional groups, and an individual may change its affiliation to different functional groups with time. Several misunderstandings of the use of functional groups can also be caused by species having similar morphological characteristics but distinct environmental tolerances (morpho-functional groups) (Padisak et al., 2009; Salmaso et al., 2015). Hence, the allocation of species in functional groups may lead to a loss of information or to a weakness in the explanation of ecosystem processes (Villéger et al., 2010). Recently, the study of the intraspecific variability of trait-based functional diversity of phytoplankton has been initiated both at the local scale within the lagoon of Venice (Aubry et al., 2017) and at a global scale (Roselli & Litchman, 2017), focusing on the size and shape of the cells. We proposed to extend this individual-based approach, using a panel of functional traits and indices developed in aquatic ecology. The selection of traits is decisive in the determination of the functional distance between species, so this choice must be well considered (McGill et al., 2006; Litchman & Klausmeier, 2008; Pavoine et al., 2013). For phytoplankton, relevant functional traits driving the performance of species reflect essential processes such as growth, sedimentation, grazing losses, and nutrient acquisition (Weithoff, 2003; Litchman & Klausmeier, 2008).

To study the potentialities of the individual-based multidimensional functional approach, alone and in combination with the taxonomic approach, for understanding the structure of phytoplankton communities, we selected three brackish Mediterranean coastal lagoons with different eutrophication status. The multidimensional functional diversity framework, using many indices such as the functional richness (FRic), the functional evenness (FEve), and the functional divergence (FDiv), has already been used to describe fish communities in coastal lagoons (Villéger et al., 2010), aquatic vegetation in shallow eutrophic lakes (Arthaud et al., 2012), and marine zooplankton (Pomerleau et al., 2015). The functional approach based on the traits of the individual phytoplankton organisms may improve the descriptions of community assembly and some other important aspects of ecosystem functioning in coastal lagoons. Very small cells can represent the major part of the phytoplankton communities in coastal lagoons (Bec et al., 2005; Glibert et al., 2010). By optical microscopy, these cells are often difficult to identify to the species level and sometimes even to the genus level. Therefore, they have often been overlooked in classical taxonomic studies or been lumped together as morphotypes (Bec et al., 2011; Palffy et al., 2013). The functional approach thus allows to consider these latter morphotypes in a meaningful way and complements the taxonomic approach in ecological studies.

In this study, we taxonomically and functionally described the phytoplankton diversity in three coastal lagoons with different eutrophication status, and during contrasting environmental periods that are supposed to differently shape phytoplankton communities. For this purpose, we used five functional traits (i.e., the cell size, the trophic regime, the mobility, the ability to form colonies, and the pelagic/benthic life mode) of phytoplankton individuals related to trophic adaptations to functionally describe the phytoplankton communities. We especially tested if the multidimensional functional approach represents an effective way for the study of phytoplankton communities in these very dynamic systems. In addition, the comparison between changes of taxonomic and functional indices values was used to understand ecological processes that structure the phytoplankton communities.

Methods

Study sites

Brackish Mediterranean coastal lagoons are transitional and shallow aquatic environments at the land–sea interface. French Mediterranean lagoons have been submitted to variable degrees of eutrophication for the last decades, leading to a strong alteration of their autotrophic communities (Souchu et al., 2010; Bec et al., 2011; Le Fur et al., 2018). We focused the study on three shallow coastal lagoons (less than 1 m average depth) with contrasting eutrophication status located on the French Mediterranean coastline (Fig. 1). The Ingril and Méjean lagoons are located at the southwest and the northeast of the palavasian complex, close to the Montpellier city district. This complex comprises 8 lagoons due to compartmentalization of an original large coastal lagoon. The entire complex was submitted to strong anthropogenic inputs during several decades from the Montpellier city district wastewater treatment through the Lez River. The river mouth located northeast of the complex caused a gradual eutrophication of the lagoons, ranging from hypertrophy nearby, to mesotrophy at the southwestern extreme (Souchu et al., 2010). A drastic reduction of anthropogenic nutrient inputs has been achieved since 2005 as a result of the implementation of a new wastewater treatment plant and the diversion of its effluents, leading to the beginning of a re-oligotrophication process (Leruste et al., 2016). But despite this recent ecological restoration process, nutrient stocks in sediment still remain important and represent an internal loading (Souchu et al., 2010). The two selected lagoons are located landward under watershed influence, and communicate only indirectly with the Mediterranean Sea through the Rhone-to-Sète canal (Leruste et al., 2016). West Méjean (MW), directly connected to the Lez River is hypertrophic. North Ingril (IN) located farthest from the Lez River is mesotrophic. Phytoplankton community dominated the primary producers in West Méjean (Bec et al., 2011), although it is increasingly colonized by macroalgae since the beginning of the ecological restoration process (De Wit et al., 2017). North Ingril hosts a huge diversity of autotrophic organisms, including phytoplankton, macroalgae, and seagrasses. The third coastal lagoon, Ayrolle (AY) is oligotrophic. Ayrolle lagoon is directly connected to the

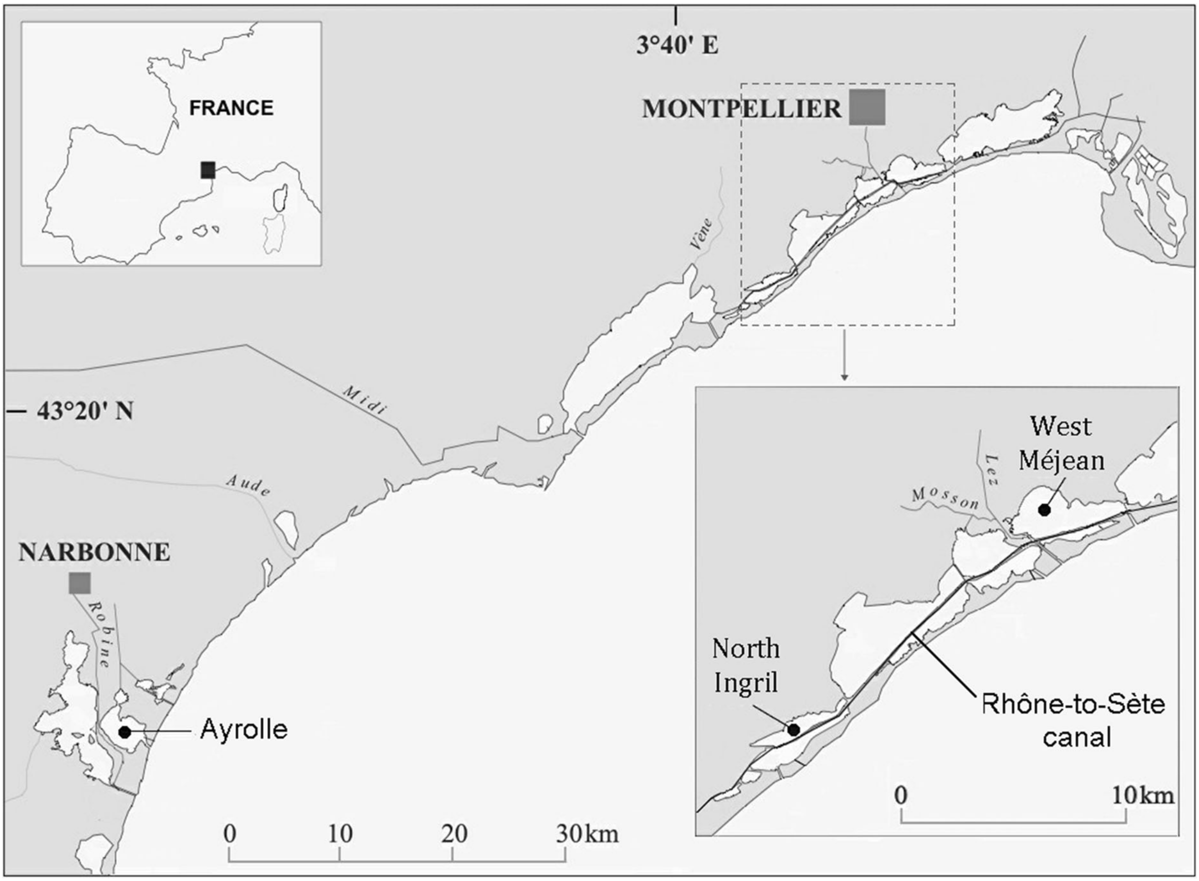

Fig. 1 Location of the 3 sampling stations in the South of France. North Ingril (IN) and West Méjean (MW) lagoons are located in the same complex of coastal lagoons next to the Montpellier city. Ayrolle (AY) lagoon is near the Narbonne city

Mediterranean Sea through an inlet and receives few nutrient inputs from it small watershed (104 km^2). The autotrophic community of Ayrolle is mainly dominated by *Zostera noltei* Hornemann (1832) seagrasses, covering more than 80% of the lagoon bed (De Wit et al., 2017), while phytoplankton biomasses remain generally low, under 5 µg Chl*a* l^{-1} (Bec et al., 2011) (see Souchu et al., 2010 for the main limnological characteristics of the three lagoons).

Mediterranean coastal lagoons are characterized by strong intra-annual variations related to their shallowness and to the specificities of the Mediterranean climate. The latter comprises a dry hot summer period and a wet period, including a cold winter. During the wet period, these lagoons receive freshwater inputs carrying nutrients from their catchments, thus resulting in lower salinities and increased nutrient availability. During summer, rainfalls are scarce and temperatures are high. As a result, their salinities increase, and the delivery of nutrients for growth mainly rely on the internal sources within the lagoons. This strong environmental variability contributes to drive phytoplankton community structure and diversity (Perez-Ruzafa et al., 2002; Bec et al., 2005).

Sampling procedure and environmental parameter measurement

Sampling was carried out during autumn 2013, spring and summer 2014, from 14 to 21 November, 15 to 23 April, and 25 August to 4 September, respectively (Table 1). Samples have been taken in one site per lagoon, located on the following coordinates: AY: 43°4′17.832″N–3°3′44.638″E; IN: 43°26′59.208″N–3°47′9.275″E; MW: 43°32′39.552″N–3°55′22.512″E. These stations were selected close to the center of the lagoon representing the larger part of the lagoon water body that is well-mixed on a spatial scale and less

Table 1 Details of the environmental parameters of the three lagoons in samplings of autumn 2013, spring and summer 2014

Sampling periods	Date	Stations	T (°C)	Salinity	TN (µM)	NH_4^+	NO_3^-	NO_2^-	DIN	TP	PO_4^{3-}	Chl *a* (µg l^{-1})	S	H_E
Autumn	14/11/13	AY	10.2	37.2	45.2	0.83	BD	0.14	0.97	2.75	0.18	5.60 ± 0.46	20.0 ± 0.0	5.1 ± 0.1
	19/11/13	IN	11.3	37.2	12.9	2.08	1.01	0.23	3.32	0.50	0.11	0.86 ± 0.02	26.3 ± 2.0	5.4 ± 1.1
	21/11/13	MW	6.2	27.4	38.4	1.60	0.90	0.14	2.64	1.25	0.46	1.70 ± 0.02	10.3 ± 0.5	2.2 ± 0.2
Spring	15/04/14	AY	17.7	33.6	25.8	0.59	0.00	0.00	0.59	0.59	0.06	0.54 ± 0.01	37.0 ± 0.8	14.1 ± 2.6
	22/04/14	IN	15.3	37	18.6	1.70	0.35	0.05	2.1	0.56	0.04	0.90 ± 0.03	42.7 ± 1.2	9.4 ± 2.2
	17/04/14	MW	15.6	21.9	42.9	3.24	0.00	0.05	3.29	1.37	0.76	3.02 ± 0.44	18.3 ± 1.7	6.8 ± 0.3
Summer	03/09/14	AY	21	30.9	32.5	0.62	0.00	0.07	0.69	0.45	0.12	0.23 ± 0.01	22.3 ± 1.7	9.6 ± 1.1
	25/08/14	IN	20.8	41.5	28.2	0.99	0.15	0.07	1.21	0.89	0.21	0.98 ± 0.04	30.7 ± 1.9	7.6 ± 2.2
	27/08/14	MW	23.2	36.6	123.6	0.68	0.00	0.37	1.05	8.72	2.48	36.1 ± 0.68	25.0 ± 0.8	1.3 ± 0.0

Chl *a* concentrations, specific richness (S), and Shannon index into effective number of species (H_E) expressed as mean and standard deviation of triplicates values

BD below the detection limit, *AY* sampling station in the oligotrophic Ayrolle lagoon, *IN* sampling station in the mesotrophic North Ingril lagoon, *MW* sampling station in the Méjean lagoon (see methods and Fig. 1)

influenced by the local conditions along the shorelines. Salinity and temperature were measured in situ with a conductivity meter. In each lagoon, 70 l of water prefiltered through 1000 µm mesh were sampled in sub-surface (20 cm depth). For each sample, concentrations of NH_4^+ and of PO_4^{3-}, NO_3^-, NO_2^-, TN, and TP (µM) were measured according standard fluorimetric and colorimetric methods, respectively (Aminot & Chaussepied, 1983).

Phytoplankton community composition

Phytoplankton biomass was estimated by quantification of the chlorophyll *a* concentration (µg Chl*a* l^{-1}). Triplicate water volumes of maximum 100 ml (depending on phytoplankton biomass) were filtered using the protocol described in Bec et al. (2011). Pigments were measured by spectrofluorimetry with a Perkin-Elmer LS50 B (Neveux & Lantoine, 1993).

Taxonomic identification and abundances of nano- (5–20 µm) and microphytoplankton individuals (> 20 µm) were done using optical microscopy at × 400 or × 600 depending on phytoplankton cell size. Triplicate of 1 l of samples fixed with formaldehyde (5% final concentration) were stored in the dark prior to analysis. Because of the weak biomasses in the oligotrophic AY lagoon, the method from Utermöhl (1931) was adapted. After at least 4 days of sedimentation, samples were concentrated by taking off 800 ml of supernatant. This step was repeated until a sufficiently concentrated volume to count phytoplankton. Using 50 µl of the concentrated sample, cells were counted with an optical microscope in fields or along transects depending on their density. At least 200 cells per sample were counted to obtain a relevant assessment of the assemblage. Taxonomic resolution was realized at species level whenever possible (Bourrelly, 1990; Tomas, 1997a, b; Bérard-Therriault et al., 1999; Loir, 2004; Bellinger & Sigee, 2015), and taxonomy was verified using several databases such as the World Register of Marine Species (http://www.marinespecies.org/, databases available online).

Abundances of picocyanobacteria (< 1 µm), autotrophic picoeukaryotes (< 3 µm), and ultraphytoplankton individuals (3–5 µm) were estimated using a FACSCalibur flow cytometer (Becton–Dickinson) according to Bec et al. (2011). The two eukaryotic groups were distinguished on the basis of optical properties including forward side scatter (FSC), related to cell size, and red fluorescence emissions (FL3), a proxy for Chl *a*-content. Among picocyanobacteria, phycoerythrin-rich (PE-cyan) and phycocyanin-rich (PC-cyan) populations were identified and distinguished by their orange and/or red fluorescence emissions, but they were not identified at a more precise taxonomic level.

Taxonomic diversity was calculated using species richness and Shannon index. Species richness (S) was measured as the total number of species observed in each community. The Shannon index expressed into the effective number of species (H_E) was calculated according Jost (2006): $H_E = \mathrm{Exp}(H')$where H′ represents the Shannon entropy ($H' = -\Sigma P_i \ln P_i$), P_i the relative biomass of species "*i*," calculated as the biovolume of this species to the biovolume of the entire algal community (Longhi & Beisner, 2010). Permanova assessed if the taxonomic composition varied among lagoons and different samplings (Anderson, 2001), and two-ways Anovas assessed if the species richness and the Shannon index significantly varied among lagoons and samplings. Tukey's multiple comparisons were used to compare the nine communities side-by-side ($\alpha = 0.05$).

Functional traits

To take intraspecific trait variability (Violle et al., 2012; Carmona et al., 2016) into account, each phytoplankton organisms was individually described using the five selected functional traits described hereafter. The phytoplankton communities of these lagoons mainly comprised unicellular organisms. Colony-forming organisms being less frequently observed in these communities have been taken into account with the "coloniality" trait.

(i) **Cell size** is the most important morphological trait for phytoplankton, affecting all other traits and functions (Irwin et al., 2006; Litchman et al., 2009). It determines the surface-to-volume ratio, which for unicellular organisms constrains light harvesting, nutrient affinity and acquisition, and growth rate of phytoplankton (Chisholm, 1992), and susceptibility towards grazing (Litchman et al., 2010). Cell size was measured by microscopy using the greatest axial linear dimension (µm). Subsequently, the cells were classified into five ordered size classes: microphytoplankton (> 20 µm) less sensitive to predation, less efficient to acquire nutrient in depleted conditions, but potentially able to store nutrients (Litchman et al., 2009, 2010), nanophytoplankton (5–20 µm), ultraphytoplankton (3–5 µm), picoeukaryotes (1–3 µm), and picocyanobacteria (< 1 µm). The three smallest groups are more competitive to acquire and use scarcely available nutrients and low light (Bec et al., 2011). While confronted to such a large range of cell sizes, we decided to quantify the functional entities in terms of biovolume rather than abundance. This is expected to give a less distorted image of relative proportions than cell abundance, which is biased in favor of small cells. The use of biovolume appears particularly important for phytoplankton communities in lagoons, which are often characterized by high abundances of small-sized species (Bec et al., 2011). Hence, for each morphotype in each size class, abundance was converted to biovolume. Accordingly, depending on their species/morphotype (e.g., centric or pennate diatoms, dinophytes), biovolumes were estimated for each cell using mathematical formulae corresponding to the closest and simplest three-dimensional geometric form to their shape, i.e., spherical, spheroid, and cylindrical forms (Vadrucci et al., 2007). All the cells < 5 µm were associated to spherical form, using the diameter measured for each cell, from 1 µm for picocyanobacteria to 5 µm for autotrophic ultraeukaryotes. This relative biovolume is considered in the following pages as an equivalent of the relative biomass of species or morphotypes.

(ii) The **trophic mode**, i.e., strict autotrophy, heterotrophy, or mixotrophy is also relevant since heterotrophy, which can be based on uptake of dissolved organic compounds or ingestion of cells or particles by phagocytosis, may represent a competitive advantage under low nutrient availability (Litchman & Klausmeier, 2008). For the study of flagellates < 20 µm, 30 ml of water fixed with formaldehyde (4% final concentration) have been sampled. For the Dinophytes > 20 µm, 250 ml of water fixed with glutaraldehyde (0,4% final concentration) have been sampled. Samples were stored at 4°C in the dark before analysis. Epifluorescence microscopy was used to distinguish strictly heterotrophic organisms from those capable of autotrophy by sensing the red fluorescence of the chlorophyll *a* (Seoane et al., 2011). Hence, this categorical trait was binary at the individual scale (capable of autotrophy *vs.* heterotrophy).

(iii) **Coloniality** was considered for all cells grouping together, including filaments, arborescent colonies, coenobia, and colonies embedded in mucus. The nutrient acquisition, sedimentation, and susceptibility to predation of colonial organisms are different from what it would if their single cells occurred as free-living unicellulars. Coloniality, thus also constitutes important aspects of functional adaptations. While colony formation reduces nutrient uptake capacities of individual cells (Longhi & Beisner, 2010), it increases resistance to grazers (Litchman et al., 2010). Coloniality was coded as a categorical trait thus opposing the colonial organisms aggregated as chains from unicellular organisms, because no other kind of morphology (round, with or without mucilage, layers, or arborescent colonies) was recorded in these samples.

(iv) **Motility** offers the ability to regulate vertical position depending on resources requirements (light, nutrients) and to migrate into favorable patches in three-dimensional space (Salmaso & Padisák, 2007; Weithoff et al., 2015). Motility was based on the presence or absence of flagella, and was classified as a categorical trait with two modalities: motile or non-motile.

(v) **Life cycle** in the water column is an essential parameter in coastal lagoons, where the shallowness involves a strong benthic–pelagic coupling. Hence, many species occurring frequently in the phytoplankton assembly display benthic and pelagic life stages, or are benthic but stirred up in the water column due to repeated sediment re-suspension (Marcus & Boero, 1998). This trait was coded as an ordered trait with three categories: benthic, bentho-pelagic, or pelagic life mode, based on the scientific literature (Tomas, 1997a, b; Loir, 2004).

Functional space and diversity indices

Computing functional diversity indices for a set of communities requires building a multidimensional functional space to place all the taxa according to their respective trait values (Mouillot et al., 2013). We used the procedure detailed in Maire et al. (2015) to choose the best functional space. First, as all traits were categorical or ordered, individual organisms with the same combination of trait values were grouped into functional entities (FEs). Second, pairwise functional distances between FEs were computed using the Gower's distance, allowing equal weight for the different types of variables. Third, a Principal Coordinate Analysis (PCoA) was performed on this dissimilarity matrix, and its 3 first axes were kept to build a functional space faithfully representing functional distance between taxa [i.e., mean SD < 0.01, (Maire et al., 2015)].

As functional diversity is multifaceted, each phytoplankton community was described using complementary indices that assessed the distribution of FEs and of their abundances (converted as cell biovolume) within the functional space (Mouillot et al., 2013).

- Functional identity (FId) is the average position for the sampled community in the multidimensional functional space calculated from the biomass-weighted position of all the individuals.
- Functional richness (FRic) measures the volume of the convex hull shaping all the FEs in the multidimensional functional space, i.e., the proportion of the functional space filled by a community. It is low when FEs have similar trait values and tends to 1 when all the most extremes FEs from the global pool of Fes are present.
- Functional evenness (FEve) measures the regularity of the repartition of FEs and of their abundances in the multidimensional functional space (Villéger et al., 2008). If abundances are regularly spaced, FEve tends to 1, while if the dominant FEs are very close, FEve tends to 0.
- Functional divergence (FDiv) measures the proportion of total biomass supported by FEs with the

most extreme trait values. This index is low when the most abundant FEs are located near the center of the functional space occupied by the community, and increase when the most abundant FEs are on the edges of the convex hull.

These three latter indices are a priori independent from each other (Villéger et al., 2008). They have been proven to be relevant to disentangle community assembly rules (Mouchet et al., 2010) and to assess impacts of disturbances on community structure (Mouillot et al., 2013).

FEs relative abundances were log-transformed (x + 1) prior to analysis. Taxonomic and functional β-diversities between pairs of communities from different lagoons and/or samplings were calculated using Bray–Curtis distances, using the relative biomass of taxa and FEs, respectively. The two distance matrices were compared with the non-parametric Mantel test [Vegan package, (Oksanen et al., 2016)], which determined the linear correlation between two matrices. All data analyses were performed on R (R Core Team, 2013). Permanova assessed if the functional composition varied among lagoons and sampling periods (Anderson, 2001).

Results

Environmental variables

Environmental variables observed in the three lagoons during the samplings are listed in Table 1. Temperature varied from 6.2°C in the MW lagoon in autumn to 23.2°C in summer, the lowest temperature being observed in autumn and the highest in summer for the three lagoons (Table 1). Salinity varied from 21.9 in the hypertrophic MW lagoon in spring to 41.5 in the mesotrophic IN lagoon in summer (Table 1). DIN concentrations were always below 1 µM in the oligotrophic AY lagoon, while higher values (up to 3.3 µM) were observed during the wet period (samplings in autumn and spring) in the mesotrophic IN and the hypertrophic MW lagoons. In all lagoons and all seasons, DIN was dominated by NH_4^+. The eutrophication gradient was particularly well reflected by Chl *a* concentrations, TP, TN, and PO_4^{3-} concentrations in summer. Indeed, TP concentrations were nine times higher in the hypertrophic MW (8.72 µM) than in the mesotrophic IN (0.89 µM) lagoons, and 99 times higher in MW than in the oligotrophic AY (0.45 µM) lagoon. PO_4^{3-} concentration was eleven times higher in MW (2.5 µM) than in IN (0.21), and twenty times higher in MW than in AY (0.12 µM). In the mesotrophic IN and hypertrophic MW lagoons, maximum Chl *a* concentrations were observed during the sampling in summer, reaching 0.98 and 36.1 µg Chl*a* l^{-1}, respectively. In contrast, in the oligotrophic AY lagoon, TN, TP, and Chl *a* concentrations were highest during the sampling in autumn.

Taxonomic α-diversity

A total of 104 taxa, identified at the species level when possible, and 3 morphotypes of unknown taxonomic affiliation were observed in the 3 studied lagoons. The taxa belonged to 11 classes, i.e., Bacillariophyceae, Chlorophyceae, Cryptophyceae, Cyanophyceae, Chrysophyceae, Dinophyceae, Dictychophyceae, Euglenoideae, Prasinophyceae, Prymnesiophyceae, and Trebouxiophyceae. The morphotypes comprised (1) *Chlorella*-like cells, (2) autotrophic picoflagellates, and (3) heterotrophic picoflagellates. With the microscope techniques used, in some cases, it was not possible to identify diatom species to the genus level. Hence, different morphotypes were identified within Coscinodiscophycidae (centric diatoms) and Fragilariophycidae (pennate diatoms) subclasses, assuming that each of these morphotypes represented a distinct species.

The observed species richness (S) varied among lagoons (two-ways Anova, df = 2, $F = 27.0$, $P < 0.0001$) and among samplings (df = 2, $F = 24.7$, $P < 0.0001$). The species richness (S) in the hypertrophic MW lagoon ranged from 10 to 25 among periods (Table 1). In this lagoon, phytoplankton communities were mainly dominated by Euglenoideae during autumn (76.4%), and by *Chlorella*-like cells in spring and summer (40.4 and 94.8%, respectively). The species richness (S) in the oligotrophic AY lagoon ranged from 20 to 37, and Bacillariophyceae dominated phytoplankton communities for all three periods (ranging from 63.8% in spring to 96.7% in autumn). Species richness (S) was systematically highest in the mesotrophic IN lagoon and ranged from 26 to 43. Its phytoplankton communities were dominated by *Chlorella*-like cells (ranging from 38% in summer to 60% in autumn) associated with species from other classes (Dinophyceae, Bacillariophyceae, Cryptophyceae, Prymnesiophyceae). The Shannon index expressed into equivalent number of species (H_E) significantly varied among the nine communities (two-ways ANOVA, df = 2, $F = 15.5$, $P < 0.05$ between samplings, df = 2, $F = 26.4$, $P < 0.05$ between lagoons). The lowest value of 1.3 was observed during the summer sampling in the hypertrophic MW lagoon, while the highest value of 14.1 was observed in the oligotrophic AY lagoon in the spring sampling (Table 1). For all three lagoons, H_E values were significantly higher for the samplings in spring compared to the autumn samplings. The hypertrophic MW lagoon always showed the lowest H_E value (Tukey's multiple comparisons, $P < 0.05$), especially during the summer sampling when its community was dominated by the blooming of *Chlorella*-like cells.

Individual-based functional entities

The individual phytoplankton cells were grouped in 20 functional entities (FEs) based on their values for the 5 functional traits (Table 2). Among them, 16 FEs corresponded to unicellular organisms and 4 FEs corresponded to colonial organisms (*s*, *t*, *h*, *i*). Different FEs encompassed a single taxon or only one morphotype (*a, b, c, d, e, g, i, k*). These FEs or those composed of a low number of taxa or morphotypes were mostly associated with small cells < 5 µm (*Synechococcus*-like cyanobacteria for *a*, *Chlorella*-like cells for *b*, heterotrophic flagellated cells < 3 µm for *d*, autotrophic flagellated cells < 3 µm for *c*, *Ollicola vangoorii* Vørs (1992) cells for *g*), but a few number of them were composed of larger cells, such as the *i* FE-containing nanoplanktonic *Scenedesmus* sp. cells, or the *o* FE-containing microplanktonic *Peridinium quinquecorne* Abé (1927) cells. Globally, the FEs with larger cells > 20 µm generally included more taxa, with a maximum of 24 taxa in the *n* FE, than those containing smaller cells. The latter FEs comprised taxa that were phylogenetically closely related, and in some cases together with taxa that were highly divergent (Table 2). For example, the *p* FE comprised many dinophytes cells, e.g., *Scrippsiella* sp., *Kryptoperidinium foliaceum* Lindemann (1924), and *Gymnodinium sanguineum* Hirasaka (1922), together with Euglenoids (e.g., *Eutreptiella* sp.). The *j* FE was another taxonomically diverse FE that included several diatom species and filamentous cyanobacteria (Table 2). On the other hand, some individuals of the same species emerged in different FEs (Table 2). For example, the dinophyte *Prorocentrum micans* Ehrenberg (1834) occurred in *n* and *p* FEs, as well as *Heterocapsa minima* Pomroy (1989) in the *l* and *m* FEs. In these two species, individuals were identified as either having autotrophic capacities (*l* and *p* FEs) or strictly heterotrophic modes (*m* and *n* FEs) (Table 2).

Functional α-diversity

Among the communities observed in the three lagoons during the three sampling periods, the number of FEs ranged from 11 to 16, in the hypertrophic MW lagoon (autumn sampling) and in the mesotrophic IN lagoon (spring and summer samplings), respectively (Table 3). On all sampling occasions, FE richness was the highest in the mesotrophic IN lagoon. The communities comprised assemblages of FEs that were differently distributed in the functional space during the three periods, which resulted in different functional identity (FId, Table 3, Fig. 2). Three communities (AY sampled in autumn and MW sampled in autumn and in summer, Fig. 2) were dominated by one or two FEs (*r* and *q* in AY, *p* in MW in autumn, and *b* in MW in summer, Table 2). The others communities were more evenly composed of a higher number of abundant FEs. The *b* FE, i.e., *Chlorella*-like cells, was often very dominant in the two palavasian lagoons (IN and MW).

The functional richness (FRic) of the nine communities (Fig. 3) is represented by the colored polygons reflecting which parts of the functional space was actually filled by each community (projections of the colored polygons in other planes are presented in Figs. S1 and S2). The FRic varied from 0.327 in the mesotrophic IN lagoon sampled in autumn to 0.894 in the mesotrophic IN lagoon sampled in spring (Table 3). During the autumn sampling, the hypertrophic MW lagoon displayed the highest FRic value (0.596), while the mesotrophic lagoon displayed the lowest (0.327). Inversely, in spring and summer samples, the mesotrophic IN lagoon displayed the highest FRic values (0.894 and 0.852 respectively), while the hypertrophic MW lagoon displayed the lowest (0.367 and 0.467, respectively).

Table 2 Functional entities (FEs) showing the values of their functional traits and a selection of representative species occurring in the different FEs

Class size	Trophic regime	Mobility	Colony forming	Habitat	Typical representatives	Label
Pico < 1 μm	Auto-	No	No	Pelagic	*Synechococcus*-like Cyanobacteria	*a*
Pico < 3 μm	Auto-	No	No	Pelagic	nn. id., small autotrophic rounded cells, *Chlorella*-like	*b*
Pico < 3 μm	Auto-	Yes	No	Pelagic	nn. id., small autotrophic flagellates	*c*
Pico < 3 μm	Hetero-	Yes	No	Pelagic	nn. id., small heterotrophic flagellates	*d*
Ultra < 5 μm	Auto-	No	No	Benthic	Centric diatoms morphotypes C2 and C3	*e*
Ultra < 5 μm	Auto-	Yes	No	Pelagic	*Pyramimonas* sp.	*f*
Ultra < 5 μm	Hetero-	Yes	No	Pelagic	*Ollicola vangoorii* Vørs (1992)	*g*
5 < nano < 20 μm	Auto-	No	Yes	Benthic	*Cyanobacteria* sp. 2	*h*
5 < nano < 20 μm	Auto-	No	Yes	Pelagic	*Scenedesmus* sp.	*i*
5 < nano < 20 μm	Auto-	No	No	Benthic	Centric diatoms cf *Thalassiosira* sp., morphotype 2, *Navicula* sp. cf *gregaria,* Pennate diatom sp. morphotypes P1, P2, P3, nanoplanktonic coccobacillus-shaped cyanobacteria *Synechococcus*-like	*j*
5 < nano < 20 μm	Auto-	Yes	No	Bentho-pelagic	*Prorocentrum* sp. cf *mexicanum*	*k*
5 < nano < 20 μm	Auto-	Yes	No	Pelagic	*Apedinella spinifera* Throndsen (1971), *Cryptomonas* sp., *Plagioselmis* sp., *Prorocentrum minimum* Schiller (1933), *Heterocapsa minima* Pomroy (1989), *Tetraselmis* sp.	*l*
5 < nano < 20 μm	Hetero-	Yes	No	Pelagic	*Heterocapsa minima, H. niei* Morrill & Loeblich III (1981), *Protoperidinium* sp. cf *steinii*	*m*
Micro > 20 μm	Hetero-	Yes	No	Pelagic	*Dinophysis acuminata* Claparède & Lachmann (1859), *Gonyaulax* sp. cf. *spinifera, Gyrodinium* sp., *Kryptoperidinium foliaceum* Lindemann (1924), *Oxyphysis Oxytoxoides* Kofoid (1926), *Protoperidinium* sp. cf *americanum, Protoperidinium* sp. cf *bipes, Prorocentrum micans* Ehrenberg (1834), Taxa Oblea, *Vulcanodinium* sp.	*n*
Micro > 20 μm	Hetero-	Yes	No	Bentho-pelagic	*Peridinium quinquecorne* Abé (1927)	*o*
Micro > 20 μm	Auto-	Yes	No	Pelagic	*Eutreptiella* sp., *Scrippsiella* sp., *Kryptoperidinium foliaceum, Prorocentrum micans, Scrippsiella* sp., *Gymnodinium sanguineum* Hirasaka (1922), *Eutreptiella* sp., *Syracosphaera* sp.	*p*
Micro > 20 μm	Auto-	No	No	Bentho-pelagic	*Ceratoneis closterium* Ehrenberg (1839), *Amphiprora* sp., *Pleurosigma* sp.	*q*
Micro > 20 μm	Auto-	No	No	Benthic	*Gyrosigma* sp., *Diploneis* sp., *Cocconeis* sp., Centric diatoms morphotype C2	*r*
Micro > 20 μm	Auto-	No	Yes	Benthic	*Licmophora* sp., *Bacillaria* sp. cf *paxillifer*	*s*
Micro > 20 μm	Auto-	No	Yes	Pelagic	*Chaetoceros* sp. (5 different species)	*t*

Labels are those used in figures and in the main text

Non-taxonomically identified morphotypes are indicated by "nn id."

Functional evenness (FEve) was always < 0.6 (Table 3), revealing an uneven distribution of FEs abundances in the functional space (Fig. 2). The lowest FEve value was observed in the mesotrophic IN lagoon in the spring sampling (0.167), while the hypertrophic MW lagoon displayed the lowest FEve values the two other sampling periods (Table 3). During the autumn sampling, the mesotrophic IN

Table 3 Functional diversity of phytoplanktonic assemblages in the three lagoons during three samplings

Sampling periods	Stations	Nb FEs	FId-1	FId-2	FId-3	FRic	FEve	FDiv
Autumn	AY	12	− 0.212	0.100	0.146	0.497	0.431	0.728
	IN	14	− 0.046	− 0.074	0.123	0.327	0.592	0.896
	MW	11	0.051	− 0.060	0.034	0.596	0.207	0.659
Spring	AY	15	− 0.110	0.009	0.015	0.709	0.503	0.680
	IN	16	0.061	− 0.080	0.083	0.894	0.167	0.639
	MW	12	0.094	− 0.076	0.055	0.367	0.260	0.668
Summer	AY	13	− 0.178	0.060	0.121	0.525	0.555	0.839
	IN	16	− 0.058	− 0.031	0.068	0.852	0.418	0.635
	MW	15	− 0.076	− 0.162	0.167	0.467	0.204	0.829

Number of functional entities (Nb FEs), of functional identity in the 3-dimensional functional space (FId-1-3), and values of functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv)

lagoon displayed the highest FEve value (0.592), while in spring and summer samplings, the oligotrophic lagoon displayed the highest (0.503 and 0.555, respectively).

Functional divergence (FDiv) varied within lagoons among samplings. In the mesotrophic IN lagoon, it ranged from 0.635 in summer to 0.896 in autumn, and in the oligotrophic AY lagoon, from 0.680 in spring to 0.839 in summer (Table 3). Overall, FDiv showed no clear pattern with eutrophication. In spring, the mesotrophic IN lagoon displayed the highest FDiv value (0.896) while the hypertrophic MW lagoon displayed the lowest (0.659). During spring and summer samplings, the mesotrophic lagoon displayed the lowest FDiv values (0.639 and 0.635, respectively), while the oligotrophic AY lagoon displayed the highest (0.680 and 0.839, respectively).

Taxonomic β-diversity

Taxonomic structure expressed by the relative biovolume of taxa differed among lagoons (Permanova, df = 2, $F = 1.1$, $P = 0.036$) and but not among periods (df = 2, $F = 1.0$, $P = 0.198$) (Fig. 4A, Table S1A). The taxonomic β-diversity averaged 87% (mean of Bray–Curtis distances: 0.87 ± 0.18). The lowest taxonomic β-diversity (37%) was observed between the communities of the mesotrophic IN lagoon sampled in spring and in summer. The highest value (99%) was observed between the communities of hypertrophic MW lagoon and of the oligotrophic AY lagoon sampled summer. The mesotrophic IN lagoon showed the lowest variability of the taxonomic diversity among the three periods (Fig. 4A).

Functional β-diversity

The functional structure expressed by the relative biovolume of FEs has not significantly changed among lagoons (Permanova, df = 2, $F = 1.4$, $P = 0.074$) and among periods (df = 2, $F = 0.9$, $P = 0.722$) while the functional identity changed it position in the functional space (Fig. 2). The functional ß-diversity averaged 67% (mean of Bray–Curtis distances: 0.67 ± 0.22, Table S1B). With the exception of the autumn sampling in the hypertrophic MW lagoon, the communities of the two palavasian lagoons, i.e., the mesotrophic IN and hypertrophic MW lagoons were grouped together. These 5 communities were thus functionally different from those of the oligotrophic AY lagoon, which formed another group (Fig. 4B). The lowest functional ß-diversity (21.6%) was observed between the communities sampled in spring in the mesotrophic IN and hypertrophic MW lagoons. The highest functional ß-diversity was observed between the communities sampled in autumn in the oligotrophic AY lagoon and the hypertrophic MW lagoon (Fig. 4B, Table S1B).

Functional and taxonomic β-diversities of the nine communities were correlated (Mantel Test, $r = 0.69$, P value = 0.005) although mismatches between levels of taxonomic (Fig. 4A) and functional (Fig. 4B) β-diversities were observed. For instance, the hypertrophic MW lagoon in autumn was functionally very dissimilar to other communities (Table S2, from 76.7

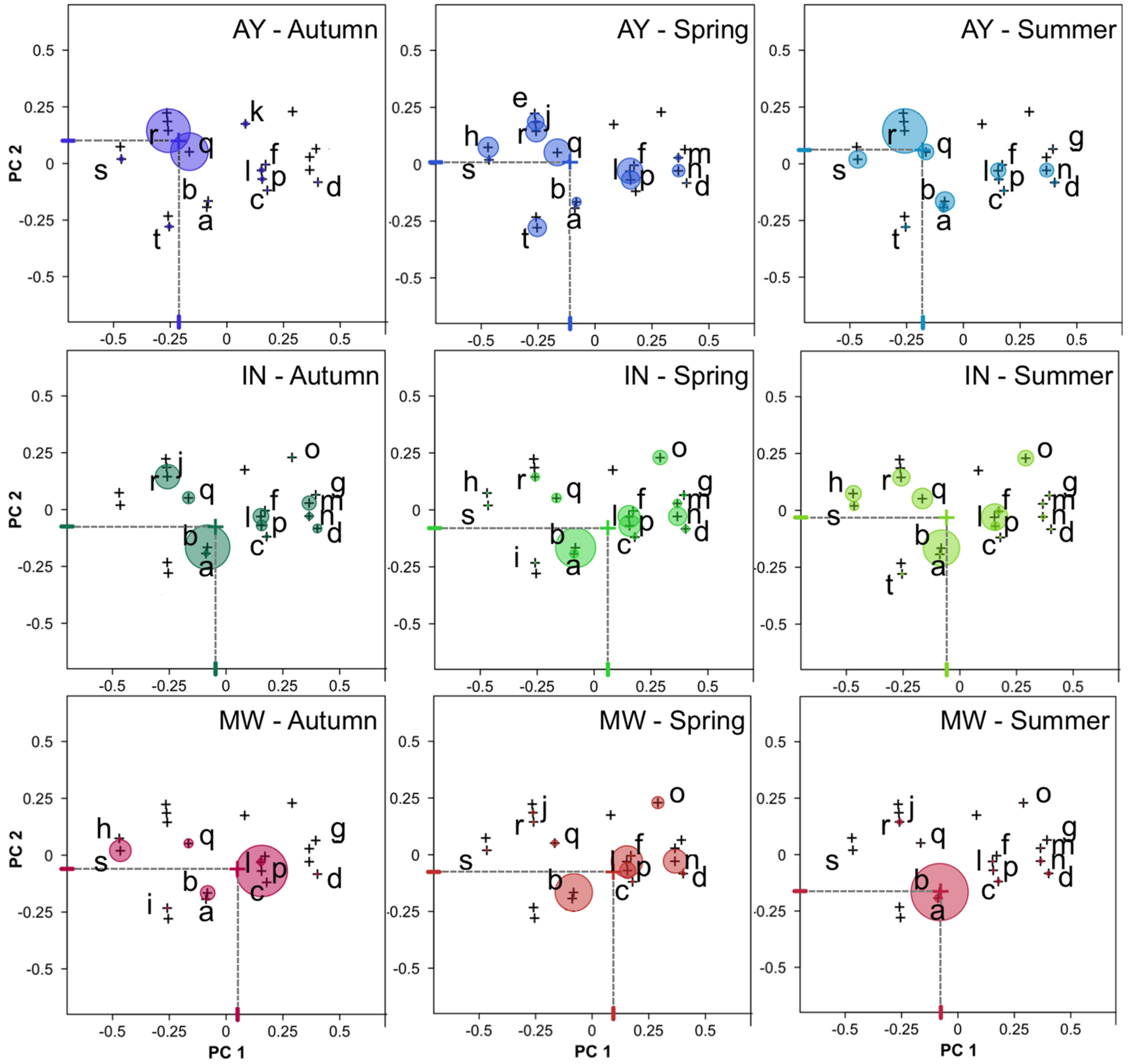

Fig. 2 Functional diversity of phytoplankton communities of the three lagoons (AY, IN, and MW) in autumn 2013, spring and summer 2014 samplings in the three-dimensional functional space, illustrated with the first two principal coordinate axes 1 and 2. The 20 functional entities (FEs) are represented with crosses. The FEs present in each community are indicated by colored circles with their labels (i.e., letters, their corresponding trait values are detailed in Table 2); their relative biomasses are represented proportional to the surfaces. The functional identity (FId) of each community corresponding to the biovolume-weighted average of the combination of traits is indicated using a large colored cross symbol with stippled-line projections (Table 3 list all the coordinates of the FId's in the 3-dimensional functional space)

to 95.7%), while in term of species composition, it was similar to the oligotrophic AY lagoon sampled in summer.

Discussion

In this study, we applied a functional classification of the individual phytoplankton organisms based on five selected traits, and we explored the potential of the multidimensional functional approach for the study of phytoplankton in highly variable coastal lagoons of

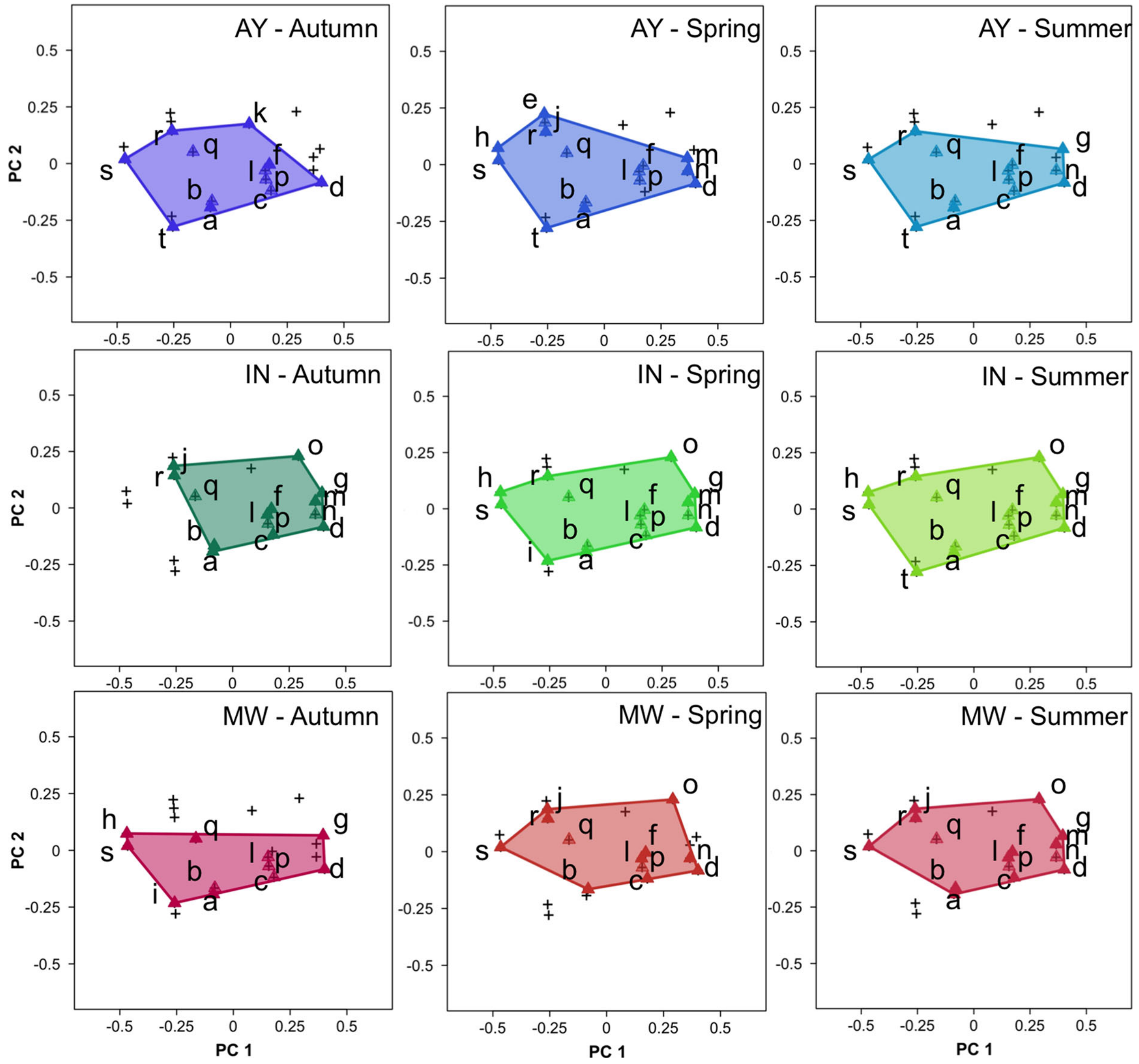

Fig. 3 Functional richness of the three lagoons (AY, IN, and MW) in autumn 2013, spring, and summer 2014 samplings in the three-dimensional functional space, illustrated with the first two principal coordinate axes 1 and 2. The 20 functional entities (FEs) are represented with crosses. FEs present in a community are represented by triangles and those being vertices in the three-dimensional space are filled. Projections of the convex hull computed in 3-dimensions on this 2-dimensional space are illustrated by polygons

different eutrophication status. This functional approach was combined with the taxonomic approach to reveal the complementarities of both approaches, and highlight how when combined, these approaches can improve our understanding of the structuration of phytoplankton communities.

Taxonomic and functional diversity in the oligo-, meso-, and hypertrophic lagoons

Our results show how the total biomass (Chl *a*) and the taxonomic and functional composition of phytoplankton reflected the different environmental conditions, i.e., temperature, salinity, forms, and origins of nutrients during the three samplings in the three lagoons. Total biomasses reflected the eutrophication

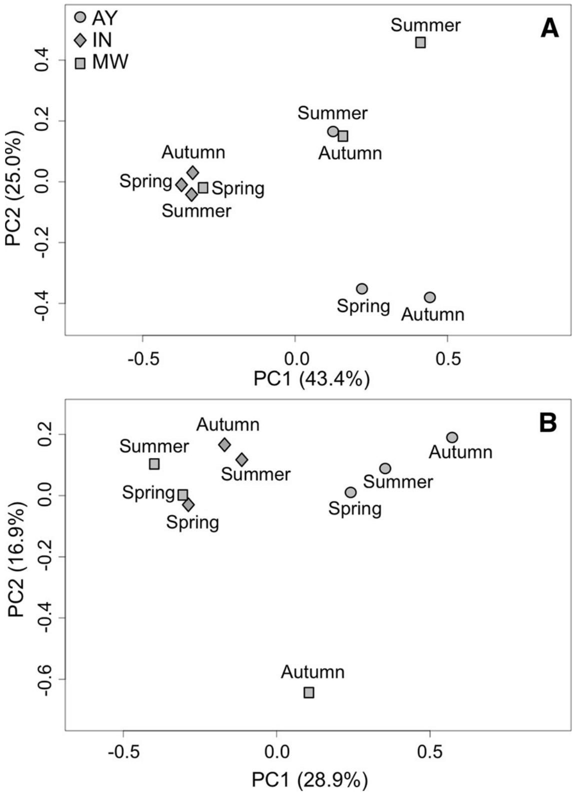

Fig. 4 Representation of Bray–Curtis dissimilarity distances matrix, with Principal Coordinates Analysis (PCoA) between the nine communities (3 lagoons × 3 samplings) according to **A** relative biomass of phytoplankton taxa, **B** relative biomass of functional entities. Symbols represent the lagoons (circle for the oligotrophic AY, diamond for the mesotrophic IN, and square for the hypertrophic MW lagoons)

gradient in the three lagoons, particularly during summer, when light and temperature were optimal for phytoplankton growth (Souchu et al., 2010; Bec et al., 2011; Leruste et al., 2016). However, in the summer sampling, concomitant with these high phytoplankton biomasses, the availability of inorganic nutrients in the water column was low as these nutrients had been depleted by fast consumption (Bec et al., 2011; Domingues et al., 2015).

With the total biomass, the taxonomic diversity also changed according to the eutrophication status of the lagoons and the sampling periods. The species richness (S) was systematically highest in the mesotrophic lagoon. This is in agreement with literature reports (Padisak, 1993; Reynolds et al., 1993), showing maximum taxonomic diversity at an intermediate level of productivity, and a strong drop of taxonomic diversity at higher productivity (Duarte et al., 2006; Longhi & Beisner, 2010). Taxonomic diversity was also highest in the communities sampled in the lagoons during spring. This season is characterized by an improvement of environmental conditions for phytoplankton growth after a cold winter. Indeed, both temperature rise and frequent rainfall events result in nutrient inputs. Nutrient availability and potential limitation can act as a strong pressure, filtering out species and changing the functional structure of a community (Conley, 1999). This has led to a strong dominance of taxa and functions having competitive advantages to cope with the reduced availability of inorganic nutrients.

The hypertrophic MW lagoon was characterized by the highest variability of environmental parameters (salinity, nutrient concentrations, Table 1). This highly productive lagoon, strongly degraded by nutrient enrichment (Souchu et al., 2010; Leruste et al., 2016), presented the highest biomasses in spring and summer samplings, and was characterized by low taxonomic and functional diversities. These communities were dominated by only two main functional entities, i.e., the *p* FE prevailing in autumn, and the *b* FE dominating in spring and summer samplings. Both *p* and *b* FEs contained species characterized by a colonist strategy (e.g., *Eutreptiella* sp., *Chlorella*-like cells) that have already been associated to shallow, highly nutrient-enriched habitats, often mesohaline and with reduced water-mass exchanges (Smayda & Reynolds, 2001; Reynolds et al., 2002; Salmaso & Padisák, 2007; Alves-de-Souza et al., 2008; Leruste et al., 2016). The main morphotype, i.e., small *Chlorella*-like cells, is particularly competitive to acquire and use nutrients, especially ammonium that represented the main N resource during the three sampling periods. In summer, these nutrients mainly came from the remineralization of sedimentary organic matter, freshwater inputs from rainfall being scarce (Collos et al., 2003). The high stocks of organic matter in sediments may also have sustained heterotrophy (*o* and *n* FEs), which appears particularly important in the communities sampled in autumn and spring. Moreover, several dinophyte species such as *Prorocentrum micans* emerged in the same samples both as individuals with autotrophic capacities and others as strict heterotrophs (e.g., in autumn), suggesting that the former had potential mixotrophic ability. Several dinophytes and Euglenoids species observed in this lagoon are known to have mixotrophic abilities (phagotrophy or osmotrophy), that may have allowed them to exploit the high amount of organic matter in this lagoon (Brandt, 2001; Ismael, 2003). Indeed, mixotrophy may allow them to access the essential N and P nutrients when their inorganic forms are unavailable (Ismael, 2003; Glibert et al., 2016). Hence, the occurrence of heterotrophy and mixotrophy would be favored during re-oligotrophication processes, as highlighted by the emergence of dinophytes after the nutrient reduction in the palavasian lagoons (Leruste et al., 2016).

The mesotrophic IN lagoon exhibited a lower environmental variability (Table 1) and their communities were composed of more FEs and taxa than the oligo- and the hypertrophic lagoons. This high number of FEs may reflect ecological niche differentiation. The wide diversity of taxa and FEs that coexisted during the three samplings included generalist taxa having intermediate trait values e.g., in the *l* and *m* FEs, as well as others mainly having extreme trait values, reflecting different and specific adaptations to the lagoon functioning e.g., in the *a*, *q*, *r*, *p*, and *o* FEs. As in the hypertrophic lagoon, the *b* FE represented an important part of the communities, reflecting the occurrence of nutrient pulses and a common eutrophication history (Leruste et al., 2016). We also observed in these communities several taxa having adaptation to cope with a low nutrient availability in the water column, such as benthic diatoms (*q*, *r* FEs). Large benthic diatoms are able to take up nutrients at the sediment–water interface and to store them for use during prolonged residence in the water column where nutrient can be unavailable (Litchman et al., 2007). We also observed Prymnesiophyceae (*p* FE) that can use phagotrophy on picoplanktonic particles including bacteria and algae (Reynolds, 2006). These communities also occasionally contained *Synecochoccus*-like cyanobacteria (*a* FE). These small cells < 1 µm contained phycoerythrin pigment, which absorbs the green light that dominates in brackish clear waters (Stomp et al., 2007). These small cells are also highly competitive for nutrient acquisition, especially under low N and P availability (Hyenstrand et al., 2000; Moore et al., 2002).

The oligotrophic AY lagoon exhibited the lowest nutrient concentrations and an intermediate variability of salinity. This shallow oligotrophic system is mainly dominated by seagrasses, and the carrying capacity for phytoplankton is reduced. These conditions of nutrient depletion and competition may have triggered a lower diversity in comparison with the mesotrophic lagoon (Reynolds et al., 1993). Ayrolle lagoon exhibited the lowest biomass during spring and summer samplings, in contrast with the autumn sampling when it displayed the highest Chl *a* concentration. This shallow lagoon is characterized by frequent wind events that have two main consequences on phytoplankton. Firstly, they cause the re-suspension of sediment containing seagrasses debris and degradation products that change the light availability in the water column. Secondly, they also lead to a re-suspension of benthic autotrophic organisms (Padisák et al., 2009), increasing the estimated phytoplankton biomass, as observed in autumn with the dominance of benthic or bentho-pelagic species (*r*, *q*, *s* FEs containing large diatoms). As in Ingril lagoon, these communities present functional adaptations to cope with the low nutrient availability in the water column, such as the highest relative proportions of large benthic diatoms dominating the three samplings. In the spring sampling, small diatoms in the *e* and *j* FEs display very fast growing ability and can outcompete other phytoplanktonic species, especially for nitrate inputs from run-off (Glibert et al., 2016). We observed other functional adaptations to fit with the environmental conditions of the lagoon. Most of the *Synechococcus*-like cyanobacteria (*a* FE) contained phycocyanin, allowing them to thrive at the low light intensities in these inorganically turbid waters. Their presence may also reflect the strong nutrient depletion and the variability of salinity in this lagoon (Stomp et al., 2007). In addition, heterotrophic and mixotrophic species able to use alternative nutrient resources were also present in spring and summer samplings (*p*, *m*, *n* FEs).

Multiple facets of functional diversity in the three coastal lagoons

The multidimensional functional approach can contribute to meaningful descriptions of community assembly and some other important aspects of ecosystem functioning in coastal lagoons, through the different indices that highlight complementary information about community structure. The highest values of FDiv were observed in summer in the oligotrophic and the hypertrophic lagoons (0.839 and 0.829). These high FDiv values show that the predominant FEs displayed extreme trait values (FEs located at or close to vertices of the convex hulls, Fig. 3), which can be taken as an indication for the specialization of FEs, reflecting the occurrence of specialists in the community. These values reflect the high relative abundances of small cells (< 3 µm) and big cells (> 20 µm) in these communities, each specialized to cope with different patterns of nutrient availabilities, origins, and forms. Strikingly, during summer these two lagoons strongly differed in FEve, with values being 0.555 and 0.204, in the oligotrophic and hypertrophic lagoons, respectively. This indicates a very unbalanced distribution of FEs in the hypertrophic lagoon where some FEs dominated the community and other FEs were rare. The pattern is less unbalanced in the oligotrophic lagoon. Concomitantly, the two lagoons showed minor differences in FRic (0.525 and 0.467, respectively) with values close to 0.5 showing that each community occupied about half of the functional space defined for the ensemble of the 9 communities. Hence, this example clearly illustrates the usefulness of the multidimensional functional approach as the three different indices reveal different aspects each, which are not correlated (Villéger et al., 2010).

Functional evenness (FEve) was systematically low in the hypertrophic lagoon (0.204–0.260), although its lowest value was observed in the mesotrophic lagoon during the spring sampling (0.167). This very low FEve reflects an extremely unbalanced distribution of the FE abundances in the functional space. This indicates that most individuals shared some closer trait values, while some other traits were not represented at all, and this may reflect a high functional redundancy during this period. Between spring and summer samplings, the FEve increased to 0.418 in the mesotrophic lagoon, revealing a less unbalanced distribution of trait values in the functional space. Functional divergence (FDiv) values in this lagoon during spring and summer samplings were quite high (0.639 and 0.635), indicating an important proportion of individuals having extreme values of traits in the community, albeit to a lesser extent than during summer in the oligo- and hypertrophic lagoons. Concomitantly, the FRic values were very high (0.894 and 0.852) indicating that its FEs present occupied more than 85% of the functional space defined for the ensemble of the nine communities. This suggests that during spring and summer, the mesotrophic lagoon provided a wide array of niches, and that FEs did not wane because of a very low variability of salinities and Chl *a* concentrations between spring and summer compared with that observed in the other lagoons. However, surprisingly in this mesotrophic lagoon, the FRic was low in autumn, concomitant with a very high FDiv value. This suggests that the community was strongly dominated by some specialists FEs having extreme values of traits, while many of the FEs representative for the ensemble of the nine communities were poorly presented in this specific community.

Comparing and combining the taxonomic and the multidimensional functional approach

The Principal Coordinates Analyses of the taxonomic and functional β-diversity strikingly showed that differences in eutrophication status among the three lagoons were better reflected by the functional β-diversity than by the taxonomic β-diversity (Fig. 4). This suggests that the ordering of organisms in 20 functional entities allows a better understanding of phytoplankton community responses to the main environmental conditions than the identification of more than 100 taxa.

Moreover, from the functional point of view, the communities of the hypertrophic and mesotrophic lagoons from the palavasian complex were more similar than those of the oligotrophic AY lagoon despite their different eutrophication status. Eutrophication was thus not the only driver of phytoplankton diversity, since the dynamics of biotic and abiotic parameters was also involved in the assembly of communities. Indeed, the hypertrophic and mesotrophic lagoons share comparable hydrological functioning as they belong to the same complex. Moreover, they were historically eutrophicated by the same main nutrient source and are currently exposed to a re-oligotrophication process since the diversion of this main anthropogenic input in 2005 (Leruste et al., 2016).

It has already been reported that the grouping of species according to their trait values most likely better reflected phytoplankton responses to their main environmental drivers than the taxonomic affiliation of cells (Longhi & Beisner, 2010; Weithoff et al., 2015). Many taxa were grouped in the same FEs, assuming that these taxa responded to the same environmental conditions. For example, the *p* FE, grouping dinophytes and Euglenoids cells (Table 2), was dominant in the hypertrophic lagoon during the autumn sampling. All these species share colonist strategy and environmental *preferendum*, i.e., shallow, highly nutrient-enriched habitats, often mesohaline with reduced water-mass exchanges (Reynolds et al., 2002; Salmaso & Padisák, 2007). Another taxonomically diverse FE was *j* that grouped several diatom species together with nanoplanktonic coccobacillus-shaped cyanobacteria (Table 2). Thus, some FEs comprised species that were deeply divergent from the taxonomic and phylogenetic points of view. In contrast, some species, e.g., the dinophytes *Prorocentrum micans* and *Kryptoperidinium foliaceum* occurred in *n* and *p* FEs, as well as *Heterocapsa minima* in the *l* and *m* FEs. Individuals of these species were identified as either being capable of autotrophy (*l* and *p* FEs) or showing strict heterotrophy (*m* and *n* FEs). This shows that the phenotypic plasticity of phytoplankton species can lead to the presence of the same species under different environmental conditions and even in very diverse ecosystems due to their wide range of phenotypic adaptations (Padisak et al., 2009). Our study confirms that it is indeed important to take phenotypic plasticity and intraspecific variability of functional traits into account (Violle et al., 2007). Moreover, the fact that the same species occurred in the same sample both as strict heterotrophs and as cells capable of autotrophy suggests that the latter may possess mixotrophic abilities (Flynn et al., 2013).

The combination of the taxonomic and the functional approaches is particularly interesting because it highlights several ecological mechanisms of community assembly (Weithoff et al., 2015). For example, comparing the communities sampled in spring and summer in the hypertrophic lagoon, some functional indices such as the number of FEs and the FRic were highest in summer, which matched with species richness (S). In contrast, the taxonomic diversity (H_E) and functional evenness (FEve) showed the opposite (Tables 1, 3). An increase of functional diversity combined with a decrease of taxonomic diversity indicates environmental changes that may have led to the potential establishment of new functional niches between the spring and summer samplings. This difference also suggests that competitive exclusion occurs within functional entities (Weithoff et al., 2015). In this case, however, the lower FEve and H_E were explained by the dramatic increase of the relative importance of the *b* FE. *Chlorella*-like cells composing this FE are most competitive to use ammonium and grow fast under higher temperatures, so they were, therefore, particularly well adapted to the hypertrophic conditions in summer. Their blooming in the lagoon thus introduces a highly uneven distribution reflected both by a lower taxonomic $\mathrm{H_E}$ index as well as by a lower FEve. Comparing the spring and summer samplings in the mesotrophic lagoon, the FRic was high and comparable for both communities. In contrast, the taxonomic diversity (S and H_E) was lower in summer. This could reflect competitive exclusion within groups of functionally similar species during summer, while different combination of trait values still represented effective strategies to cope with environmental conditions allowing high FRic values (Weithoff et al., 2015).

Conclusion

We proposed an objective way to assign individual phytoplankton cells to FEs. As shown in the discussion, this compares quite well to previous approaches where taxa have been assigned to functional groups (e.g., Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010; Litchman et al., 2010). Nevertheless, our individual-based approach has highlighted that some species may emerge in different FEs, showing that it is important to take intraspecific variability and phenotypic plasticity into account. This approach especially highlighted the importance of heterotrophic or mixotrophic dinophytes (*m* and *n* FEs) in the three lagoons. These abilities have emerged since the beginning of the re-oligotrophication process in the palavasian lagoons (Leruste et al., 2016) because of their competitive advantage in case of inorganic nutrient depletion.

The taxonomic approach gains a lot of potential when it is combined with the individual-based multidimensional functional approach. Firstly, this is an asset when the taxonomic approach alone falls short due to the occurrence of phytoplankton cells that cannot be determined taxonomically. This is typically the case in many Mediterranean coastal lagoons, where small cells often represent the majority in the community (Bec et al., 2011). In this respect, the functional approach allows integrating these small cells in the same way as other taxa by using their trait values. Secondly, this multidimensional approach allows choosing the pertinent functional traits (categorial, ordered or not), that are likely to reflect as closely as possible the functional adaptations of the communities in response to ecosystem functioning. For the analysis at the community level, it is, however, important to consider the different facets of the functional diversity. In the multidimensional functional approach, these are taken into account by the different indices. Together with the taxonomy, this can show specialization, functional redundancy, or competitive exclusion in the communities. Hence, combining this functional approach with a taxonomic approach collectively provides even better insights into community structuration and other aspects of ecosystem functioning that need to be further explored in the future. Therefore, more frequent sampling of these highly variable environments will allow to better describe how the changing environmental conditions impact the structuring and functioning of their phytoplankton communities.

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