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Introducing mixotrophy into a biogeochemical model describing an eutrophied coastal ecosystem: The Southern North Sea

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Running page head: Mixotrophy in a biogeochemical model

Abstract:

Most biogeochemical/ecological models divide planktonic protists between phototrophs (phytoplankton) and heterotrophs (zooplankton). However, a large number of planktonic protists are able to combine several mechanisms of carbon and nutrient acquisition. Not representing these multiple mechanisms in biogeochemical/ecological models describing eutrophied coastal ecosystems can potentially lead to different conclusions regarding ecosystem functioning, especially regarding the success of harmful algae, which are often reported as mixotrophic. This modelling study investigates the implications for trophic dynamics of including 3 contrasting forms of mixotrophy, namely osmotrophy (using alkaline phosphatase activity, APA), non-constitutive mixotrophy (acquired phototrophy by microzooplankton) and also constitutive mixotrophy. The application is in the Southern North Sea, an ecosystem that faced, between 1985 and 2005, a significant increase in the nutrient supply N:P ratio (from 31 to 81 mole N:P). The comparison with a traditional model shows that, when the winter N:P ratio in the Southern North Sea is above 22 molN molP^{-1} (as occurred from mid-1990s), APA allows a 3 to 32% increase of annual gross primary production (GPP). In result of the higher GPP, the annual sedimentation increases as well as the bacterial production. By contrast, APA does not affect the export of matter to higher trophic levels because the increased GPP is mainly due to *Phaeocystis* colonies, which are not grazed by copepods. Under high irradiance, non-constitutive mixotrophy appreciably increases annual GPP, transfer to higher trophic levels, sedimentation, and nutrient remineralisation. In this ecosystem, non-constitutive mixotrophy is also observed to have an indirect stimulating effect on diatoms. Constitutive mixotrophy in nanoflagellates appears to have little influence on this ecosystem functioning. An important conclusion from this work is that contrasting forms of mixotrophy have different impacts on system dynamics and, due to the complex interactions in the ecosystem, their combined effect is not exactly the addition of the effects individually observed. It is thus important to describe such contrasting forms in an appropriate fashion.

(314 words)

1. Introduction

Since the second half of the 20th century, nitrogen (N) and phosphorus (P) river loads to coastal marine systems have substantially increased worldwide (ca. 18% N and 13% P between 1970 and 2000; Seitzinger et al. 2010) due to land use change, population growth, economic development, and the expansion of the crop/animal production systems. As a result, most of these coastal ecosystems show symptoms of eutrophication: increase of primary production and algal biomass (Smayda, 2008), leading to high ratios of organic to inorganic nutrients (GEOHAB, 2006). As N loading is often disproportionate relative to P and silicon (Si), coastal ecosystems are also generally nutrient unbalanced compared to phytoplankton requirements (Glibert and Burkholder, 2006).

In these conditions, the ability of some planktonic protists to acquire nutrients and carbon from several sources can be very advantageous. Mixotrophy, i.e., the ability to combine phototrophy and heterotrophy in one organism, is now recognized to be widespread among planktonic protists. Mixotrophs are frequently reported in oligotrophic systems (Bird and Kalff, 1987; Arenovski et al., 1995; Zubkov and Tarran, 2008; Hartmann et al., 2012) and several reports also highlight their significance in eutrophied ecosystems where nutrient and/or light limitation often occurs due to nutrient loads imbalance or associated with extended periods of stratification (Nygaard and Tobiesen, 1993; Havskum and Riemann, 1996; Burkholder et al., 2008). In particular, it has been shown that most harmful algal blooms (HABs) in eutrophied coastal ecosystems are composed of mixotrophic species (Stoecker et al., 2006; Burkholder et al., 2008; Jeong et al., 2010). A wide diversity exists among mixotrophs and several classifications have been proposed. Jones (1997) and Stoecker (1998) suggested classifications aligning with the dominant trophic mode (phototrophy or heterotrophy) and the primary nutrient limitation (carbon, nutrient or other essential substances). More recently, Mitra et al. (2016) have proposed a classification according to the mixotroph functional types. In their classification, the first criterion of distinction between mixotrophs is based on the origin of their chloroplasts: either constitutive (constitutive mixotrophs; CMs) or acquired from ingested phototrophic prey (non-constitutive mixotrophs; NCMs) (see Mitra et al., 2016 for further details on mixotroph classification).

While the term "mixotroph" may be reserved for protists combining phototrophy and phagotrophy (as discussed in Flynn et al., 2013), all phototrophic protists may be recognised as mixotrophs by virtue of an ability to acquire nutrients from dissolved organic matter by osmotrophy (Glibert and Legrand, 2006; Burkholder et al., 2008). Some dissolved organic nutrients can be directly taken up (e.g., urea, amino acids) while others need to be oxidised (e.g., combined amino acids, proteins) or hydrolysed (e.g., dissolved organic phosphate,

DOP) outside the cell (Palenik and Morel, 1990; Glibert and Legrand, 2006). The hydrolysis of DOP by extracellular enzymes is an ecologically important mechanism of P acquisition by phototrophic protists (Kuenzler and Perras, 1965; Cembella et al., 1984; Flynn et al., 1986; Hoppe; 2003). Under phosphate (PO_4^{3-}) limitation, many marine phototrophic protists are indeed able to express alkaline phosphatase activity (APA). Just as mixotrophy (phototrophy + phagotrophy) may support harmful species (e.g., *Karenia brevis*, *Prymnesium parvum*, among many others; Burkholder et al., 2008) in developing HABs, APA is suspected to aid the growth of massive blooms of *Phaeocystis globosa* colonies when phosphate is limiting in the Southern North Sea (Veldhuis et al., 1987; Rousseau et al., 2004).

To describe the functioning of eutrophied coastal ecosystems in a more realistic way (in particular, regarding the development and control of HABs), it is important that ecological/biogeochemical models take account of the various mechanisms of nutrient acquisition deployed by planktonic protists. This is, however, hardly ever the case; traditionally, "phytoplankton" are only described as using inorganic nutrients and performing photosynthesis, while "microzooplankton" only engage in heterotrophy.

In this paper, we explore how the 3 contrasting forms of mixotrophy (i.e., osmotrophy using APA, constitutive mixotrophy and non-constitutive mixotrophy) affect the functioning of the planktonic ecosystem of the Belgian coastal zone (BCZ) located in the Southern North Sea by introducing these mechanisms of nutrient/carbon acquisition in the MIRO ecosystem model of Lancelot et al. (2005). This coastal ecosystem is subjected to direct and indirect inputs of anthropogenic nutrients discharged by rivers with watersheds characterized by high human population density and intense agricultural and industrial activities (Billen et al., 2005). However, after the introduction of nutrient control policies in the late 1980s, that were more successful in decreasing P inputs than N inputs, the annual molar N:P ratio of river nutrient inputs increased from 31 to 81 between 1985 and 2005 (e.g., Brion et al., 2006; Passy et al., 2013). Such high molar N:P ratio of nutrient inputs may lead to conditions of P-stress (Flynn, 2010). Such P-stress is known to promote the synthesis of high-N toxins, such as paralytic shellfish toxins (John and Flynn, 2002), and also excess production of fixed carbon that may support bacterial growth or amass as mucilage. Properly simulating the ecophysiology of the plankton under such conditions is essential, else predictive capability might be lost (Flynn, 2005).

2. Method

2.1. Model description

The base of model used for this work develops from the MIRO model, which describes carbon (C), N, P and Si cycling through constituents of the English Channel and the Southern North Sea, over seasons and years, in response to physical and nutrient forcing (Lancelot et al., 2005). MIRO was adapted to consider the 3 forms of mixotrophy (osmotrophy using APA, constitutive mixotrophy and non-constitutive mixotrophy). The revised model describes the dynamics of bacteria, planktonic protists (four groups), copepods, organic matter degradation, and inorganic nutrient (nitrate, NO_3^-; ammonium, NH_4^+; PO_4^{3-}; and dissolved silica, DSi) regeneration in the water column and the sediment (Fig. 1).
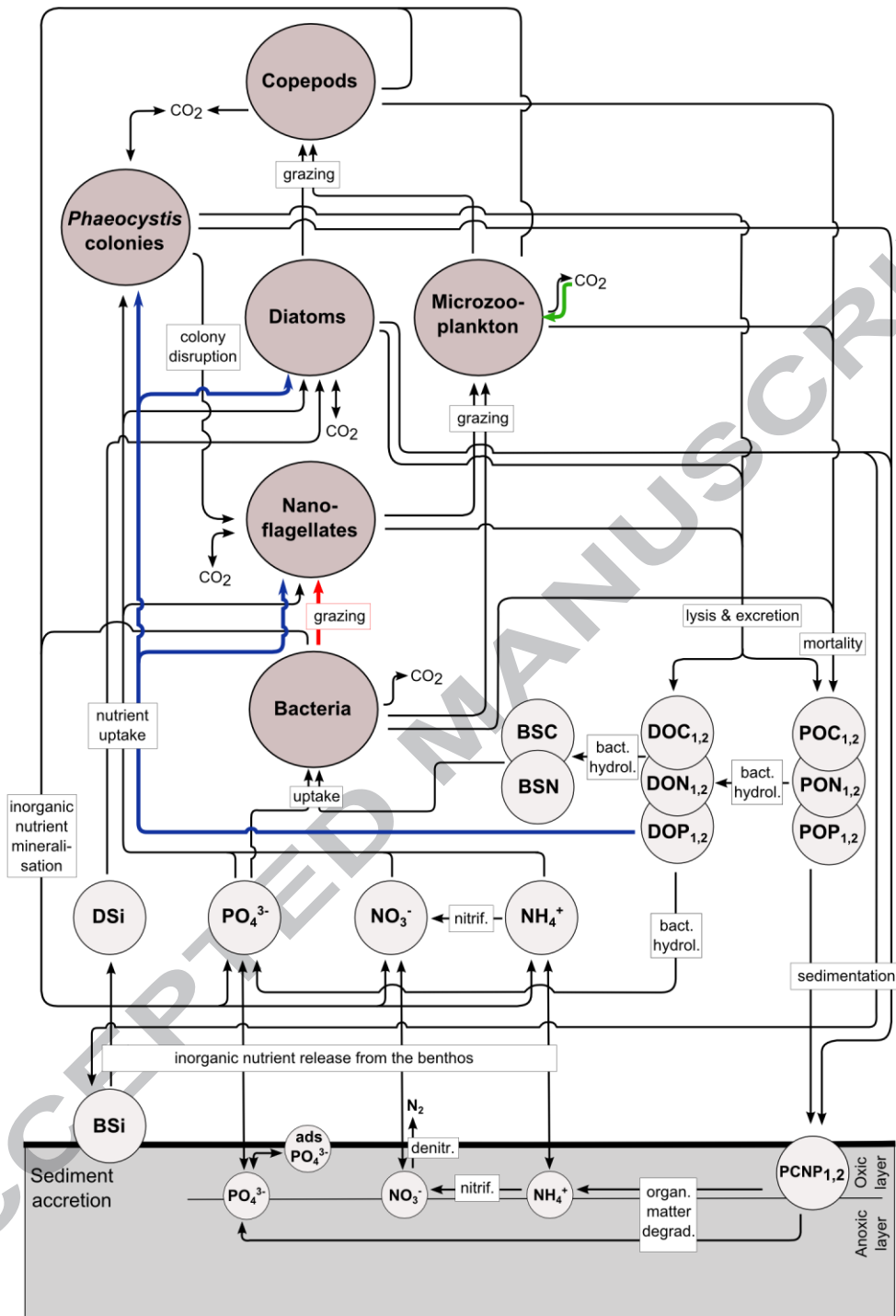
Figure 1: Structure of the modified MIRO model taking into account (i) the ability of the three phytoplanktonic groups (nanoflagellates, diatoms, *Phaeocystis* colonies) to use dissolved organic phosphorus (DOP) by alkaline phosphatase activity (APA) when PO_4^{3-} is limiting (blue arrows), (ii) constitutive mixotrophy in nanoflagellates (red arrow), and (iii) non-constitutive mixotrophy in microzooplankton (green arrow). Photosynthesis in microzooplankton is allowed by plastids acquired from nanoflagellates.

The four functional groups of planktonic protists are: diatoms, *Phaeocystis* colonies, nanoflagellates, and microzooplankton. The three first are considered as primarily phototrophs. Their growth is described by an adaptation of the AQUAPHY model (Lancelot et al., 1991; Ghyoot et al., 2015), which includes five state variables describing intracellular components chosen on the basis of their function: F^{phot}, the functional and structural metabolites (e.g., DNA, RNA, proteins) related to phototrophy; S_C, carbon monomers (i.e., early products of photosynthesis); R_C, carbon reserves (i.e., carbohydrates, fatty acids); S_P, soluble phosphate; and S_N, soluble inorganic nitrogen. For *Phaeocystis* colonies, a 6th state variable is added to describe the mucus (M) surrounding colony cells. The total C-cell biomass in each functional group (mgC m^{-3}) is given by $F^{phot} + S_C + R_C$. The component F^{phot} is assumed to have a fixed C:N:P stoichiometry but variable cellular stoichiometry in the whole organism is enabled by considering the additional C, P and N accumulated as carbon monomers (S_C), carbon reserves (R_C), soluble inorganic phosphorus (S_P) and inorganic nitrogen (S_N) reserves.

Diatoms, *Phaeocystis* colonies and nanoflagellates are able to express APA for using DOP when PO_4^{3-} is limiting (Kuenzler and Perras, 1965; Flynn et al., 1986; van Boekel and Veldhuis, 1990). The model describes two classes of DOP differing with respect to their biodegradability (Lancelot et al., 2005): the rapidly biodegraded (DOP_1) and the slowly biodegraded (DOP_2). The APA is implicitly considered by allowing the direct uptake of $DOP_{1,2}$ when PO_4^{3-} becomes limiting (Ghyoot et al., 2015). The amount of $DOP_{1,2}$ that is taken up is controlled by an hyperbolic function depending on the external PO_4^{3-} concentration, so that $DOP_{1,2}$ uptake is at its maximum when PO_4^{3-} is depleted and $DOP_{1,2}$ uptake is zero when PO_4^{3-} is abundant (details in Ghyoot et al., 2015).

Among the three functional groups that would traditionally be labelled as "phytoplankton", phagotrophy has only been reported for nanoflagellates (Sanders and Porter, 1988; Havskum and Riemann, 1996). Nanoflagellates are here assumed to be constitutive mixotrophs (CMs); that is their ability to perform photosynthesis is constitutive (Mitra et al. 2016). In this instance phototrophy is considered as obligatory (i.e., a minimum level of phototrophy is required to enable cell growth) but phagotrophy upon bacteria is possible and is triggered by nutrient and/or light limitation. Structural and functional metabolites related to these heterotrophic processes are described by an additional state variable (F^{het}; according to the description of constitutive mixotrophy given in Ghyoot et al. (2017)).

The last group of planktonic protists considered by the model is the microzooplankton. These are described as primarily phago-heterotrophs (feeding on bacteria and nanoflagellates) but are also able to photosynthesize using chloroplasts acquired from

ingested nanoflagellates. In this capacity they conform to the description of being non-constitutive mixotrophs (NCMs). They are assumed to be generalist NCMs, in that they can acquire chloroplasts from a broad range of phototrophic species rather than from specific prey (as do specialists NCMs; Mitra et al., 2016). Our assumption here is that these generalist NCMs do not require a minimum level of phototrophy to grow and are unable to take up inorganic nutrients (the inorganic nutrients regenerated through heterotrophic activity are, in this model, directly recycled for phototrophic growth). This form of mixotrophy is described by the same state variables as for the mixotrophic nanoflagellates (i.e., F^{phot}, F^{het}, S_C, R_C, S_N, S_P) but the processes describing state variable dynamics differ. Here, F^{phot} is exclusively supplied by the grazing on nanoflagellates. As long as F^{phot} and light are available, NCMs have phototrophic growth; this operates as a function of F^{phot}, S_C limitation (depending on light limitation) and S_N/S_P limitation (nutrient limitation). The phototrophic components acquired by NCMs are continuously digested at a constant rate; a fraction of this digested material is used to synthesize new F^{het} (heterotrophic growth) while the other fraction is egested or respired (see Ghyoot et al. (2017) for full description of NCMs dynamics).

Copepods are represented by a simplified description (one state variable). They feed on diatoms and on mixotrophic microzooplankton, and they excrete compounds with varying stoichiometry in order to maintain a constant body C:N:P. Second-order mortality (to account for natural mortality and also predation by higher trophic levels) is described in what constitutes the MIRO closure term.

2.2. Model parameterisation

Model parameters that are not related to mixotrophy and APA processes were taken from Lancelot et al. (2005), while parameters related to APA were those detailed in Ghyoot et al. (2015). As physiological parameters related to mixotrophy are in very short supply in the literature, we performed a sensitivity analysis to identify parameters to which the model was most sensitive and then, we tuned those parameters values to optimise model output against observations collected between 1989 and 1999 at Station 330: 51°26.05 N; 002°48.50 E in the Southern North Sea. The field dataset included biomass of bacteria, nanoflagellates, diatoms, microzooplankton, *Phaeocystis* colonies, and concentration of NO_3^-, NH_4^+, PO_4^{3-} and DSi (Lancelot et al., 2005).

We used the "Differential Evolution" algorithm (Storn and Price, 1997) as a search method, with the three control variables of this algorithm set according to the recommendations given in Storn and Price (1997) to speed up the tuning process (i.e., the size of the population = 5-7 times the number of parameters to tuned; weighting factor = 0.5; crossover constant =

0.9). The objective function used to decide if a parameter vector is better than another was the maximum log-likelihood, assuming a normal distribution of model-observations residuals (Terseleer et al., 2014).

2.3. Dynamic sensitivity analysis

To study to what extent the parameter values affect the model results (and more specifically, the conclusion regarding the importance of considering mixotrophy in a biogeochemical model), we conducted a global dynamic sensitivity analysis using the method described in Mitra et al. (2014b). The model was run 500 times with all physiological parameters varying randomly, assuming a normal distribution around their mean value (Appendix A). In the absence of information to indicate the true variability of parameter values, we assumed a standard deviation of 10% to the mean value. The random parameter values were generated by using the Latin Hypercube sampling method from the experimental design package for python (pyDOE).

2.4. Model runs

The model was implemented in a multi-box framework constructed to accord with the hydrological regime (as in Lancelot et al., 2005). The Southern North Sea was divided into three boxes: the 'oceanic' Western Channel (WCH), the French coastal zone (FCZ), and the Belgium Coastal Zone (BCZ). Each box is assumed to be homogeneous and has its own characteristics (see Fig.1 and Table 1 in Lancelot et al., 2005). Each box receives waters from the South-West adjacent box and exports waters to the North-East adjacent box (taking account of the cumulated nutrient enrichment of Atlantic waters by the Seine and Scheldt rivers). The model is therefore run successively in the WCH, the FCZ (receiving the Seine loads and Atlantic waters from the WCH), and the BCZ (receiving the Scheldt loads and Atlantic waters from the FCZ).

Two types of simulations were performed on the basis of their forcings. The first simulation period described a climatological average between 1989 and 1999, and was obtained with climatological forcing corresponding to the same period (nutrient river loads, incident light and temperature; see details in Lancelot et al., 2005). The second simulation described ecosystem evolution between 1985 and 2005 and was, therefore, obtained with observed forcing's for each year (Gypens et al., 2007). For both simulation-types, we performed five different runs corresponding to different versions of the model: (i) the reference model (i.e., without APA and mixotrophy), (ii) the model with APA, (iii) the model with constitutive mixotrophy operated by nanoflagellates, (iv) the model with non-constitutive mixotrophy operated by microzooplankton, and (v) the full model (i.e., including APA and the two forms

of mixotrophy). Hereafter, the different model versions are named respectively: $MIRO_{ref}$, $MIRO_{APA}$, $MIRO_{CM}$, $MIRO_{NCM}$, and $MIRO_{mixo}$.

3. Results

3.1. Effect of mixotrophy on plankton and nutrient climatology dynamics

Figure 2 compares the simulated climatological seasonal cycle of plankton and nutrients in the BCZ obtained with $MIRO_{ref}$ and $MIRO_{mixo}$ (average for the period 1989-1999) with relevant observations reported for the BCZ.
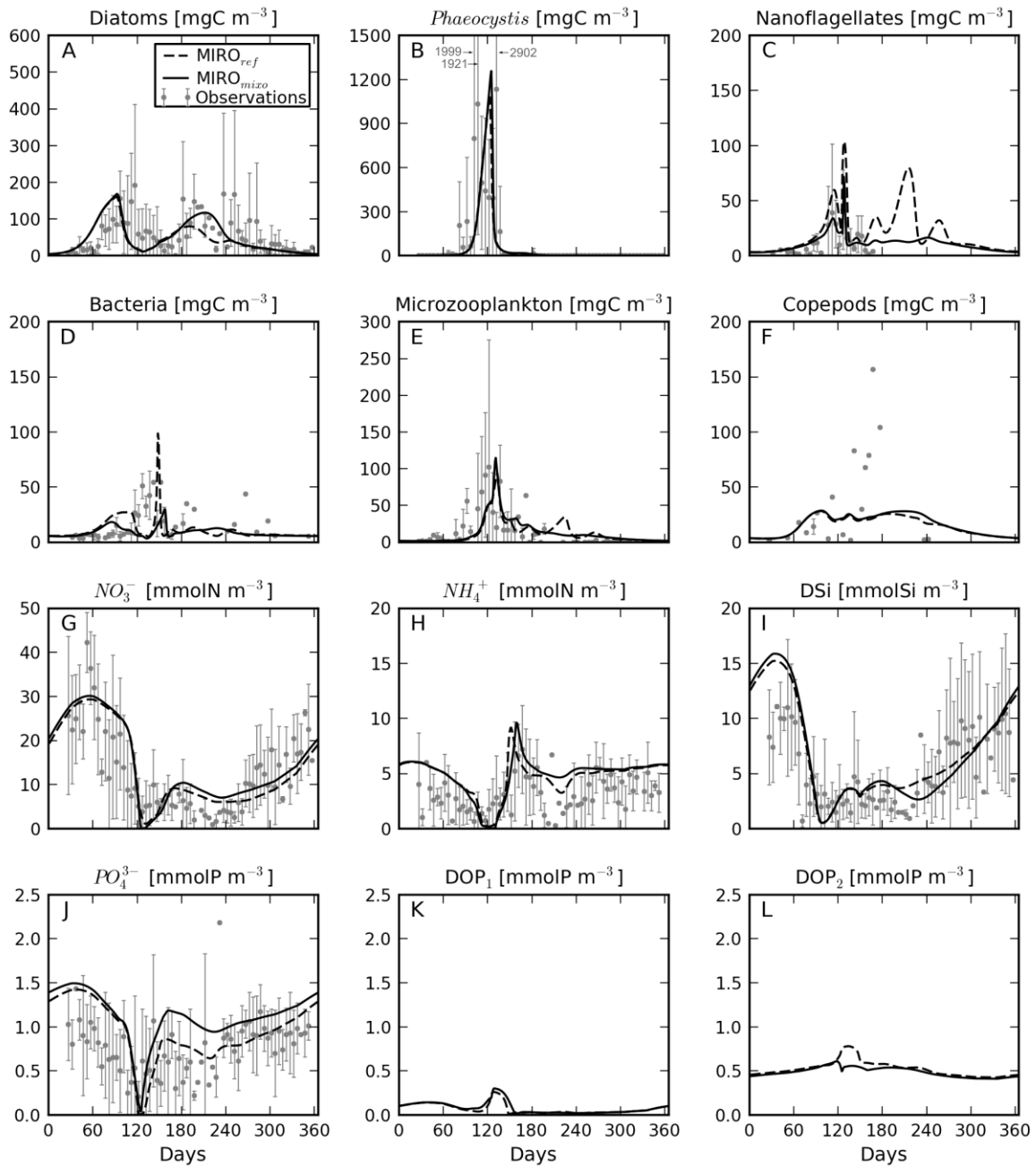
Figure 2: Seasonal planktonic and nutrient concentrations in the Belgian coastal zone (BCZ) simulated with MIRO_ref (dashed line) and MIRO_mixo (continuous line). The year described here is a climatological average corresponding to the period 1989-1999 (with climatological physical and nutrient forcing). MIRO_mixo includes (i) APA ability for nanoflagellates, diatoms, and *Phaeocystis* colonies, (ii) constitutive mixotrophy in nanoflagellates, and (iii) non-constitutive mixotrophy in the microzooplankton. MIRO_ref does not consider any of these mechanisms of nutrient acquisition. See Fig. 1 for model schematic. Results are compared to 1989-1999 observations (5 days mean ± SD; see Lancelot et al. 2005).

Overall, both simulations reproduce the main features of the plankton dynamics observed in the area, although some noticeable differences between the two simulations can be seen.

Both models simulate the massive spring bloom of *Phaeocystis* colonies (Fig. 2B) as well as the early spring and summer diatom blooms (with however a slight phase shift when compared to the observations; Fig. 2A). However, the maximum biomass reached by *Phaeocystis* colonies and summer diatoms are higher with $MIRO_{mixo}$ than with $MIRO_{ref}$ by 17% and 11% respectively; the performance of $MIRO_{mixo}$ is more consistent with the observations (Fig. 2A,B)). Regarding nanoflagellates, both models simulate a small bloom in spring, which agrees with the available observations (Fig. 2C). $MIRO_{ref}$ simulates generally higher nanoflagellates biomass than $MIRO_{mixo}$ and also simulates a second bloom in summer, which cannot be confirmed with observations. Bacteria growth is simulated to develop after that of *Phaeocystis* colonies, sustained by the large release of organic matter (Fig. 2D). $MIRO_{ref}$ simulates two peaks of bacteria growth: a first peak in early spring (which is not consistent with the observations) and a high narrow peak that overestimates the observed bacterial biomass. With $MIRO_{mixo}$, the early spring and summer peaks are simulated but with significantly lower amplitudes that are more consistent with the observations, although noticeably phase shifted. Both models simulate the increase of microzooplankton when nanoflagellates and bacteria are growing (Fig. 2E) with, however, a short delay compared to observations. Finally, the annual evolution of copepods follows closely those of diatoms and microzooplankton, but the two simulations underestimate the observed biomass (Fig. 2F).

Regarding nutrient dynamics, the two models described the nutrient consumption related to phototrophic growth and the nutrient remineralisation related to heterotrophic activity by bacteria, microzooplankton, and copepods (Fig. 2G-J). As $MIRO_{mixo}$ described higher summer nutrient concentrations than did $MIRO_{ref}$, these values were higher than those observed (except for DSi; Fig. 2I). In spring, the higher PO_4^{3-} concentration simulated with $MIRO_{mixo}$ is balanced by a lower DOP_2 concentration (Fig. 2L).

On the basis of this climatological seasonal cycle, it can thus be seen that considering mixotrophy in the model does not resolve all the discrepancies between simulations and observations (e.g., the phase shift for diatoms, bacteria, and microzooplankton, and the underestimation of copepods biomass) but it resolves some of them, i.e., the underestimation of diatoms biomass in summer, the overestimation of nanoflagellates biomass in summer, and the low consumption of DSi in summer.

3.2. Significance of APA and mixotrophy in a period of decreasing P availability (1985-2005)

Figure 3 shows the annual gross primary production (GPP; i.e., the annual C-fixation by photosynthesis; gC m^{-2} yr^{-1}) simulated with $MIRO_{ref}$ over the period ranging from 1985 to

2005 (Fig. 3A) and the relative change in GPP induced by inclusion of APA (Fig. 3B), inclusion of constitutive mixotrophy in nanoflagellates (Fig. 3C), inclusion of non-constitutive mixotrophy in the microzooplankton (Fig. 3D), and by inclusion of all three mixotrophic mechanisms considered together (Fig. 3E).
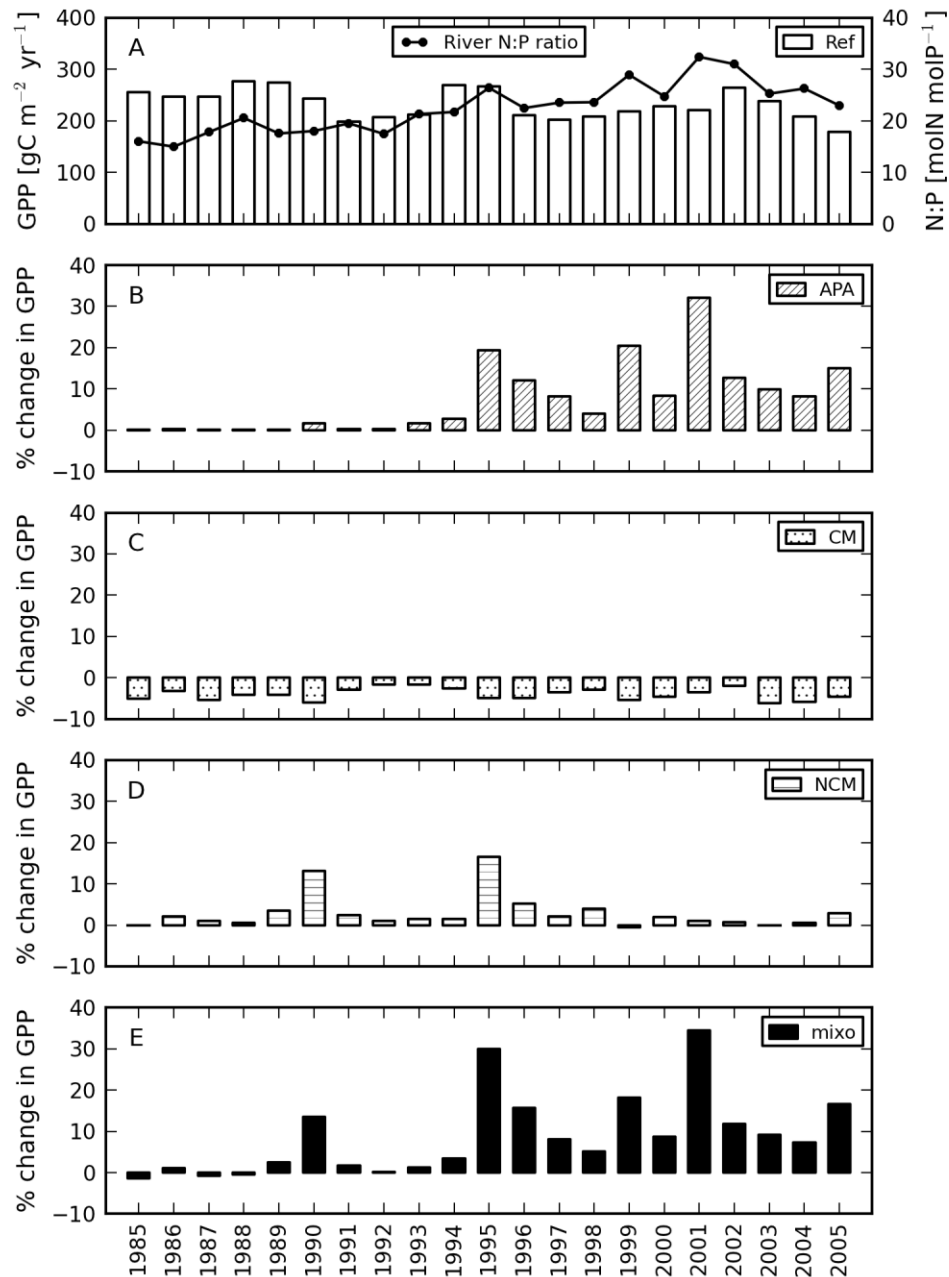
Figure 3: Annual areal gross primary production (GPP; gC m^{-2} yr^{-1}) obtained with the reference simulation (MIRO$_{ref}$) over the period ranging from 1985 and 2005 (A; bars) and, the percentage difference from that reference induced by explicit inclusion of APA (B), of constitutive mixotrophy in nanoflagellates (C), of non-constitutive mixotrophy in the microzooplankton (D), or of the combination of all these three mixotrophic mechanisms (D). The winter molar N:P ratio of inorganic nutrients simulated in the Belgian coastal zone (BCZ) with MIRO$_{ref}$ is also shown (A; dots).

The annual GPP simulated with $MIRO_{ref}$ varies between years (Fig. 3A), driven by external forcing, i.e., river nutrient loads, incident light, and seawater temperature (as shown in Gypens et al., 2007). The impact of including APA on the annual GPP shows a clear temporal trend which compares well with the observed increase of winter N:P ratio of inorganic nutrients (the winter N:P ratio being an indicator of the N:P ratio in the initial stock of nutrients available for phototrophic growth) in the BCZ (Fig. 3A,B). By providing an additional source of P via the cleavage of DOP, APA allows higher GPP from the mid-1990s, i.e., when the winter N:P ratio is such that phototrophic protists become PO_4^{3-} limited in spring (not shown). By contrast, enabling constitutive mixotrophy in nanoflagellates results in a slight decrease (< 7%) of the annual GPP (Fig. 3C) with little variation between years. The increasing N:P ratio does not influence the amplitude of the effect of constitutive mixotrophy on the annual GPP because, compared to APA which responds only to PO_4^{3-} limitation, constitutive mixotrophy can also be stimulated by a lack of C (i.e., of light for photosynthesis) and/or by dissolved inorganic nitrogen (DIN) limitation. Analysis of seasonal evolution in the simulations shows that, in winter, nanoflagellates use phagotrophy to support C acquisition, but only at a very low level (heterotrophic growth only contributes between 1 and 2% to the total growth). By contrast, in spring, nanoflagellates use phagotrophy to support nutrient (N or P) acquisition; in that case, nutrients from bacteria contribute between 11 and 22% to the total nutrient assimilation. Simulations also show that, before 1990, spring phagotrophy is always induced by a DIN limitation while, from 1993, PO_4^{3-} limitation is the triggering factor (not shown).

Allowing expression of non-constitutive mixotrophy in microzooplankton has little effect (< 6%) on GPP except for some years (1990: 13%; 1995: 17%; Fig. 3D). In those exception years the average incident light at the period of microzooplankton growth (between day 100 and day 240) is high (respectively 6 and 14% higher than the average light between day 100 and day 240 computed from the 21 years between 1985 and 2005).

When all mechanisms of nutrient acquisition are taken into account, the combined effects are mainly triggered by APA and result in an increase of the annual GPP from 1990 and, more systematically, from 1995 (Fig. 3E) of the order of 10-30%. In order to understand how the contrasting mechanisms of nutrient acquisition affect the functioning of the coastal ecosystem submitted to river inputs of increasing N:P, we compare the independent effects of APA, constitutive or non-constitutive mixotrophy on the annual biological activities for two years, i.e., 1985 and 1995. These two years have been chosen as they reflect the qualitative trend for the studied period (averaged values for the 10 year periods) and display the most contrasting effects on the ecosystem GPP (Fig. 3). The biological activities described here

(Fig. 4) are: GPP, copepod grazing (which represents the C transfer to higher trophic levels), NH_4^+ and PO_4^{3-} remineralisation by all heterotrophs, and C sedimentation.
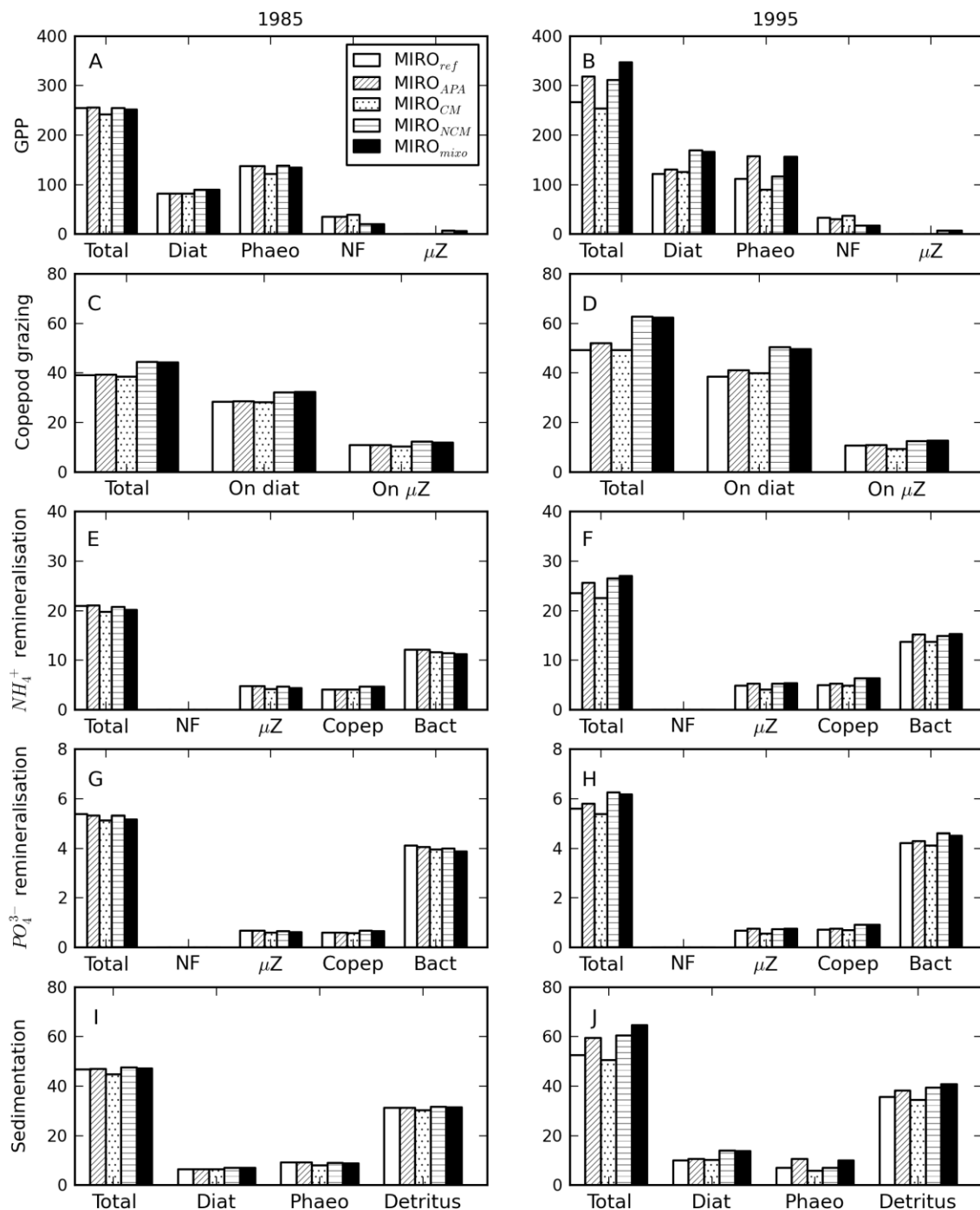
Figure 4: Comparison of the annual fluxes obtained with the five different model versions between two contrasting years of the period ranging from 1985 to 2005: 1985 (left column) and 1995 (right column). The annual areal fluxes investigated are: (A-B) gross primary production (GPP; i.e., C fixation by photosynthesis; gC m^{-2} yr^{-1}), (C-D) copepod grazing (gC m^{-2} yr^{-1}), (E-F) NH$_4^+$ remineralisation (gN m^{-2} yr^{-1}); (G-H) PO$_4^{3-}$ remineralisation (gP m^{-2} yr^{-1}); and sedimentation (gC m^{-2} yr^{-1}). The key to different model forms in shown in panel A. See also the schematic in Fig. 1.

APA has little effect on the different annual biological activities in 1985 (Fig. 4; left panels). In contrast, in 1995 (Fig. 4; right panels) APA allows *Phaeocystis* and summer diatoms to increase their annual GPP by respectively 41% and 7 % (Fig. 4B) and this explains the simulated 20% increase of the total annual GPP (Fig. 2B). In consequence of higher *Phaeocystis* biomass accumulation (and diatoms biomass to a lesser extent), the total C sedimentation increases by 13% (Fig. 4J). Enabling expression of APA also increases, by 20%, the bacterial production following the bloom of *Phaeocystis* (not shown), which results in a higher NH_4^+ remineralisation (9%) by mainly bacteria, but also by the microzooplankton and copepods (Fig. 4F). The PO_4^{3-} remineralisation is also higher (4%) but the contribution of bacteria is less than for NH_4^+ remineralisation because the $DOP_{1,2}$ used by *Phaeocystis* colonies and summer diatoms is then less available for bacterial hydrolysis (Fig. 4H). Despite the 20% increase of total GPP, the copepod grazing only increases by 6% because the model assumes that *Phaeocystis* colonies are too large to be grazed by copepods, and the diatoms and the microzooplankton are little affected by APA (Fig. 4D).

Adding constitutive mixotrophy in nanoflagellates has little effect on biological activities whatever the year (Fig. 4; left vs right panels). When nanoflagellates are able to feed on bacteria, their GPP increases by 10-13% but in consequence the GPP of *Phaeocystis* colonies then also decreases by 12-19% because of the increased competition for nutrients between these phototrophic groups (Fig. 4A,B). In total, this results in a 5% decrease of the annual GPP (Fig. 3C) and a 4% decrease of the C sedimentation (Fig. 4I, J). When nanoflagellates are mixotrophs, a competition also takes place between microzooplankton and nanoflagellates for bacterial prey. This induces a decrease of the total microzooplankton grazing (by 9-16%) and the microzooplankton consumes proportionally more nanoflagellates than bacteria. As the C:N and C:P of nanoflagellates are higher than those of bacteria, the nutrient remineralisation by the microzooplankton decreases by 12-17% (Fig. 4E, F, G, H). Bacteria, being ingested by both microzooplankton and nanoflagellates, release less nutrients by remineralisation. Mixotrophic nanoflagellates, using the regenerated nutrients for their phototrophic growth, never contribute significantly to the total nutrient remineralisation in this ecosystem. The resulting total nutrient remineralisation rate decreases thus by 4-6%.

As shown in Fig. 3, the effect of non-constitutive mixotrophy in the microzooplankton is variable between years and apparently not affected by the N:P increase over the period 1985-2005. The difference observed between 1985 and 1995 is the maximum difference observed over the whole period and appears to be related to the different light intensities observed between day 100 and day 240 (corresponding to period of microzooplankton development) for the two years; the average irradiance was 17% higher in 1995 than in 1985. It allows the mixotrophic microzooplankton to increase photosynthesis by 6% thanks

to the chloroplasts of the ingested nanoflagellates. In addition, due to the close interactions between phototrophic and heterotrophic activities within mixotrophs, the grazing on bacteria and nanoflagellates is also increased (by 9%) compared to that simulated in 1985. As a result of the higher grazing rate, the nutrient remineralisation by the mixotrophic microzooplankton is ~12% higher in 1995 than in 1985 (Fig. 4E, F, G, H) and allows the summer diatoms to increase their GPP by 17% (Fig. 4B) and to reach a higher maximum biomass (cf. Fig. 2A). This higher diatom production has a stimulating effect on several biological activities in the ecosystem: (i) copepod grazing increases by 27% (Fig. 4D) and, therefore, the nutrient remineralisation by copepods also increases (Fig. 4F, H), (ii) C sedimentation increases by 15% (Fig. 4J), and (ii) the organic matter released by diatoms lysis support a higher bacterial activity, leading to higher nutrient remineralisation (Fig. 4F, H).

The combination of the 3 forms of mixotrophy (osmotrophy using APA, constitutive mixotrophy and non-constitutive mixotrophy) has a stimulating effect on all biological activities compared to the reference simulation (Fig. 4). However, because of the complex interactions between the different compartments of the ecosystem, the resulting effect in terms of GPP, copepod grazing, nutrient remineralisation and sedimentation is slightly lower than the addition of the effects observed for the three nutritional mechanisms separately.

4. Discussion

4.1. Model assessment

Mixotrophy is recognised to be widespread in aquatic ecosystems and many studies highlight their potential impact on ecosystem structure and functioning (Bird and Kalff, 1986; Estep, 1986; Hall et al. 1993; Nygaard and Tobiesen, 1993; Arenovski et al., 1995; Havskum and Riemann, 1996; Stoecker et al., 1997; Stickney et al., 2000; Labry et al., 2005; Carvalho and Granéli, 2010; Duhamel et al., 2010; Hartmann et al. 2012; Mitra et al., 2014a). However, most biogeochemical/ecological models still conform to the classic view that aquatic protists can be strictly divided into phototrophic phytoplankton and heterotrophic (phagotrophic) microzooplankton (as per Flynn et al., 2013). Those models that have included mixotrophy typically consider only one type, and that usually conforms to the behaviour of constitutive mixotrophy (i.e., to phototrophic organisms that may engage in heterotrophy). Very few have explored non-constitutive mixotrophy enacted through the acquisition of photosystems from their prey (Flynn and Hansen, 2013; Moeller et al., 2016). Here, different mixotrophic forms were implemented within the same ecological framework. We implemented a form of photo-osmotrophic activity via APA, and two contrasting forms of

photo-phagotrophy via constitutive and non-constitutive mixotrophy. These were operated within the established MIRO model describing the planktonic ecosystem of the Southern North Sea.

Climatological simulations have shown that some features of the Southern North Sea ecosystem dynamics are better described when APA and mixotrophy are considered in MIRO: the summer diatoms attained a higher biomass, which better corresponds with the observations (Fig. 2A); the unrealistic summer bloom of nanoflagellates simulated with $MIRO_{ref}$ disappears with $MIRO_{mixo}$ (Fig. 2C); and the high narrow peak of bacteria simulated in spring decreases to give a smoother seasonal evolution (Fig. 2D).

Due to the large number of physiological parameters used in the model and the uncertainty regarding the value of some of these parameters, an error in the estimation of several parameter values could change the model dynamics and affect the extent to which annual biological activities are influenced by APA and mixotrophy. Although the global sensitivity conducted on the $MIRO_{mixo}$ model (climatological run) shows that the model to be rather sensitive to changes in certain parameters values (Fig. 5), it should be noted that the surface area delimited by the 5th and 95th percentiles (black lines in Fig. 5) is generally similar to that covered by the observations reported in the BCZ and their standard deviations (cf. Fig. 2). While the temporal succession is generally conserved, the amplitude of the different blooms can be very different. For example, the summer bloom of diatoms ranges from effectively being absent to large blooms approaching 500 mgC m^{-3} (Fig. 5A). Similarly, large variabilities can be observed for blooms of *Phaeocystis* colonies (Fig. 5B). When mixotrophy is considered in the model, the ranges of variabilities for each state variable (and particularly for the nanoflagellates biomass) are generally smaller than those observed with $MIRO_{ref}$ (not shown).
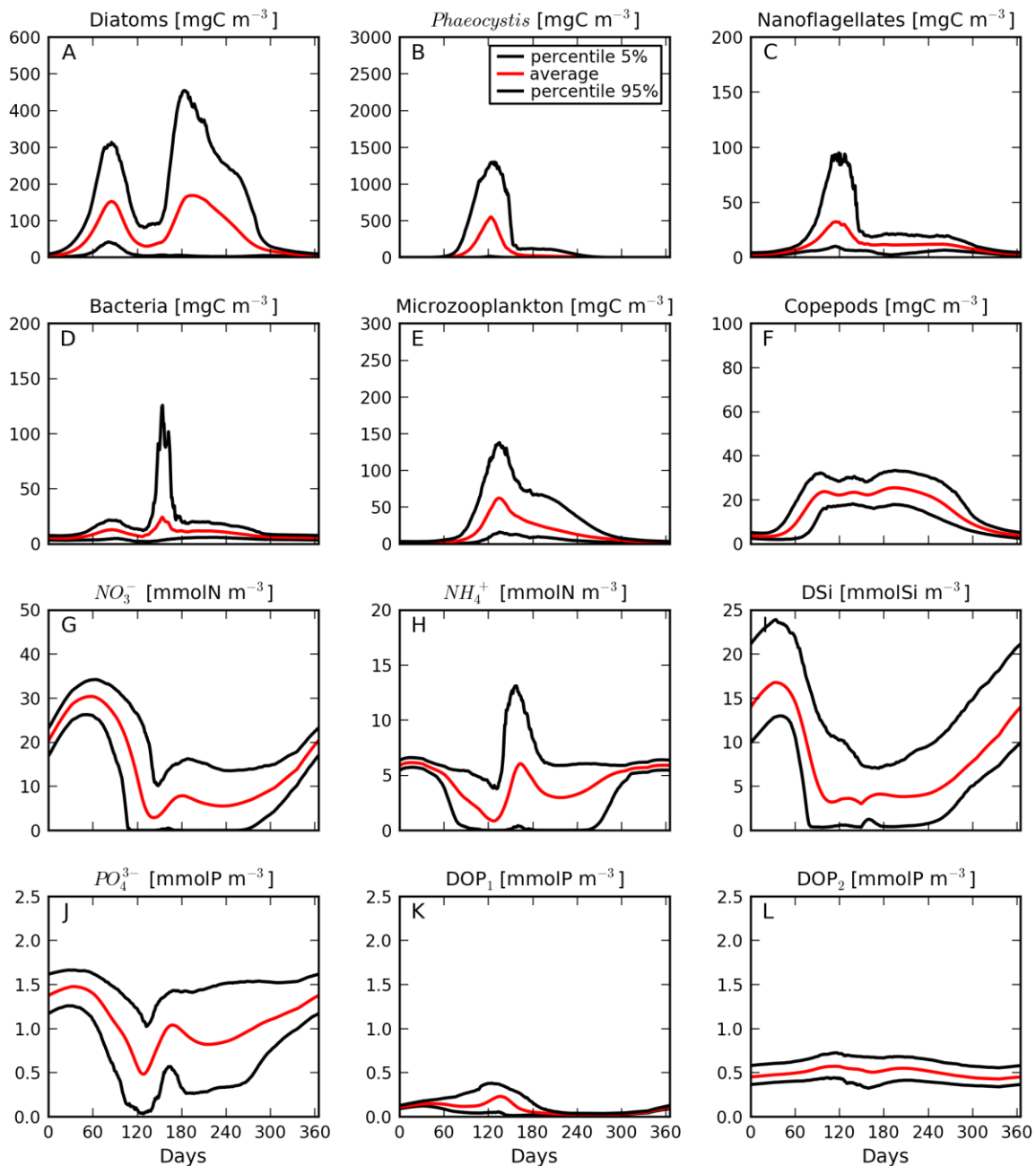
Figure 5: Seasonal planktonic and nutrient concentrations in the Belgian coastal zone (BCZ) obtained from the dynamic global sensitivity analysis conducted on MIRO$_{mixo}$. Red line shows the mean outputs of the 500 runs differing by the values of the physiological parameters (all parameter values were simultaneously randomly varied around their reference value, assuming a standard deviation of 10%). Black lines show the 5th and 95th percentiles. See Methods for full description of the approach.

Despite the close link between the model results and the physiological parameter values, the qualitative changes induced by APA and mixotrophy are generally maintained, irrespective of the parameters values (Fig. 6). From the dynamic sensitivity analyses, the percentage of change in annual copepod grazing between MIRO$_{mixo}$ and MIRO$_{ref}$ is always positive (the 5th and 95th percentiles being respectively 0.02 and 49%) and have a large variability. This

suggests that the copepod grazing is the most impacted of the five biological activities, which is perhaps not surprising given that copepods constitute the higher trophic level and closure term of the model. The percentages of change in annual GPP and sedimentation are positive for most of the runs conducted in the sensitivity analysis, but generally lower than those calculated for the annual copepod grazing. Nutrient remineralisation (NH_4^+ and PO_4^{3-}) is similarly affected by changes in physiological parameter values; most of the sensitivity runs shows a decrease of NH_4^+ and PO_4^{3-} remineralisation when mixotrophy and APA are introduced into the model, consistent with nutrient cycling internal to the mixotrophic plankton (rather than external as per the traditional non-mixotrophic paradigm).
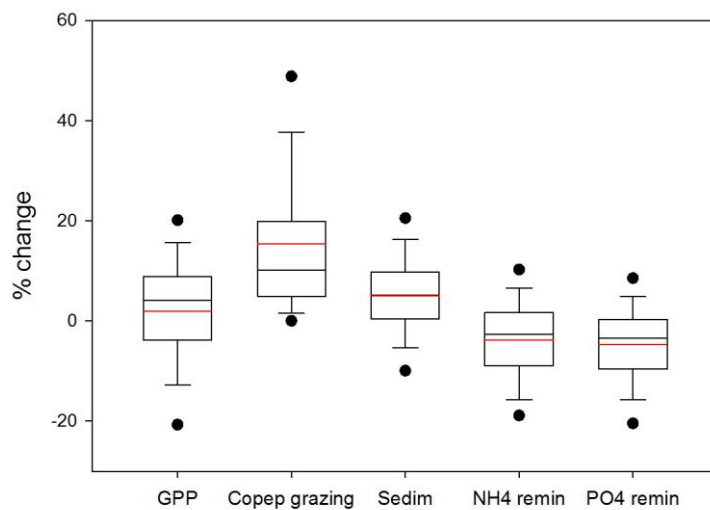
Figure 6: Variability in the percentage of change between the annual fluxes obtained with MIRO_{mixo} versus the reference model without mixotrophy (MIRO_{ref}). Red line shows the mean percentage computed from the 500 runs differing by the values of the physiological parameters (all parameter values were simultaneously randomly varied around their reference value, assuming a standard deviation of 10%; see Methods). The boundaries of the box indicate the 25th and 75th percentiles while the black line inside the box show the median. Whiskers indicate the 10th and 90th percentiles, and dots indicate the 5th and 95th percentiles.

4.2.   Significance of the three forms of mixotrophy

To properly describe the multiple interactions linking the different components of eutrophied coastal ecosystems, it is necessary that ecological and biogeochemical models take account of the various mechanisms of carbon and nutrient acquisition deployed in reality by planktonic protists. It is also important that they do so making appropriate recognition of the underlying physiology. All mixotrophs are not the same; their modes of operation differ significantly (Jones, 1997; Stoecker, 1998; Mitra et al., 2016) and considering that they do operate in a similar fashion (typically conforming to the pattern of constitutive mixotrophy), risks misrepresenting reality as much as ignoring mixotrophy completely, as is the traditional approach to model. While other studies (Stickney et al., 2000; Hammer and Pitchford, 2005;

Mitra et al., 2016) demonstrated the potential for differential impacts between different forms of mixotrophy (phototrophy + phagotrophy) in simple conceptual frameworks, this work shows the potential of different types of mixotrophy to impact on a complex ecosystem. As coastal ecosystems are at the junction between land, ocean, and atmosphere, they are particularly sensitive to changes in nutrient loads driven by human activities. Therefore, a mechanism of nutrient acquisition that is not essential under luxury nutrient and light conditions can become essential when the surroundings are less favourable.

Our inter-annual results suggested that, in the eutrophied Southern North Sea dominated by *Phaeocystis* blooms, APA had no effect on the ecosystem functioning as long as PO_4^{3-} was not the nutrient limiting phototrophic growth. By contrast, the lack of APA ability at low PO_4^{3-} would lead to an underestimation of 3 to 32% of the ecosystem GPP after the mid-1990s (Fig. 3B). In particular, this would underestimate the magnitude of the spring bloom of *Phaeocystis* colonies which are reported as harmful (Lancelot, 1995). This result is consistent with field studies showing the importance of DOP in sustaining productivity in PO_4^{3-}-limited coastal ecosystems (e.g., Nausch, 1998; Labry et al., 2005; Nicholson et al. 2006; Bogé et al., 2012). Yet, the significance of APA is not limited to coastal ecosystems but can also be important in oceanic ecosystems displaying PO_4^{3-} limitation; for example, it has been shown that DOP hydrolysis provides ~60% of the estimated annual P demand in the Sargasso Sea (Lomas et al., 2010) and supports 30% of the primary production in the North Atlantic gyre during the boreal spring (Mather et al., 2008).

We also showed that, as a function of light conditions, expression of non-constitutive mixotrophy (NCM) in microzooplankton can appreciably affect the ecosystem functioning as well. By photosynthesizing using chloroplasts acquired from their photosynthetic prey these mixotrophic microzooplankton can increase their grazing activity, which leads to higher nutrient remineralisation. Due to the complex trophic interactions between the ecosystem components, these changes have several important secondary effects on the ecosystem functioning: (i) it increases the GPP (up to 17%) because the higher nutrient remineralisation supports phototrophic growth of other planktonic protists (here, the summer diatoms); (ii) the resulting higher biomass of summer diatoms supports a higher copepod grazing and, therefore, a subsequent higher transfer to higher trophic levels (up to 31%); (iii) the higher planktonic biomass also increases the matter exported to the sediment (up to 15%) and (iv) the organic matter released by cell lysis supports a higher bacterial production (up to 8%).

Due to the lack of similar studies, these results regarding the effects of non-constitutive mixotrophy within a real food web setting cannot be compared with other works. However, when simulating in a mesotrophic setting a simple food-web structure composed of a strictly

phototrophic protist, a NCM protist (grazing on the phototroph), and bacteria, Mitra et al. (2016) showed that primary production by the NCM protist is limited due to its strict dependency on prey-chloroplasts; we also observed a limited primary production by the NCM in our simulations. Mitra et al. (2016), using a model with a rather different conceptual and mathematical underpinning, also showed that the inorganic nutrient regeneration was enhanced with the NCM protist; this suggests that, under inorganic nutrient limitation, the strictly phototrophic protist would benefit from this nutrient supply, as also observed in our results. As non-constitutive mixotrophy is very common amongst planktonic ciliates and dinoflagellates (Stoecker et al., 1989; Stoecker, 1999; Skovgaard, 1998) and in other respect ubiquitous in both coastal (e.g., Stoecker et al., 1989) and oligotrophic systems (e.g., Dolan and Marrasé, 1995; Pitta et al. 2001), our results suggest that the role of non-constitutive mixotrophy could be very important in many well-lit ecosystems. As such it would appear that this form of mixotrophy warrants explicit inclusion in future models.

In quantitative terms, constitutive mixotrophy in nanoflagellates has less impact on the Southern North Sea ecosystem functioning (< 7% for all processes studied here) than non-constitutive mixotrophy in microzooplankton and APA. This situation contrasts with the situation observed in oligotrophic systems. Many experimental, field and modelling studies have highlighted, for example, the importance of bacterivory in mixotrophic nanoflagellates in oligotrophic systems (Arenovski et al., 1995; Zubkov and Tarran, 2008; Hartmann et al. 2012; Mitra et al., 2014a; Unrein et al., 2014; Fischer et al., 2016). Zubkov and Tarran (2008) estimate that small algae perform 40-95% of the bacterivory in the euphotic layer of the temperate North Atlantic Ocean in summer and 37-70% in the surface waters of the tropical North-East Atlantic Ocean. Mitra et al. (2014a) showed that primary production can potentially be increased by a factor 2 in oligotrophic systems thanks to a shortened and more effective chain from nutrient regeneration to primary production, a result which has been supported by the field study of Ptacnik et al. (2016). Constitutive mixotrophy also plays an important role in ecosystems submitted to prolonged darkness. McKie-Krisberg et al. (2015) showed that bacterivory in Antarctic photosynthetic nanoflagellates is a successful survival strategy during the polar night. However, even in the Southern North Sea, where our model indicates a rather minor role for such organisms as mixotrophs, ingestion of bacteria by these nanoflagellates increases their competitiveness against the *Phaeocystis* colonies for inorganic nutrients and it also adversely affects microzooplankton by lowering bacteria availability. This type of mixotrophy could, therefore, have significance in ecosystems where nanoflagellates are dominant and where nutrients and/or light are limiting. As the model used to describe constitutive mixotrophy in the MIRO model is configured to increase phagotrophy under C limitation as well as under nutrient limitation (Ghyoot et al., 2017), it

could also capture the competitive advantage of mixotrophic nanoflagellates in light limiting ecosystems.

The three alternative mixotrophic nutritional modes investigated in this study have all been shown to be potentially involved in the development of harmful algal blooms (Burkholder et al., 2008). In the PO_4^{3-} limited ecosystems of the Southern North Sea, APA helps to sustain massive blooms of *Phaeocystis* colonies. These are considered as ecosystem disruptive events because the large gelatinous colonies are not grazed by copepods (Lancelot et al., 2009). In consequence, the eventual degradation of the ungrazed colonies in some ecosystems (though not the Southern North Sea) can lead further ecosystem damage due to deoxygenation and increased water viscosity (Steidinger and Garcés, 2006). By conferring a competitive advantage when inorganic nutrient are scarce, constitutive mixotrophy is also suspected to play an important role in the development of massive blooms of toxic species as *Prymnesium parvium* and *Chrysochromulina polylepis* (Nygaard and Tobiesen, 1993; Dahl et al., 2005; Carvalho and Granéli, 2010; Granéli et al. 2012). Similarly, some notable harmful species are reported to be NCM, e.g., the dinoflagellates *Dinophysis* sp., known for their production of diarrheic shellfish toxins, and the ciliate *Mesodinium rubrum* forming "red tides" (Hansen et al., 2013). The higher frequencies and amplitudes of HABs observed in coastal eutrophied ecosystems (Burkholder et al., 2008; Heisler et al., 2008) argues for the explicit inclusion of description of different modes of mixotrophy to reflect the alternative nutritional modes of planktonic protists in ecological/biogeochemical models.

In conclusion, our work demonstrates the important potential for mixotrophy in shaping planktonic system dynamics. By better capturing the functioning of these ecosystems, it could help to take adequate measures to prevent the undesirable effects of HABs and also better understand factors affecting biogeochemical cycling. While undoubtedly both our understanding of mixotrophy and our modelling thereof will improve in tandem, this work demonstrates the need to recognise that the different forms of mixotrophy need to be represented appropriately and explicitly in models.

Author contribution

All five authors contributed to the model conception and test design. CG conducted the model analyses and prepared the manuscript while AM, CL, KJF and NG revised it.

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Highlights:

- A biogeochemical model including 3 contrasting forms of mixotrophy is analysed
- Contrasting forms of mixotrophy have different impacts on system dynamics
- Their combined effect is not the addition of the effects individually observed
- It is important to describe different forms of mixotrophy in an appropriate fashion