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1  
2 **Modelling mixotrophic functional diversity and**  
3 **implications for ecosystem function**  
4

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16 **Abstract**

17 Mixotrophy is widespread among protist plankton displaying diverse functional forms within a  
18 wide range of sizes. However, little is known about the niches of different mixotrophs and how they  
19 affect nutrient cycling and trophodynamics in marine ecosystems. Here we built a plankton food  
20 web model incorporating mixotrophic functional diversity. A distinction was made between  
21 mixotrophs with innate capacity for photosynthesis (constitutive mixotrophs, CMs) and those which  
22 acquire phototrophy from their prey (non-constitutive mixotrophs, NCMs). We present simulations  
23 of ecosystems limited by different light and nutrient regimes. Our simulations show that strict  
24 autotrophic and heterotrophic competitors increased in relative importance in the transition from  
25 nutrient to light limitation, consistent with observed oceanic biomass ratios. Among CMs, cells < 20  
26 µm dominate in nutrient poor conditions while larger cells dominate in light-limited environments.  
27 The specificity of the prey from which NCMs acquire their phototrophic potential affects their  
28 success, with forms able to exploit diverse prey dominating under nutrient limitation. Overall,  
29 mixotrophy decreases regeneration of inorganics and boosts the trophic transfer efficiency of  
30 carbon. Our results show that mixotrophic functional diversity has the potential to radically change  
31 our understanding of the ecosystem functioning in the lower trophic levels of food webs.  
32  
33

34 **keywords:** mixotrophy, acquired phototrophy, marine plankton, functional diversity, size,  
35 ecosystem model, ERSEM

## 36 **Introduction**

37 Food webs comprise complex arrays of interactions between resources and consumers  
38 (Worm *et al.*, 2002; Araújo *et al.*, 2011). Despite the recognised importance of predation and  
39 competition in defining the ecological niches of different functional taxa (Hunter and Price 1992;  
40 Cloern and Dufford, 2005), the overall structure and dynamics of food webs are also greatly  
41 affected by additional factors, such as intraguild predation and omnivory (Polis *et al.*, 1989;  
42 Williams and Martinez, 2000; Johnson *et al.*, 2010; Granados *et al.*, 2017). Mixotrophy, defined  
43 here as the combination of phototrophy and phagotrophy in a single organism (Table I), is another  
44 ‘twist’ that can shift our understanding of ecosystem dynamics from terrestrial to aquatic  
45 environments (Tittel *et al.*, 2003; Selosse *et al.*, 2017).

46 Mixotrophy among protist plankton is near ubiquitous in the sunlit ocean and has been  
47 observed among all dominant protist classes (from the largest to the smallest), with diatoms being  
48 the only exception (Zubkov and Tarran, 2008; Flynn *et al.*, 2013; Biard *et al.*, 2016; Stoecker *et al.*,  
49 2017). Accordingly, protist plankton have been recently regrouped to better represent their  
50 physiological functionality in terms of energy and nutrient acquisition (Mitra *et al.*, 2016). A critical  
51 feature of this functionality is that mixotrophs can be divided amongst organisms with a constitutive  
52 ability to photosynthesise (the constitutive mixotrophs, CMs) and those that do not possess the  
53 innate ability to fix carbon dioxide but acquire this ability from their prey (the non-constitutive  
54 mixotrophs, NCMs) (Table I) (Mitra *et al.*, 2016). NCMs are further divided into generalist forms  
55 (GNCMs) that can exploit plastids acquired from diverse phototrophic prey, and specialist forms  
56 (SNCMs) that must acquire the phototrophic machinery from specific prey (Table I) (Stoecker *et al.*  
57 *et al.*, 2009; Johnson *et al.*, 2011; Mitra *et al.*, 2016). Thus, while conceptual food webs traditionally  
58 divide the plankton into phytoplankton or zooplankton, this dichotomy comprehensively  
59 misrepresents reality, with most protist ‘phytoplankton’ capable of grazing, and ca. half of the  
60 ‘microzooplankton’ capable of photosynthesis (Flynn *et al.*, 2013; Stoecker *et al.*, 2017).

61 There is a need to understand how mixotrophy, in its different forms, may change our  
62 understanding and simulations of food web dynamics and biogeochemical cycling in the oceans.  
63 For instance, CMs have been hypothesized to ‘farm’ bacteria in oligotrophic waters; while CMs  
64 feed on bacteria to acquire essential nutrients, they also release dissolved organic matter (DOM)  
65 which supports bacterial growth (Mitra *et al.*, 2014). Mixotrophs, compared to their heterotrophic  
66 competitors, can retain more nutrients from their prey as they can use them along with the organic  
67 carbon obtained through photosynthesis. An implication of this is, if mixotrophs outcompete strict  
68 heterotrophs in oligotrophic regions, then nutrient limitation of pure autotrophs (including  
69 cyanobacteria) may become more severe (Fischer *et al.*, 2016). Furthermore, NCMs have the clear  
70 potential to achieve higher gross growth efficiencies through acquired phototrophy, potentially

71 increasing the transfer of carbon biomass to higher trophic levels, particularly in low chlorophyll  
72 waters (Stoecker *et al.*, 2009). Taken together these studies suggest that mixotrophy has the  
73 potential to enhance both the production of large size, fast sinking particles (e.g. faecal pellets) by  
74 mesozooplankton, which may feed on mixotrophs, and the bacterial production of recalcitrant  
75 material (Jiao *et al.*, 2010; Polimene *et al.*, 2017) which may be stimulated by the enhanced  
76 production of dissolved organic carbon (DOC) (Mitra *et al.*, 2014). Both the production of particles  
77 and recalcitrant DOC are key fluxes for the global carbon cycle contributing to the ocean carbon  
78 sequestration (Legendre *et al.*, 2015).

79         So far, few studies have investigated the relevance of functional diversity within the  
80 mixotrophs on food web functioning and ecosystem properties (Flynn and Mitra, 2009; Mitra *et al.*,  
81 2016; Ghyoot *et al.*, 2017). Understanding the ecological niches of mixotrophs and their strict auto-  
82 and hetero- trophic competitors helps to identify when and where different mixotrophs are major  
83 components of plankton communities and, thus, potentially affect ecosystem properties (Fischer *et*  
84 *al.*, 2016; Leles *et al.*, 2017). Mixotrophic functional diversity is a topic of particular importance in  
85 the context of climatic and anthropogenic changes on the oceans. Consider plankton communities in  
86 two contrasting marine ecosystems, oligotrophic seas and eutrophic coastal systems, characterised  
87 by nutrient and light limitation, respectively. Global warming is expected to increase ocean  
88 stratification in the former, potentially expanding the area occupied by low productive seas  
89 (Polovina *et al.*, 2008; Behrenfeld *et al.*, 2016). In turn, the increased runoff of nutrients and organic  
90 matter in coastal waters usually promotes unbalanced (and high) nitrogen to phosphorus ratios  
91 (Burkholder *et al.*, 2008; Gomes *et al.*, 2014). In both cases, mixotrophy has been shown to be a  
92 successful strategy (Burkholder *et al.*, 2008; Zubkov and Tarran, 2008; Wilken *et al.*, 2013; Gomes  
93 *et al.*, 2014). Thus, acknowledging the role of mixotrophs can be key to predict the dynamics of  
94 plankton communities in a changing ocean.

95         While there is increasing awareness that mixotrophy is a key trait shaping biological  
96 communities, quantifying its physiological and ecological relevance is challenging (Selosse *et al.*,  
97 2017). This lack of knowledge is mainly due to the difficulty to accurately characterise the  
98 abundance and distributions of mixotrophs in the field (Anderson *et al.*, 2017). Modelling studies  
99 provide a suitable platform to investigate the effects of mixotrophs on ecosystem function by using  
100 a hypothesis testing approach. Although several studies have simulated mixotrophy (Thingstad *et*  
101 *al.*, 1996; Stickney *et al.*, 2000; Flynn and Mitra, 2009, Flynn and Hansen, 2013; Våge *et al.*, 2013;  
102 Mitra *et al.*, 2014; Mitra *et al.*, 2016; Moeller *et al.*, 2016), few have accounted for mixotrophic  
103 functional diversity and their impact on ecosystem dynamics (Ghyoot *et al.*, 2017). In addition, the  
104 structure of the mixotroph model is very important; mixotrophy does not simply reflect the additive

105 interaction between phototrophy and phagotrophy and the description of metabolic switching from  
106 one strategy to the other is important to correctly simulate metabolic rates (Mitra and Flynn, 2010).

107 Here we combined, for the first time, models of diverse types of mixotrophs across different  
108 size classes with submodels of plankton as described in the European Regional Seas Ecosystem  
109 Model (ERSEM; Baretta-Bekker *et al.*, 1995; Butenschön *et al.*, 2016). The impact of mixotrophic  
110 functional diversity on key biogeochemical fluxes and plankton trophodynamics was assessed by  
111 contrasting our model with a ‘non-mixotrophic’ plankton food web model. Since mixotrophy is  
112 expected to dominate in mature ecosystems in which resources are limiting, we simulated nutrient  
113 or light limitation scenarios, akin to conditions representative of oligotrophic seas and eutrophic  
114 coastal systems, respectively. Our theoretical framework allows the investigation of the relative  
115 importance of constitutive and non-constitutive mixotrophs (CMs and NCMs) and of their strict  
116 autotrophic and heterotrophic competitors.

117

## 118 **The Model**

### 119 *The food webs*

120 We compared two plankton food webs (named here as ‘non-mixotrophic’ and ‘mixotrophic’  
121 food webs) that differ only in the inclusion of mixotrophic functional types (Fig. 1). The community  
122 structure of the non-mixotrophic food web was defined following the conceptual framework of  
123 ERSEM (Baretta-Bekker *et al.*, 1995; Butenschön *et al.*, 2016). This non-mixotrophic food web  
124 comprised eight functional groups that differ mainly in size and trophic strategy (Fig. 1): four  
125 phototrophs (picophytoplankton, nanoflagellates, microflagellates, and diatoms), three predators  
126 (nanoflagellates, microzooplankton, and mesozooplankton), and one decomposer (heterotrophic  
127 bacteria). Here, heterotrophic nanoflagellates feed on pico- and nano- sized prey, microzooplankton  
128 feed on pico-, nano-, and micro-sized prey, and mesozooplankton feed on nano- and micro- sized  
129 prey (Fig. 1). Intraguild predation was allowed among all predators.

130 In the mixotrophic food web, nanoflagellates and microflagellates (previously perceived as  
131 strict autotrophs) were allowed to feed on diverse prey items, as supported by evidence from the  
132 literature (Zubkov and Tarran, 2008; Jeong *et al.*, 2010; Hansen, 2011; Unrein *et al.*, 2014). These  
133 constitutive mixotrophs are called hereafter as CM-nano and CM-micro, respectively (Table I).  
134 They can access the same prey as their heterotrophic competitors of same size (heterotrophic  
135 nanoflagellates and microzooplankton, respectively; Fig. 1 and Table S1). In turn, the  
136 microzooplankton group was divided into strict heterotrophic species and NCMs; previous  
137 estimates suggest that 40–60% of total microzooplankton can acquire phototrophic potential (Dolan  
138 and Pérez, 2000; Leles *et al.*, 2017). They share the same prey items and were assumed not to feed  
139 on each other (Fig. 1). Our conceptual framework accounted for GNCMs, such as oligotrich ciliates,

140 which have lower control over the acquired phototrophic machinery but can obtain it from diverse  
141 prey items, and SNCMs, such as *Mesodinium rubrum*, which have higher control over the acquired  
142 phototrophic machinery but rely on specific prey (Mitra *et al.*, 2016). SNCMs must obtain the  
143 phototrophic potential by feeding on CM-nano, while GNCMs can also obtain it feeding on CM-  
144 micro (Stoecker *et al.*, 1988-1989; Gustafson *et al.*, 2000; Johnson *et al.*, 2007; McManus *et al.*,  
145 2012). Intraguild predation was allowed within each mixotrophic functional type (Fig. 1, Table S1).

146 The model resolves the major chemical elements in the ocean, i.e. carbon, nitrogen,  
147 phosphorus, and silicate, both in organic and inorganic forms, accounting for variable stoichiometry  
148 within plankton groups (except for within mesozooplankton where C:N:P was held constant).  
149 Protist functional groups were described by a general plankton model that develops from the  
150 previous work by Flynn and Mitra (2009) and Mitra *et al.* (2016). Nutrient pools and the bacteria  
151 and mesozooplankton submodels correspond to those of ERSEM (Butenschön *et al.*, 2016) and are  
152 described further below. Overall, plankton growth dynamics result from the balance of gains  
153 through uptake of nutrients and assimilation into organic compounds and losses through respiration,  
154 excretion (non-assimilated material) and/or release of excess of nutrients (linked to stoichiometric  
155 regulation), predation, and non-predatory mortality (e.g. viral lysis). All state variables have units of  
156 element concentration (e.g. mg C m<sup>-3</sup>). Model equations and parameter values can be found in the  
157 supplementary material (Tables S2–S5). Our model was implemented in the open source Fortran-  
158 based Framework for Aquatic Biogeochemical Models (FABM) (Bruggeman and Bolding, 2014),  
159 an open platform (available at <http://fabm.net>) through which different models or submodels may be  
160 coupled in a single framework.

#### 161 *Nutrients, dissolved, and particulate organic matter*

162 Nutrient pools were divided between inorganics (nitrate, ammonium, phosphate, silicate,  
163 and dissolved inorganic carbon), dissolved organic matter (DOM), and detrital particulate organic  
164 matter (POM). DOM was divided between labile and semi-labile assuming that the former is  
165 rapidly consumed by bacteria and that the latter is more resistant to microbial degradation (Hansell,  
166 2013). Detrital POM was divided in three size-classes assuming that mesozooplankton can  
167 scavenge only on the medium size fraction. The chemical and the biological components of the food  
168 web model interact through the uptake of inorganics and the formation and recycling of organics, as  
169 described below for protists, bacteria, and mesozooplankton.

#### 170 *Protists*

171 The general protist model has the potential to simulate any protist from strict autotrophs to  
172 strict heterotrophs, including CMs and NCMs (see Supplementary Methods). The uptake of  
173 inorganics, photoacclimation, prey ingestion, and acquired phototrophy can be enabled/disabled  
174 accordingly. Here, we describe the main modifications and/or additions applied to the protist model

175 with respect to Flynn and Mitra (2009); equations can be found in the Supplementary Material. We  
176 developed the model in four main aspects:

177 i) We implemented the uptake of silicon to simulate diatoms following Flynn (2005). The  
178 representation of diatoms differs from that of other phototrophic protists due to their physiological  
179 requirement for silicon to build their frustules. In turn, silicon uptake differs fundamentally from  
180 that of nitrogen and phosphorus because the external nutrient concentration, instead of the internal  
181 (nutrient quota) concentration, ultimately affects growth; consequently, quota models are  
182 inappropriate for silicon dynamics (Flynn and Martin-Jézéquel, 2000).

183 ii) We implemented the allometric description of predation as described by Flynn and Mitra  
184 (2016). This formulation simulates the kinetics of prey capture and ingestion relating prey  
185 abundance and encounter rates to a prey-selection function controlled by satiation. In our model,  
186 prey selection is controlled by the total prey size spectrum accessible by the predator and its optimal  
187 prey size; capture is then minimum on both extremes of the prey size spectrum increasing linearly  
188 towards the optimal prey size (Flynn, 2018). This approach is very similar to the Gaussian predation  
189 kernel, but our formulation has the additional benefit of being defined directly by the observable  
190 lower and upper prey size limits.

191 iii) Acquired phototrophy was modified so that kleptochloroplasts are not digested but lost  
192 over time at a constant rate (Flynn and Hansen, 2013).

193 iv) All model equations were modified so that state variables were expressed in units of  
194 element quantity per water volume to allow model coupling with ERSEM submodels.

195 In our food webs, strict autotrophs can photoacclimate through the synthesis of chlorophyll,  
196 take up ammonium, nitrate, and phosphorus (plus silicon if diatoms), release labile DOC during  
197 photosynthesis, release labile DOM due to non-predatory mortality, and release dissolved inorganic  
198 carbon (DIC) and excess of inorganic nutrients (ammonium and phosphate) as part of respiration  
199 and stoichiometric regulation, respectively. Parameter values controlling light harvesting and  
200 nutrient uptake defined the differences between strict autotrophs in the model. In addition, CMs can  
201 engage in phagotrophy, re-assimilate inorganic nutrients released by breaking down their prey, and  
202 excrete the non-assimilated material as labile DOM. CMs must acquire a critical proportion of  
203 growth through photosynthesis and phagotrophy can be down-regulated if enough carbon is  
204 provided through phototrophy (Hansen, 2011). The model assumed that the internal re-assimilation  
205 of nutrients depends on the stoichiometric status of the mixotroph (N or P stress). We also assumed  
206 that CMs have lower maximum growth rates ( $\mu_{\max}$ ) compared to their heterotrophic competitors  
207 (Fischer *et al.*, 2016). On top of the differences related to light harvesting and nutrient uptake, CM-  
208 nano and CM-micro differ in their predation impact, with the former selecting pico-sized prey and  
209 having a narrower prey size spectrum, while the later selects for nano-sized prey (Table S1).

210 Strict heterotrophs assimilate organics through predation and release labile DOM due to  
211 non-predatory mortality, DIC through respiration, and non-assimilated material as labile DOM. The  
212 same processes were applied to NCMs, but these can fix inorganic carbon through acquired  
213 phototrophy (but do not photoacclimate), take up external inorganic nutrients (only SNCMs), and  
214 re-assimilate inorganic nutrients internally. Heterotrophic nanoflagellates select pico-size prey and  
215 have a narrower prey size spectrum while microzooplankton and NCMs select for nano-sized prey.  
216 NCMs were assumed to have the same  $\mu_{\max}$  as their heterotrophic counterparts, to select autotrophic  
217 prey, and to be positively selected by mesozooplankton compared to strict heterotrophic  
218 microzooplankton (Dolan and Pérez, 2000; Broglio *et al.*, 2004; Figueiredo *et al.*, 2007; Schoener  
219 and McManus, 2017). Among NCMs, GNCMs retain chloroplasts from their prey while SNCMs  
220 can also retain other cellular components and largely rely on photosynthesis to obtain carbon; thus,  
221 SNCMs were assumed to rely on photosynthesis for a critical proportion of growth while GNCMs  
222 were not (Stoecker *et al.*, 2009). In addition, SNCMs have better control over the acquired  
223 phototrophic machinery compared to GNCMs (Stoecker *et al.*, 2009); thus, our model assumes  
224 lower loss rate of kleptochloroplasts for the former.

#### 225 *Decomposers*

226 Bacteria were assumed to consume all forms of particulate and dissolved organic matter and  
227 to take up or release inorganic nutrients depending on the quality (i.e. N and P relative content) of  
228 the organic matter. Bacteria thus compete with phytoplankton for inorganic nutrients when organic  
229 substrates are nutrient depleted. Bacteria were assumed to release any carbon in excess to their  
230 physiological requirement (which is regulated by an ‘optimal’ cellular carbon to nutrient ratio) as  
231 semi-labile DOC. Recalcitrant DOC was also produced by the release of capsular material  
232 (Stoderegger and Herndl, 1998) which was assumed to be a fixed proportion of the carbon uptake.  
233 Overall these two fluxes imply that bacteria (especially when feeding on carbon-rich substrates)  
234 change the quality of DOM, increasing the proportion of recalcitrant DOC with respect to the labile  
235 forms. This mechanism is consistent with the microbial carbon pump concept (Jiao *et al.*, 2010;  
236 Polimene *et al.*, 2017).

#### 237 *Mesozooplankton*

238 The mesozooplankton model assumes a fixed internal nutrient to carbon ratio and the ability  
239 to scavenge on particulate organic matter. We modified the predation function from ERSEM to be  
240 consistent with that used in the protist model but through a simpler description; clearance rate is  
241 prey specific and was defined by the biomass of prey multiplied by the slope of the relationship  
242 between the abundance of prey and capture (Flynn and Mitra, 2016). Prey preference was based on  
243 size and depends on functional type. Mesozooplankton release excess nutrients as ammonium and



244 phosphate and contribute both to the pool of dissolved and particulates through mortality and  
245 excretion (e.g. faecal pellets).

#### 246 *Model set-up*

247 The food webs were simulated through chemostat-like modelling experiments. The model  
248 assumes plankton biomass and nutrients to vary over time within a homogeneous “box” that  
249 receives a constant input of inorganic nutrients (nitrate, phosphate, silicate) through a constant  
250 dilution rate. The same dilution rate also washes out residual nutrients and other dissolved and  
251 particulate organics (including plankton) and inorganics from the system. This construct is thus akin  
252 to a mixed layer environment which is subjected to an input of nutrients from a steady deeper layer,  
253 i.e. which does not accumulate properties over time. We assumed a fixed depth of 10 m, 12:12  
254 hours light-dark cycle, a constant temperature of 10°C, and a constant dilution rate of 0.01 day<sup>-1</sup>.  
255 The photosynthetically active radiation (PAR) was computed from the shortwave radiation in the  
256 surface ( $I_{\text{surf}}$ ), which was assumed to be constant through the period of the simulation, and an  
257 attenuation coefficient dependent on the concentration of plankton and particulate organic matter.  
258 The concentration of inorganics (e.g. dissolved inorganic nitrogen – DIN) entering the system (akin  
259 to concentrations below the mixed layer) was constant throughout a given simulation.

260 We simulated two different scenarios: low light-high nutrient ( $I_{\text{surf}} = 50 \text{ W m}^{-2}$  or 228  $\mu\text{mol}$   
261  $\text{photon m}^{-2} \text{ s}^{-1}$ ; DIN = 20  $\mu\text{M}$  nitrate) and high light-low nutrient ( $I_{\text{surf}} = 250 \text{ W m}^{-2}$  or 1,140  $\mu\text{mol}$   
262  $\text{photon m}^{-2} \text{ s}^{-1}$ ; DIN = 4  $\mu\text{M}$  nitrate). These irradiance and nutrient concentrations were chosen to  
263 induce light limitation or nutrient limitation among phototrophs. Light limitation was assessed  
264 through the relative rate of photosynthesis (i.e. the ratio between the actual photosynthesis rate and  
265 the maximum photosynthesis rate) while nutrient limitation was assessed through the normalised  
266 nutrient to carbon quotas. We assumed an input 16:1 mole ratio of dissolved inorganic nitrogen  
267 (DIN; nitrate) to phosphorus, and a 1:1 mole ratio of DIN to silicon in all simulations.

268 Models output are presented herein through the average of the last year of simulation.  
269 Dynamic plots can be found in the supplementary material (Figs. S2–S6). We compared: i) the total  
270 ammonium regeneration, ii) the trophic transfer efficiency, and iii) the total production of labile  
271 DOC between the non-mixotrophic and the mixotrophic food webs. These metrics were chosen to  
272 test the hypotheses that mixotrophy decreases the overall regeneration of inorganics, increases the  
273 transfer of biomass to higher trophic levels promoting the accumulation of biomass in larger size-  
274 classes and increases DOC production. The ratio between the total amount of food ingested by  
275 mesozooplankton and the total gross primary productivity (GPP) was used as a measure of trophic  
276 transfer efficiency. We assumed that all organic carbon released by phytoplankton through primary  
277 production, egestion of unassimilated prey (mainly by protists), and natural mortality contributed to  
278 the pool of labile DOC. The contribution of different functional groups to each of the processes was

279 also investigated. Finally, the relative biomasses of mixotrophs and their respective autotrophic and  
280 heterotrophic competitors were evaluated in the transition from light to nutrient limitation. Thus, a  
281 third modelling experiment was conducted to simulate intermediate conditions of light and nutrient  
282 limitation ( $I_{\text{surf}} = 100 \text{ W m}^{-2}$  or  $457 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ,  $\text{DIN} = 16 \mu\text{M}$  nitrate).

### 283 *Sensitivity analyses for mixotrophic food web*

284 Sensitivity analyses were performed to evaluate how the ecological processes described  
285 above (i.e. ammonium regeneration, trophic transfer efficiency, and total production of labile DOC)  
286 are affected by the choice of parameter values and nutrient concentrations in the chemostat medium  
287 for the mixotrophic food web. We evaluated the sensitivity of the mixotrophic food web for both the  
288 nutrient-limited and the light-limited scenarios. The main parameters that define functional diversity  
289 within our conceptual food web, such as those related to phototrophy, nutrient uptake, predation,  
290 respiration, and mixotrophic potential (following previous sections) were selected for the analyses.

291 We used an approach based on the Monte-Carlo ensemble technique to rank the importance  
292 of the input parameters (Saltelli *et al.*, 2008; Sankar *et al.*, 2018). This technique allows the  
293 detection of the parameters (and thus of the respective processes and functional types) that each  
294 targeted output is most sensitive to. Even if several input parameters are included in the analysis, a  
295 few input parameters often account for most of the variation observed in model output (Saltelli *et*  
296 *al.*, 2008). The method generates a number  $n$  of realizations based on the probability density  
297 functions of  $m$  input factors  $x_i$  (i.e. model parameters), assumed to be uniformly distributed and  
298 independent from each other. Each realization produces a vector containing values randomly  
299 sampled from the distributions of all input parameters. Each vector of parameters is then used to run  
300 a model simulation and compute the output  $y$ . The output of  $n$  realizations and model runs is  
301 subsequently represented by a multiple linear regression:

$$302 \quad y = b_0 + \sum_{i=1}^m b_i x_i + \text{residuals} \quad (1)$$

303 The standardized regression coefficients ( $\beta_i$  computed from  $b_i$ ) were used as global  
304 sensitivity indices of the input factors (Saltelli *et al.*, 2008):

$$305 \quad \beta_i = \frac{b_i \sigma_{x_i}}{\sigma_y} \quad (2)$$

306 where  $\sigma_{x_i}$  and  $\sigma_y$  are the standard deviations of the realizations of the input factor  $x_i$  and of the  
307 model output  $y$ , respectively. Thus, each parameter included in the analysis is associated to a  
308 sensitivity coefficient which indicates whether an increase in the value of the parameter has a  
309 positive or negative effect on the targeted output (i.e. increase or decrease the output value,  
310 respectively). Since the validity of the results depends on the fraction of the model output variability  
311 that is explained by the multiple linear regression (Saltelli *et al.*, 2000), we estimated the overall

312 fraction of explained variance ( $R^2$ ) and the significance of the standardized regression coefficients  
313 ( $\beta_i$ ).

314 A total of  $m = 116$  input parameters were included in the sensitivity analyses. We performed  
315  $n = 2320$  realizations assuming 20 realizations for each input parameter as a rule of thumb (Hair *et*  
316 *al.*, 2006). Random values were generated assuming a range of  $\pm 30\%$  of the reference value of the  
317 input parameters (e.g. Sankar *et al.*, 2018). The analyses were performed using a Python code  
318 developed for the purpose. In addition to these analyses, we conducted an extra sensitivity test to  
319 confirm that averaged model outputs during the last year of the simulation were independent from  
320 initial conditions; methods and results from this analysis can be found in the Supplementary  
321 Material. Model output and graphical visualization was processed/performed in R software (R Core  
322 Team, 2018) using the packages ‘netcdf4’, ‘ggplot2’, ‘gridExtra’, and ‘plyr’.

323

## 324 **Results**

### 325 *Light-limited scenario*

326 Ecosystem properties differed between the non-mixotrophic and the mixotrophic food webs  
327 in the light-limited scenario (Fig. 2). Ammonium regeneration was higher in the non-mixotrophic  
328 food web, mainly due to the activity of heterotrophic protists (Fig. 2a). Once mixotrophs were  
329 included, they competed with their heterotrophic counterparts and down-regulated the biomass of  
330 heterotrophic nanoflagellates (Fig. 3). Mixotrophs did not contribute as much to the regeneration of  
331 ammonium but supported a higher trophic transfer efficiency of carbon biomass to higher trophic  
332 levels (Fig. 2b). This is explained by changes in community composition, from smaller (in the non-  
333 mixotrophic food web) to larger (in the mixotrophic food web) phototrophs, since mesozooplankton  
334 exhibit a preference for larger prey items. In the absence of mixotrophs, autotrophic nanoflagellates  
335 and microflagellates were outcompeted by picophytoplankton and diatoms (Fig. 3), with only the  
336 latter having a cell size large enough to serve as food for mesozooplankton (Fig. 2b). In the  
337 mixotrophic food web, CMs thrived, with mixotrophs contributing significantly to the diet of  
338 mesozooplankton (Fig. 2b). In turn, the production of DOC was higher in the non-mixotrophic food  
339 web (Fig. 2c). This was mainly due to the higher total GPP (Fig. S7), reflecting the high biomass  
340 levels attained by picophytoplankton and diatoms (Fig. 3), and due to higher mortality following the  
341 overall increase in carbon biomass (Fig. S8). Relative to that of phototrophs, the production of DOC  
342 by phagotrophic protists was minor in the non-mixotrophic framework while being more significant  
343 in the presence of mixotrophs (Fig. 2c).

### 344 *Nutrient-limited scenario*

345 Mixotrophy was more successful under the high-light and low-nutrient condition, with  
346 mixotrophs outcompeting their strict autotrophic and strict heterotrophic counterparts, respectively  
347 (Fig. 3). As a result, ecosystem properties differed substantially between the non-mixotrophic and  
348 the mixotrophic food webs in this scenario (Figs. 3 and 4). Similar to the light limited-scenario,  
349 ammonium regeneration was lower in the mixotrophic food web (Fig. 4a) and NCMs could  
350 outcompete their heterotrophic counterparts due to limited prey availability (Fig. 3). As mixotrophs  
351 did not contribute to the regeneration of ammonium (Fig. 4a), this in turn decreased the availability  
352 of inorganic nutrients, which favoured CMs (mainly CM-nano) over strict autotrophs (Fig. 3).

353 The role of mixotrophy in the trophic transfer efficiency was even more pronounced in the  
354 nutrient-limited scenario (Fig. 4b). Indeed, while in the non-mixotrophic food web  
355 mesozooplankton was limited by the paucity of suitable prey, in the mixotrophic food web,  
356 mesozooplankton could rely on NCMs which in turn were supported by the CM-nano biomass  
357 feeding on picophytoplankton (Figs. 3 and 4b). Contrary to the light-limited scenario, mixotrophy  
358 also boosted the production of labile DOC under nutrient limitation. This was mainly related to a  
359 greater fraction of ingested prey remaining unassimilated (i.e. more inefficient predators due to  
360 lower prey quality). The main contributors to the production of labile DOC on this scenario were  
361 CM-nano and GNCMs (Fig. 4c). The direct effect of increased availability of labile DOC is the  
362 stimulation of bacterial metabolism, which in turn leads to enhanced production of recalcitrant DOC  
363 (Fig. 4c). Accordingly, production of recalcitrant DOC was considerably lower in the non-  
364 mixotrophic food web (Fig. 4c).

365 The individual and additional effects of mixotrophic diversity were also investigated by  
366 including one or more mixotrophic types at a time (Fig. S9). The additional simulations were  
367 performed under nutrient limitation due to the higher importance of mixotrophy on this scenario.  
368 We evaluated the changes on community structure (in terms of carbon biomass considering  
369 mixotrophs and their strict auto- and hetero- trophic competitors), ammonium regeneration, and  
370 trophic transfer efficiency relative to the non-mixotrophic food web. When only one mixotrophic  
371 type was considered, changes on community structure were more significant for CM-nano which,  
372 outcompeting picophytoplankton, were the only mixotrophic type to enable the growth of  
373 mesozooplankton alone (Fig. S9). On the other hand, CM-micro down-regulated the biomass of  
374 strict heterotrophs, allowing higher picophytoplankton biomass and decreasing the overall  
375 regeneration of ammonium by ~ 70% (Fig. S9). The individual impact of GNCMs or SNCMs was  
376 small; in fact, SNCMs did not survive because they depend on nanophytoplankton to obtain their  
377 phototrophic capacity and this group was outcompeted by picophytoplankton (Fig. S9). Differences  
378 were more pronounced once CM-nano and GNCMs or SNCMs were included in the model because

379 CM-nano supports the biomass of NCMs which, in turn, is transferred to mesozooplankton (Fig.  
380 S9). Finally, including all mixotrophic types increased the extent of the overall niche for  
381 mixotrophy, enhancing its overall impact (Fig. S9).

### 382 *Sensitivity analyses for mixotrophic food web*

383 The overall fraction of variance explained by the multiple linear regression on the 116  
384 selected parameters was high for all three targeted outputs in both limitation scenarios ( $R^2 > 0.9$ ).  
385 Here we present the first 8 parameters ranked by highest sensitivity (Tables II and III); the full  
386 ranking can be found in the supplementary material (Table S6). The sensitivity coefficients of all  
387 the parameters reported here were statistically significant. A positive coefficient (in Tables II and  
388 III) indicates that an increase in the parameter value led to an increase in the output value and vice-  
389 versa.

390 In the light-limited scenario, all targeted outputs were most sensitive to photosynthetic  
391 parameters (Table II). Ammonium regeneration was promoted by increasing the efficiency of  
392 diatoms and picophytoplankton in harvesting light ( $\alpha_{\text{Chl}}$  and  $\text{ChlC}_{\text{abs}}$ , positive coefficients in Table  
393 II) and decreased if higher maximum nitrogen to carbon quotas were considered ( $\text{NC}_{\text{max}}$ , negative  
394 coefficients in Table II). In turn, the trophic transfer efficiency was most sensitive to the optimal  
395 prey size ( $S_{\text{opt}}$ ) of CM-nano (Table II). Increasing their optimal prey size increased the intraguild  
396 predation within this group, resulting in higher growth rates but lower population biomass. A  
397 cascade effect is then observed, because less prey would be available for CM-micro, which are an  
398 important prey item for mesozooplankton in this scenario ( $\text{Cr}_{\text{CM-micro}}$ ). On the other hand, increasing  
399  $\alpha_{\text{Chl}}$  and  $\text{ChlC}_{\text{abs}}$  among CMs and diatoms supported higher trophic transfer efficiency, since these  
400 were the main prey supporting mesozooplankton biomass (positive coefficients in Table II).  
401 Mesozooplankton intraguild predation ( $\text{Cr}_{\text{mesozoo}}$ ) was also important and negatively impacted (i.e.  
402 decreased) the trophic transfer efficiency (ranked 5<sup>th</sup>). Regarding the production of labile DOC, the  
403 contribution of the major phototrophs (diatoms, picophytoplankton, and CM-nano) was the main  
404 source of DOC in the light-limited scenario, mainly driven by parameters controlling their  
405 phototrophic potential ( $\alpha_{\text{Chl}}$  and  $\text{ChlC}_{\text{abs}}$ ; positive coefficients in Table II). The optimal prey size of  
406 CM-nano was also important, although to a lesser extent ( $S_{\text{opt}}$ ).

407 In the nutrient-limited scenario, the parameterisation of bacteria and mixotrophs was more  
408 important (Table III). Ammonium regeneration was negatively impacted by increasing the  
409 maximum prey size accessible by CM-nano ( $S_{\text{max}}$ ), as well as its preferred prey size ( $S_{\text{opt}}$ ) (negative  
410 coefficients in Table III), because it favours the success of CM-nano relative to their strict  
411 heterotrophic competitors. However, increases in the maximum phototrophic growth rate ( $\mu_{\text{phot}}$ ) of

412 CM-nano counterbalanced the negative effect of  $S_{opt}$ . Maximum internal N or P to carbon quotas  
413 were also important (ranked 2<sup>nd</sup>, 3<sup>rd</sup>, and 8<sup>th</sup>). Similar to the light-limited scenario, the trophic  
414 transfer efficiency was inversely related to the optimal prey size ( $S_{opt}$ ) of CM-nano, resulting in less  
415 prey for GNCMs, which were an important prey for mesozooplankton under nutrient limitation  
416 (ranked 1<sup>st</sup> and 3<sup>rd</sup>, respectively). A similar negative effect was observed when increasing  $S_{max}$   
417 among mixotrophs (negative coefficients in Table II ). Parameters associated with  
418 picophytoplankton and bacteria were also important ( $\mu_{phot}$  and  $NC_{max}$ , respectively), but to a lesser  
419 extent than the previous ones (Table III). The production of labile DOC was positively related (i.e.  
420 increased) with the maximum phototrophic growth rate of CM-nano ( $\mu_{phot}$ ) and with parameters  
421 controlling the predation by NCMs ( $S_{max}$ ; positive coefficients in Table II). The internal  
422 stoichiometry regulation of mixotrophs and bacteria was also important, with a negative effect  
423 associated with N to C ratios (ranked 3<sup>rd</sup>, 4<sup>th</sup>, and 6<sup>th</sup>) and a positive effect associated with P to C  
424 ratios (ranked 7<sup>th</sup> and 8<sup>th</sup>).

425

## 426 Discussion

427 Our study suggests that the interpretations and predictions of the functioning of the marine  
428 planktonic ecosystem could radically change if we consider mixotrophic functional diversity in  
429 ocean models, with mixotrophy impacting nutrient availability, mass and energy transfer to higher  
430 trophic levels, and the microbial loop (Figs. 2–4). Our simulations show that the relative dominance  
431 of different mixotrophic functional groups can shape the planktonic ecosystem in different ways  
432 depending on light and nutrient regimes. Size was shown to be important to determine the success  
433 of mixotrophs with an innate capacity for photosynthesis; while small cells dominated under  
434 nutrient limitation, larger cells were more important under light limitation (Fig. 5a). Among  
435 acquired phototrophs, the specificity of the prey from which kleptochloroplasts are obtained  
436 affected their success, with generalist forms dominating under nutrient limitation and specialist  
437 forms showing maximal contribution in intermediate conditions of light and nutrients and under  
438 nutrient limitation (Fig. 5b).

439 The results from our simulations appear consistent with empirical observations. The nano-  
440 CMs and GNCMs (e.g. oligotrich ciliates) have been reported to be important members within  
441 oligotrophic gyres and during summer within temperate seas (Stoecker *et al.*, 1987; Zubkov and  
442 Tarran, 2008; Hartmann *et al.*, 2012; Unrein *et al.*, 2014; Haraguchi *et al.*, 2018). In contrast, micro-  
443 CMs and SNCMs (e.g. *Mesodinium rubrum*) can be major components of plankton assemblages in  
444 eutrophic coastal environments and during winter within temperate seas (Burkholder *et al.*, 2008;  
445 Jeong *et al.*, 2010, Hansen, 2011; Johnson *et al.*, 2013). Our simulations also produced realistic  
446 estimates of the biomass ratios between NCMs and their heterotrophic competitors. In the light-

447 limited scenario, our model predicted coexistence of NCMs and their heterotrophic counterparts,  
448 with the latter comprising half of the total assemblage (Fig. 5b). These results were consistent with  
449 previous observations showing that strict heterotrophs comprise on average 60% of total ciliate  
450 biomass during winter within coastal temperate seas (Nielsen and Kiørboe, 1994; Leles *et al.*,  
451 2017). Once limited by prey availability, strict heterotrophs survived at a very low biomass only  
452 accounting for 5% of the total assemblage (Fig. 5b). Overall, this value is lower than expected  
453 during summer (Leles *et al.*, 2017); minimum values were reported in the Mediterranean Sea and in  
454 the Northwest Atlantic Shelves, in which heterotrophic microzooplankton accounted for less than  
455 15% of total ciliate biomass (Stoecker *et al.* 1987; Modigh, 2001; Bernard and Rassoulzadegan,  
456 1994).

457 Acquired phototrophy has been suggested to stabilise coexistence between NCMs and the  
458 prey that provides their phototrophic potential (Moeller *et al.*, 2016). However, the nature of this  
459 coexistence would depend on light availability, with the amplitude of repeating biomass cycles  
460 increasing with irradiance (Moeller *et al.*, 2016). In a food web considering bottom-up (nutrients)  
461 and top-down (higher predators) controls, we found that the amplitude of repeating cycles was  
462 considerably lower under high-light and low-nutrient conditions, approaching a constant steady-  
463 state (Fig. S3). In addition, when we assumed trophic interactions between SNCMs, GNCMs, and  
464 strict heterotrophs; the model became more unstable with one group slowly outcompeting the  
465 others. Defining the differences between these groups is challenging. For instance, experimental  
466 evidence found similar maximum growth rates and inorganic N uptake between strict heterotrophs  
467 and GNCMs (Schoener and McManus, 2017). Although our assumptions were based on the current  
468 literature, there is little quantitative information on the costs and benefits associated to acquired  
469 phototrophy (Dolan and Pérez, 2000; Stoecker *et al.*, 2009; McManus *et al.*, 2012). Our sensitivity  
470 experiments suggest that defining the prey size spectrum accessible and selected by each of these  
471 groups significantly impact the targeted outputs (Tables II and III).

472 Our model predicted that the dominance of mixotrophs over their strict autotrophic and  
473 heterotrophic counterparts increases in the transition from light to nutrient limitation (Fig. 5).  
474 Mixotrophs can outcompete strict autotrophs and strict heterotrophs by using nutrients more  
475 efficiently. Indeed, when nutrient-rich prey are ingested any surplus of N and P may be combined  
476 with newly fixed carbon instead of being excreted outside the cell (Rothhaupt, 1997; Flynn and  
477 Mitra, 2009; Fischer *et al.*, 2016). In addition, certain species of acquired phototrophs can take up  
478 inorganic nutrients (Hattenrath-Lehmann and Gobler, 2015; Qiu *et al.*, 2016). These features  
479 minimise the remineralization of nutrients to the environment enhancing the bottom-up control of  
480 strict autotrophs under nutrient limitation and favouring mixotrophs over strict heterotrophs under  
481 low prey availability (Fig. 5). Thus, the simulations indicate that the presence of not only CMs but

482 also NCMs can decrease nutrient regeneration. This is consistent with previous findings showing  
483 that the dinoflagellate *Dinophysis acuminata* and the ciliate *M. rubrum* take up inorganic nutrients;  
484 these two species are classifiable as SNCMs as they must acquire kleptochloroplasts from *M.*  
485 *rubrum* and red cryptophyte algal prey, respectively (Hattenrath-Lehmann and Gobler, 2015; Qiu *et*  
486 *al.*, 2016). Recent evidence also shows that oligotrich ciliates (i.e. GNCMs) can take up inorganic  
487 nutrients, although it might not contribute significantly to their growth (Schoener and McManus,  
488 2017).

489 Mixotrophs have been previously suggested to increase the trophic transfer efficiency to  
490 higher trophic levels (Stoecker *et al.*, 2009; Stoecker *et al.*, 2017). Our simulations support and  
491 expand this ecological concept by considering the functional diversity among mixotrophs and their  
492 strict autotrophic and heterotrophic competitors. Our results suggest that CMs have a competitive  
493 advantage over strict autotrophic competitors, particularly under nutrient limitation, allowing the  
494 accumulation of biomass in larger prey instead of in pico-sized prey, which are too small to be  
495 consumed by higher consumers. In turn, CMs provide photosynthetic potential to NCMs, which  
496 composed the bulk of biomass that sustained higher trophic levels (Fig. 4b). In fact, crustacean  
497 zooplankton and fish larvae preferentially prey on NCMs, such as oligotrich ciliates and *M. rubrum*,  
498 rather than on their strict auto- and hetero- trophic competitors (Broglio *et al.*, 2004; Figueiredo *et*  
499 *al.*, 2007). Our simulations suggest that ~ 50% of mesozooplankton diet was composed by NCMs in  
500 the nutrient-limited scenario versus 20% under light limitation. These values are consistent with  
501 observations showing that the relative importance of the consumption of NCMs by copepods varies  
502 across environmental gradients, increasing towards less productive systems (Calbet and Saiz, 2005).

503 Mixed nutrition may also increase the release of labile DOC among protists (Flynn *et al.*,  
504 2008; Mitra *et al.*, 2014). The theoretical framework presented here provides a platform to explore  
505 how this might affect the production of recalcitrant DOC by bacteria. Our results showed higher  
506 production of labile DOC in the mixotrophic food web only when nutrients were limiting,  
507 stimulating bacterial production (Mitra *et al.*, 2014) and, consequently, boosting the production of  
508 recalcitrant DOC (Fig. 2c vs Fig. 4c). The main source responsible for the higher production of  
509 labile DOC on this scenario was the increased release of labile DOC by protists (Fig. S8). This  
510 release, in turn, was induced by higher prey consumption combined with an overall poor prey  
511 quality, described here by internal N:C and P:C quotas (Mitra, 2006; Polimene *et al.*, 2015).  
512 Overall, the stronger nutrient limitation in the presence of mixotrophs resulted in lower prey quality  
513 and hence less efficient microzooplankton. However, it is noteworthy that the production of labile  
514 DOC and hence of recalcitrant DOC is strongly dependent on model assumptions, particularly in  
515 the partitioning of voided material between particulate and dissolved pools. It is also important to  
516 note that our model lacked the description of osmotrophy among CMs (Ghyoot *et al.*, 2017), which



517 can decrease the net production of DOC, or even change the mixotroph from a source of DOC into  
518 a sink.

519 Our results are dependent on several assumptions and uncertain parameters, but we aimed to  
520 explore the emerging paradigm in marine ecology, in which the phytoplankton-zooplankton  
521 dichotomy no longer holds. Constitutive mixotrophy was particularly important to maintain  
522 phototrophy within nano- and micro- plankton size classes, which would be outcompeted by  
523 picophytoplankton otherwise. This result appears robust because it is mainly related to the overall  
524 predation impact, which is lower among picophytoplankton as predicted by allometric constraints.  
525 The success of different phototrophs is also dependent on their phototrophic capacities and internal  
526 stoichiometric quotas, as showed by our sensitivity tests, and these parameters are well  
527 characterised in the literature (Table S3). On the other hand, acquired phototrophs were too  
528 dominant relative to their heterotrophic competitors in our simulations. While we could compile  
529 information on their total prey size spectrum (Table S1), it seems that we still lack information on  
530 the costs associated to acquired phototrophy. Our results also suggest that NCMs may act as a sink  
531 or source of inorganic nutrients, depending on environmental conditions. Similarly, quantitative  
532 studies on the cycling of DOM by mixotrophs and consequently in the production of recalcitrant  
533 DOC by bacteria, can help to elucidate the significance of mixotrophy to the microbial carbon  
534 pump.

535 The importance of mixotrophy in the environmental setups used in our simulation  
536 experiments can have profound consequences in view of climatic and anthropogenic changes on the  
537 oceans, particularly in oligotrophic seas and eutrophic coastal systems. Warmer waters and stronger  
538 stratification have been previously hypothesised to favour mixotrophic plankton in oligotrophic seas  
539 (Polovina *et al.*, 2008; Wilken *et al.*, 2013; Behrenfeld *et al.*, 2016). In turn, increased  
540 eutrophication in coastal waters can induce light-limitation and promote the formation of harmful  
541 algal blooms, many of which are mixotrophic species (Burkholder *et al.*, 2008; Gomes *et al.*, 2014).  
542 Our findings provide the basis for the mechanisms giving competitive advantages to different  
543 mixotrophs relative to their strict auto- and hetero- trophic competitors under such environmental  
544 conditions (i.e. nutrient or light limitation). In view of our results, we believe that future studies  
545 aiming to predict the impact of environmental changes on the oceanic food webs should consider  
546 the mixotrophic potential of plankton communities.

547

## 548 **Conclusions**

549 Our investigation suggests that mixotrophic functional diversity can significantly alter our  
550 understanding of ecosystem dynamics within the lower trophic levels of marine food webs, with  
551 key groups of mixotrophs controlling nutrient regulation, trophic transfer, and the microbial loop.

552 Our model predicted predominance of nano-CMs and GNCMs in nutrient depleted conditions (akin  
553 to oligotrophic oceans), and a higher importance of micro-CMs and SNCMs under light limited  
554 conditions (e.g. eutrophic coastal systems). This is the first time that the roles of different mixotroph  
555 types have been explored simultaneously within plankton food webs. This work demonstrates the  
556 importance of deploying detailed descriptions of mixotroph physiology. Our results also show how  
557 mixotrophy interacts in the direct and indirect control of the growth of strict autotrophic and  
558 heterotrophic populations, particularly under nutrient limitation. Moreover, we demonstrated how  
559 mixotrophy can promote the transfer of carbon biomass to higher planktonic predators through the  
560 interplay between CMs and NCMs. Critically, we have constructed a food web framework for  
561 comprehensive quantitative exploration of the role of mixotrophic functional diversity in marine  
562 ecosystems, which can be readily implemented in a variety of settings: from chemostats to spatially  
563 structured models of the water column (1D) and the global ocean (3D). It thus provides a powerful  
564 tool to investigate the role of mixotrophy in a changing ocean.

565

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570

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581

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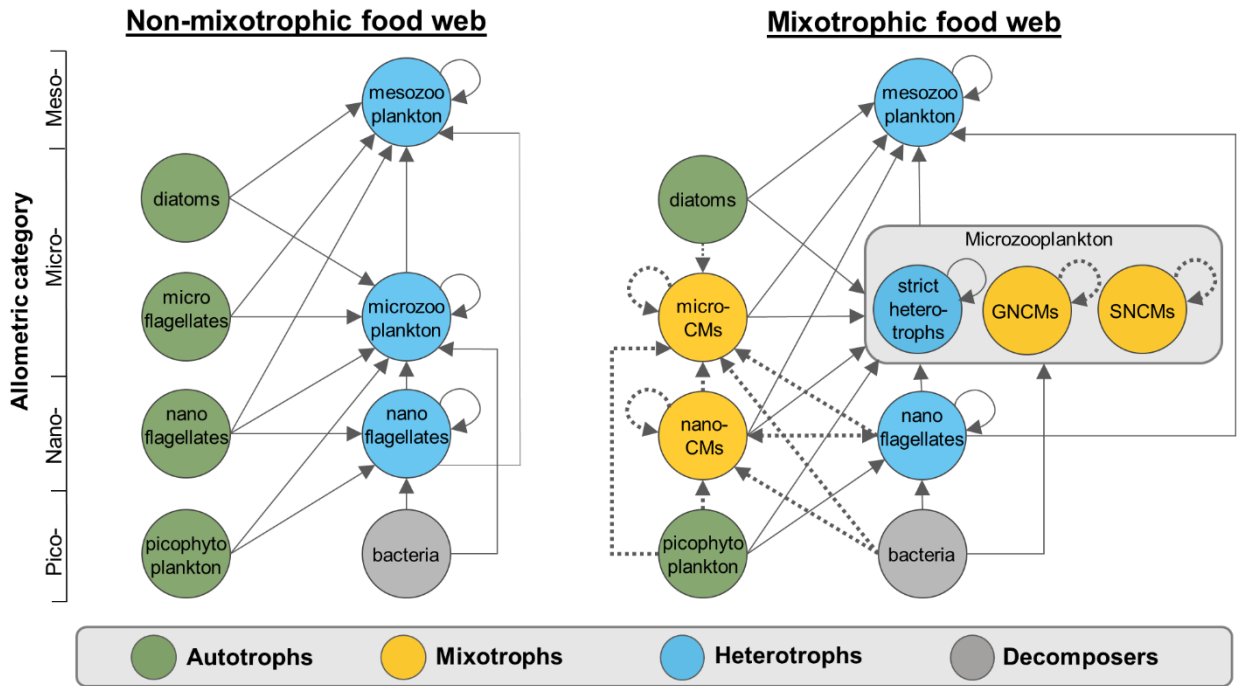
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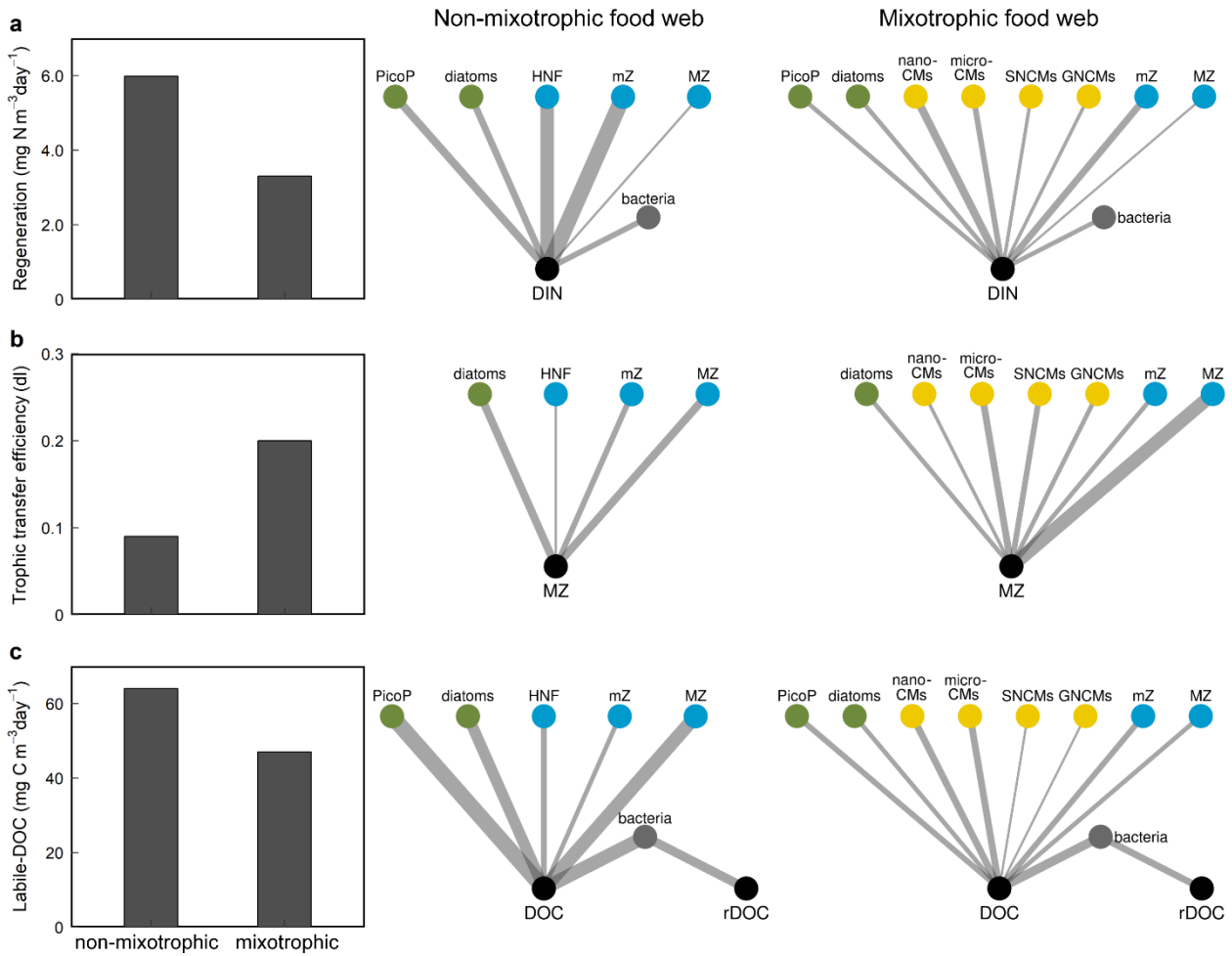


766

767 **Fig. 1** Conceptual frameworks of plankton food webs used on this study to investigate the impact of  
 768 mixotrophic diversity on different ecosystem properties. Food webs only differ in the presence of  
 769 the mixotrophic trait. Arrows indicate trophic interactions; dotted arrows correspond to new  
 770 interactions associated to mixotrophy. Abbreviations are as per Table I.

771





773 **Fig. 2** Light limited-scenario for the non-mixotrophic and the mixotrophic food webs. a)

774 ammonium (DIN) regeneration; b) trophic transfer efficiency (measured by the ratio of the total

775 amount of food ingested by mesozooplankton (MZ) by the total gross primary productivity); and c)

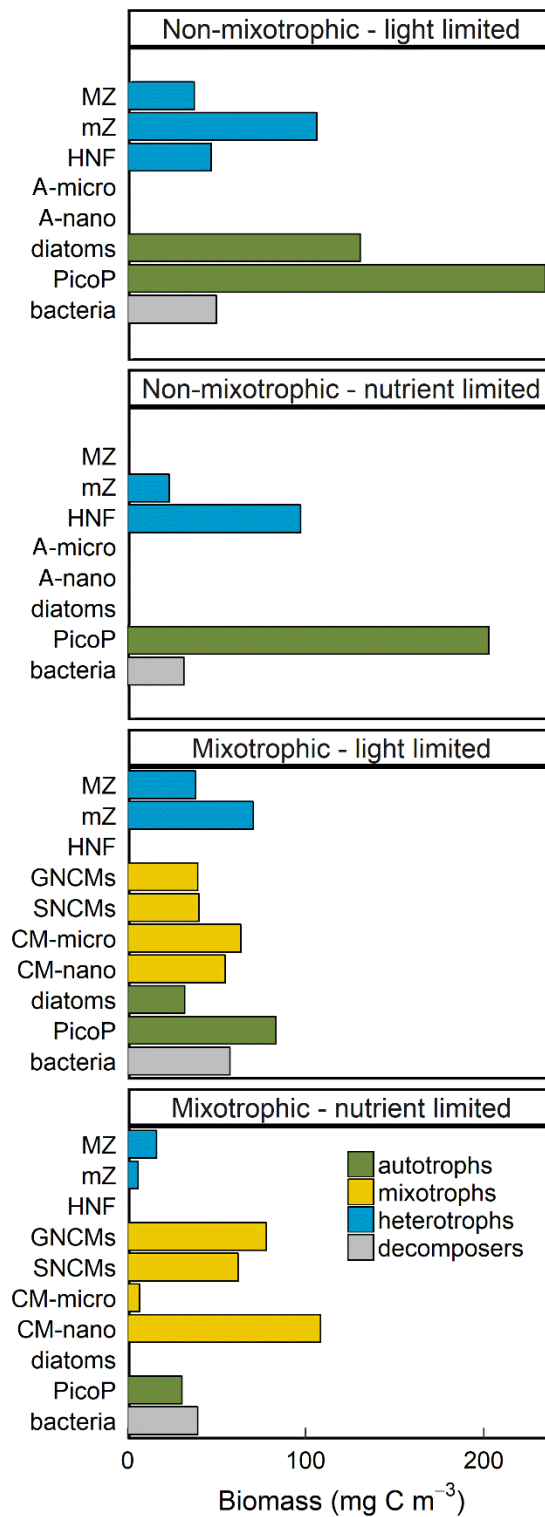
776 total production of labile dissolved organic carbon (DOC). Data were averaged for the last year of

777 simulation. Schematics show the relative contribution of functional groups (green–autotrophs,

778 yellow–mixotrophs, blue–heterotrophs, grey–decomposers) to each of the ecosystem properties

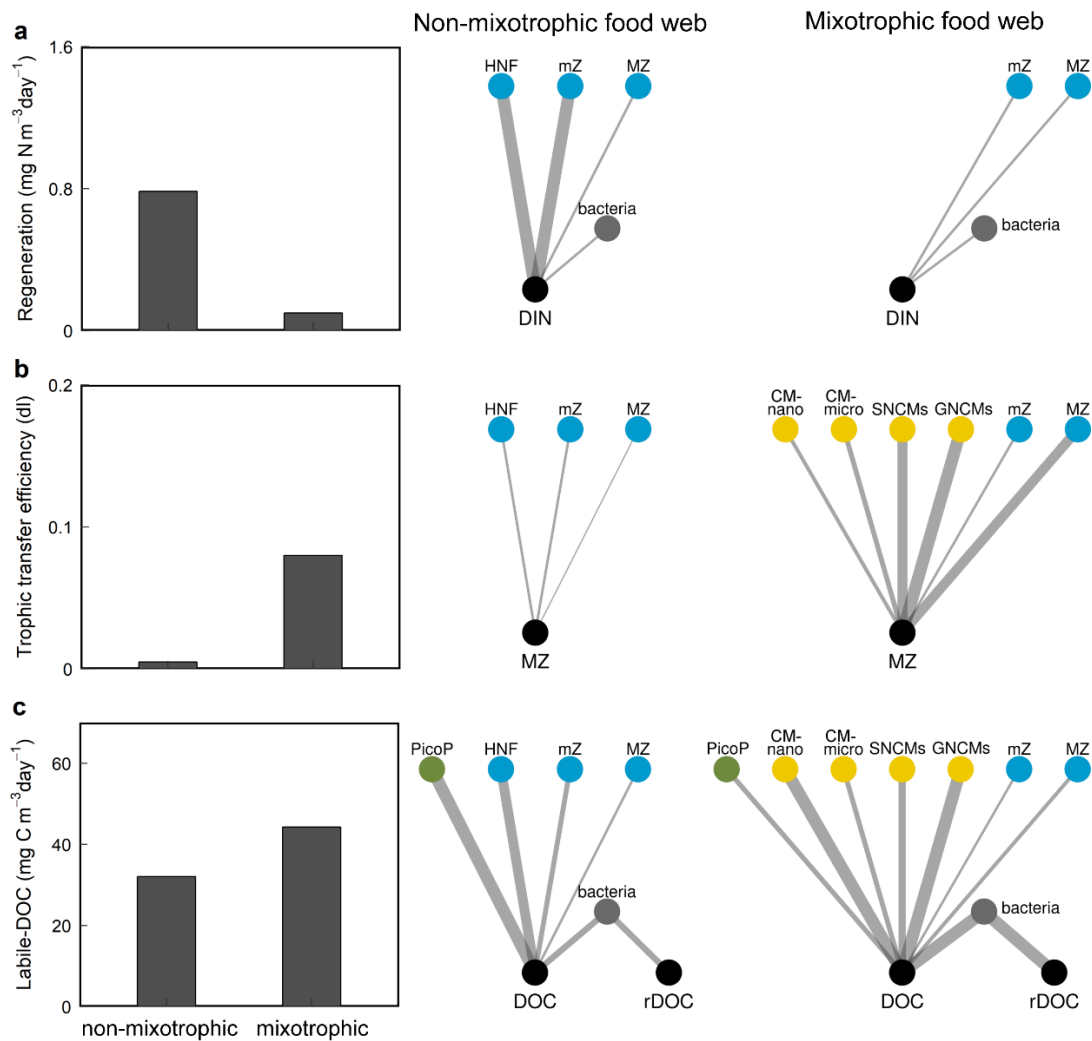
779 (black nodes). In panel b, fluxes represent the amount of food ingested by mesozooplankton.

780 rDOC–recalcitrant DOC, dl-dimensionless; for other abbreviations please refer to Table I.



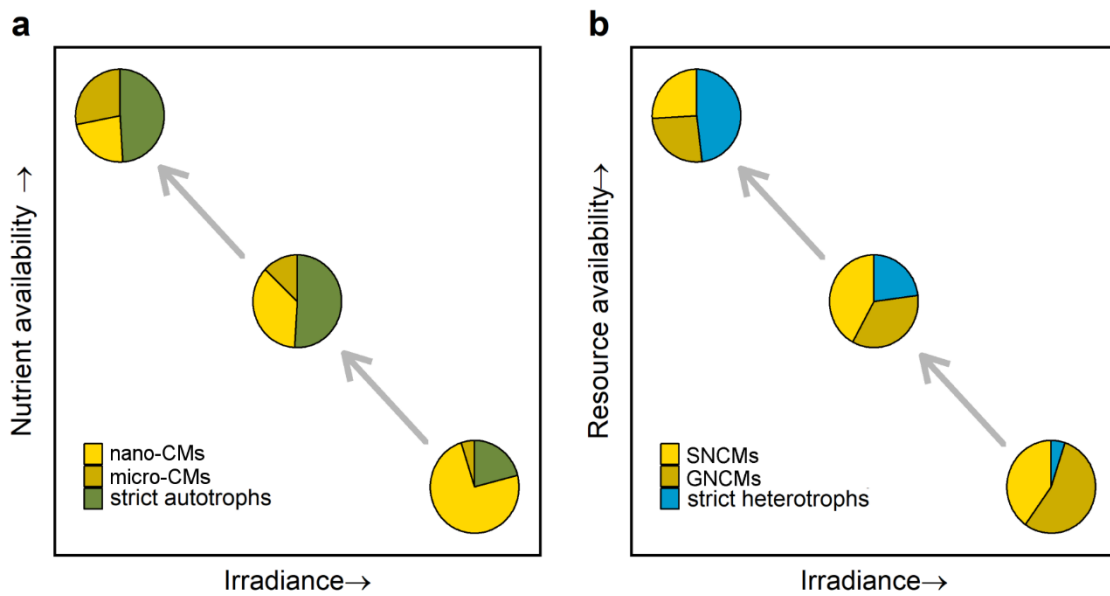
781

782 **Fig. 3** Community composition for the non-mixotrophic and the mixotrophic food webs in both the  
 783 light-limited and the nutrient-limited scenarios. Carbon biomass of the different functional groups  
 784 are given; colours indicate different trophic strategies. Data were averaged for the last year of  
 785 simulation. MZ – mesozooplankton; A-nano – autotrophic nanoflagellates; A-micro – autotrophic  
 786 microflagellates; for other abbreviations please refer to Table I.



787

788 **Fig. 4** Nutrient limited-scenario for the non-mixotrophic and the mixotrophic food webs. a)  
 789 ammonium (DIN) regeneration; b) trophic transfer efficiency (measured by the ratio of the total  
 790 amount of food ingested by mesozooplankton (MZ) by the total gross primary productivity); and c)  
 791 total production of labile dissolved organic carbon (DOC). Data were averaged for the last year of  
 792 simulation. Schematics show the relative contribution of functional groups (green–autotrophs,  
 793 yellow–mixotrophs, blue–heterotrophs, grey–decomposers) to each of the ecosystem properties  
 794 (black nodes). In panel b, fluxes represent the amount of food ingested by mesozooplankton.  
 795 rDOC–recalcitrant DOC, dl-dimensionless; for other abbreviations please refer to Table I.



796

797 **Fig. 5** Relative biomass of mixotrophic, strict autotrophic, and strict heterotrophic protists in a  
 798 gradient from nutrient to light limitation. a) constitutive mixotrophs (CMs) and their strict  
 799 autotrophic competitors; b) non-constitutive mixotrophs (NCMs) and their strict heterotrophic  
 800 competitors. Data were averaged for the last year of simulation. Note that area corresponding to  
 801 high nutrient and high irradiance (upper right corner of the panels) are potentially mutually  
 802 exclusive due to self-shading; for abbreviations please refer to Table I.

803

805 **Table I** Definitions of mixotrophic functional diversity following Mitra *et al.*, (2016) and model  
 806 organisms used in this study

Term	Definition	Model organism
Mixotrophy	The combination of phototrophy and phagotrophy in a single organism	protist plankton
Constitutive mixotrophs (CMs)	Possess their own photosystems; within the model structure these are facultative mixotrophs, i.e. do not need to feed to survive	nanoflagellates (CM-nano) and microflagellates (CM-micro)
Non-constitutive mixotrophs (NCMs)	Need to acquire phototrophic potential from their phototrophic prey and are obligate mixotrophs	specialist (SNCMs) and generalist (GNCMs) forms
Generalist non-constitutive mixotrophs (GNCMs)	NCMs that obtain their phototrophic machinery from diverse phototrophic prey and have poor control over these	oligotrich ciliates
Specialist non-constitutive mixotrophs (SNCMs)	NCMs that obtain their phototrophic machinery from specific phototrophic prey and have high control over these	e.g. <i>Mesodinium rubrum</i>
Strict autotrophic competitors	Strictly autotrophic protist plankton; within the model structure these compete with mixotrophs for light and nutrients	Picophytoplankton (PicoP) and diatoms
Strict heterotrophic competitors	Strictly heterotrophic protist plankton; within the model structure these compete with mixotrophs for prey	Heterotrophic nanoflagellates (HNF) and heterotrophic microzooplankton (mZ)

809 **Table II** Results of the Monte-Carlo sensitivity analyses for three targeted model outputs in the  
 810 mixotrophic food web within the light-limited scenario (sensitivity coefficients of all parameters  
 811 were statistically significant at  $p < 0.001$  and  $R^2 > 0.9$ ). These are ranked (most important first) with  
 812 respect to their absolute value. Coefficient signs indicate a positive or negative effect on the  
 813 targeted model outputs, i.e. increase or decrease of the output values, respectively. DOC – dissolved  
 814 organic carbon and Mesozoo – mesozooplankton; for other abbreviations please refer to Table I and  
 815 for parameter description refer to Tables S2, S4, and S5

Targeted output	Functional type	Parameter	Coefficient
Ammonium regeneration	PicoP	$\alpha_{\text{Chl}}$	0.34
	CM-nano	$\text{NC}_{\text{max}}$	-0.27
	diatoms	$\alpha_{\text{Chl}}$	0.27
	diatoms	$\text{ChlC}_{\text{abs}}$	0.27
	PicoP	$\text{ChlC}_{\text{abs}}$	0.24
	CM-micro	$\text{NC}_{\text{max}}$	-0.21
	SNCMs	$\text{NC}_{\text{max}}$	-0.18
	bacteria	$\text{NC}_{\text{max}}$	0.17
Trophic transfer efficiency	CM-nano	$S_{\text{opt}}$	-0.31
	diatoms	$\alpha_{\text{Chl}}$	0.27
	diatoms	$\text{ChlC}_{\text{abs}}$	0.22
	CM-nano	$\text{ChlC}_{\text{abs}}$	0.20
	Mesozoo	$\text{Cr}_{\text{mesozoo}}$	-0.19
	PicoP	$\text{ChlC}_{\text{abs}}$	-0.19
	Mesozoo	$\text{Cr}_{\text{CM-micro}}$	0.17
	CM-nano	$\alpha_{\text{Chl}}$	0.17
Production of labile DOC	diatoms	$\alpha_{\text{Chl}}$	0.50
	diatoms	$\text{ChlC}_{\text{abs}}$	0.40
	PicoP	$\alpha_{\text{Chl}}$	0.31
	PicoP	$\text{ChlC}_{\text{abs}}$	0.16
	CM-nano	$\alpha_{\text{Chl}}$	0.15
	CM-nano	$\text{ChlC}_{\text{abs}}$	0.12
	CM-nano	$S_{\text{opt}}$	0.11
	diatoms	BR	-0.09

$\alpha_{\text{chl}}$ , initial slope of photosynthesis-irradiance curve; BR, basal respiration rate;  
 $\text{ChlC}_{\text{abs}}$ , absolute maximum Chl:C ratio; Cr: slope of capture-prey abundance  
 curve;  $\text{NC}_{\text{max}}$ , maximum N:C ratio;  $S_{\text{opt}}$ : optimum prey size.

816

817

818 **Table III** Results of the Monte-Carlo sensitivity analyses for three targeted model outputs in the  
 819 mixotrophic food web within the nutrient-limited scenario (sensitivity coefficients of all parameters  
 820 were statistically significant at  $p < 0.001$  and  $R^2 > 0.9$ ). These are ranked (most important first) with  
 821 respect to their absolute value. Coefficient signs indicate a positive or negative effect on the  
 822 targeted model outputs, i.e. increase or decrease of the output values, respectively. DOC – dissolved  
 823 organic carbon and Mesozoo – mesozooplankton; for other abbreviations please refer to Table I and  
 824 for parameter description refer to Tables S2, S4, and S5

Targeted output	Functional type	Parameter	Coefficient
Ammonium regeneration	CM-nano	$S_{max}$	-0.46
	CM-nano	$NC_{max}$	-0.20
	bacteria	$NC_{max}$	0.14
	GNCMs	$S_{max}$	0.11
	CM-nano	$\mu_{phot}$	0.10
	CM-nano	$S_{opt}$	-0.10
	SNCMs	$S_{max}$	0.09
	SNCMs	$PC_{max}$	0.06
Trophic transfer efficiency	CM-nano	$S_{opt}$	-0.42
	CM-nano	$S_{max}$	-0.29
	Mesozoo	$Cr_{GNCMs}$	0.21
	GNCMs	$S_{max}$	-0.21
	SNCMs	$S_{max}$	-0.16
	CM-nano	$S_{min}$	-0.13
	PicoP	$\mu_{phot}$	-0.12
	bacteria	$NC_{max}$	0.11
Production of labile DOC	CM-nano	$\mu_{phot}$	0.33
	SNCMs	$S_{max}$	0.32
	CM-nano	$NC_{min}$	-0.31
	bacteria	$NC_{max}$	-0.30
	GNCMs	$S_{max}$	0.25
	CM-nano	$NC_{max}$	-0.20
	GNCMs	$PC_{max}$	0.19
	bacteria	$PC_{max}$	0.17

Cr: slope of capture-prey abundance curve;  $NC_{max}$ , maximum N:C ratio;  $NC_{min}$ , minimum N:C ratio;  $\mu_{phot}$ , maximum phototrophic growth rate;  $PC_{max}$ , maximum P:C ratio;  $S_{max}$ , maximum prey size;  $S_{min}$ , minimum prey size;  $S_{opt}$ : optimum prey size.

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826