Modelling the biogeochemical effects of heterotrophic and autotrophic N₂ fixation in the Gulf of Aqaba (Israel), Red Sea

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Abstract. Recent studies demonstrate that marine N_2 fixation can be carried out without light by heterotrophic N_2 -fixers (diazotrophs). However, direct measurements of N_2 fixation in aphotic environments are relatively scarce. Heterotrophic, as well as unicellular and colonial photoautotrophic diazotrophs, are present in the oligotrophic Gulf of Aqaba (northern Red Sea). This study evaluates the relative importance of these different diazotrophs by combining biogeochemical models with

- 15 time series measurements at a 700m-deep monitoring station in the Gulf of Aqaba. At this location, an excess of nitrate, relative to phosphate, is present throughout most of the water column and especially in deep waters during stratified conditions. A relative excess of phosphate occurs only at the water surface during nutrient-starved conditions in summer. We show that a model without N₂ fixation can replicate the observed surface chlorophyll but fails to accurately simulate inorganic nutrient concentrations throughout the water column. Models with N₂ fixation improve simulated deep nitrate by enriching sinking
- 20 organic matter in nitrogen, suggesting that N₂ fixation is necessary to explain the observations. The observed vertical structure of nutrient ratios and oxygen is reproduced best with a model that includes heterotrophic, colonial and unicellular autotrophic diazotrophs. These results suggest that heterotrophic N₂ fixation contributes to the observed excess nitrogen in deep water at this location. If heterotrophic diazotrophs are generally present in oligotrophic ocean regions, their consideration would increase current estimates of global N₂ fixation and may require explicit representation in large-scale models.

25 1 Introduction

Biological nitrogen fixation refers to the conversion of dinitrogen gas (N_2) via reduction to ammonium (NH_4) into bioavailable forms of nitrogen by a specialized group of microbes containing the nitrogenase enzyme complex. On geological timescales, the size of the oceanic reservoir of bioavailable nitrogen, and thus the ocean's capacity for exporting carbon to depth, is controlled by the balance between removal of fixed nitrogen by denitrification and input by N₂ fixation (Falkowski, 1997;

30 Haug et al., 1998; Deutsch et al., 2007; Gruber and Galloway, 2008; Fennel et al., 2005). The amount of organic matter exported from the surface to the deep ocean (i.e., export production) depends on allochthonous inputs of nitrogen (i.e., "new

nitrogen") into the euphotic zone (Eppley and Peterson, 1979). These new nitrogen inputs determine the amount of "new production", which is directly related to the exported fraction. Locally the supply of new nitrogen can occur through several mechanisms, including microbially mediated N₂ fixation, diapycnal mixing injecting deep nitrate (NO₃) into the surface, lateral transport, atmospheric sources and riverine input. While the injection of deep NO₃ is often regarded as the dominant source of

5 new nitrogen that drives the seasonal cycle of marine primary production; there is significant interest in quantifying the contribution of N₂ fixation to primary production, particularly in oligotrophic areas (Karl, 2002; Zehr and Ward, 2002; Capone et al., 2005; Luo et al., 2012).

Diazotrophs contain nitrogenase, the catalyst enzyme for N₂ reduction, which is encoded by *nif* genes. *Trichodesmium spp.*, a group of non-heterocystous filamentous cyanobacteria that form large colonies, were traditionally considered the main
contributors to N₂ fixation in the surface subtropical and tropical ocean (Carpenter and McCarthy, 1975; Capone et al., 2005). Increased sampling efforts and method improvements subsequently led to the discovery of diverse diazotroph groups including heterocystous endosymbiotic cyanobacteria (Zehr et al., 1998; Carpenter et al., 1999), free-living unicellular cyanobacteria (Zehr et al., 2001; Montoya, 2004; Moisander et al., 2010), and other cyanobacterial symbionts (Zehr et al., 2000). Most recently, genetic techniques have allowed the detection of *nif* genes in a number of anaerobic and heterotrophic phylotypes

15 (Zehr et al., 2008; Zehr, 2011; Rahav et al., 2013, 2015). The abundance of *nif* genes does not necessarily imply that these organisms are actively fixing N₂ (Zehr et al., 2000; Moisander et al., 2017); however, the correlation between bacterial productivity and N₂ fixation rates suggests that significant aphotic N₂ fixation may occur in the Red Sea (Rahav et al., 2013, 2015). The differences in size and physiology of these diverse diazotrophs also suggest that they occupy distinct niches, and thus may affect primary productivity and export production differently (Bonnet et al., 2016; Moisander et al., 2010).

- 20 Most biogeochemical models treat N₂ fixation as a purely light-dependent, autotrophic process. These models either use standard formulations of light limitation for the diazotrophic groups (e.g., Fennel et al., 2002; Moore, et al., 2004; Gregg, 2008; Dutkiewicz et al., 2012), or include theoretical considerations to introduce an empirical N₂ fixation flux in the model (e.g., Bisset et al., 1999). Some approaches neglect light limitation on diazotrophy and instead infer global N₂ fixation patterns from the distribution of dissolved inorganic nitrogen and phosphorus, and estimates of ocean circulation (e.g., Deutsch et al., 2007).
- 25 In trait-based models, diazotrophs are usually accounted for by a single functional group with biological rate parameters intended to represent either *Trichodesmium spp.*, unicellular cyanobacteria, or a generic autotrophic diazotroph. More complex models of diazotroph growth have explored cellular function and the effects of a variable cellular stoichiometry, however still limited by light (Kreuss et al., 2015; Fernández-Castro et al., 2016). Only a few modelling studies have evaluated multiple autotrophic diazotroph groups simultaneously, by considering separate groups for *Trichodesmium spp.*, unicellular
- 30 cyanobacteria and diatoms-cyanobacterial associations (e.g., Monteiro et al., 2010; Dutkiewicz et al., 2012, 2015). To our knowledge, heterotrophic N₂ fixation has not yet been considered explicitly in biogeochemical models.

Understanding the ecological dynamics of different types of diazotrophs should significantly improve predictive capabilities in biogeochemical models, and lead to more accurate estimates of global N₂ fixation rates. It has been suggested that N₂ fixation rates are underestimated globally due to limited knowledge about the distribution and characteristics of N₂ fixing organisms (Montoya, 2004; Zehr, 2011). It is also assumed that marine N₂ fixation may increase globally, as a result of ocean warming

and higher concentrations of dissolved CO₂ in sea water (Hutchins et al., 2007; Levitan et al., 2007; Dutkiewicz et al., 2015).
 To our knowledge, these laboratory experiments have only explored the response of *Trichodesmium*. Less information is available about the effects of climate trends on other diazotrophic organisms.

In this study we explore the biogeochemical signatures that result from different assumptions about the ecological niches occupied by diazotrophs. Our study area is the Gulf of Aqaba, a northern extension of the Red Sea. Aside from the reported

- 10 presence of diverse diazotroph types, the morphology of the Gulf of Aqaba limits horizontal transport of deep waters, thus allowing us to simplify the physical model's complexity and focus on the biological component. We aim to answer the following two questions: i) How important is N₂ fixation as a source of new nitrogen in the Gulf of Aqaba? ii) How important is heterotrophic, light-independent N₂ fixation? To address these questions, we implemented a one-dimensional model at a monitoring station for which monthly quality-controlled measurements of physical and biogeochemical variables are available
- 15 from 2004 onward. We then systematically tested different model assumptions about diazotrophy and calibrated selected model parameters to facilitate an objective comparison between the different biogeochemical model versions. The different assumptions about diazotrophy consider the characteristics of organisms identified in the Gulf of Aqaba, including heterotrophic fixing α and γ proteobacteria (Rahav et al., 2013), unicellular cyanobacteria, and *Trichodesmium spp*. (Post et al., 2002; Foster et al., 2009; Rubin et al., 2012). Our most important conclusion is that aphotic N₂ fixation is necessary to
- 20 reproduce the observed excess nitrogen in deep waters of the Gulf, while maintaining reasonable surface N₂ fixation rates. Our best performing biogeochemical model of the Gulf of Aqaba estimates annual N₂ fixation rates in overall agreement with local and large-scale estimates in the literature.

2 Study Area: The Gulf of Aqaba

The Gulf of Aqaba is a quasi-rectangular, 200-km long, 20-km wide, semi-enclosed basin in the northeast region of the Red 25 Sea (Figure 1). The average depth of the Gulf of Aqaba is 800 m, its deepest point approximately 1800 m, and it is surrounded by arid mountains that steer the dominantly northerly winds (Berman et al., 2003). Two shallow sills, the Bab el Mandeb (~140 m) and the Strait of Tiran (~240 m), inhibit the entrance of cold and dense deep waters from the Indian Ocean. Since inflow is restricted to warm surface waters, the Gulf's deep-water masses (>300 m) are locally formed (Wolf-Vecht et al., 1992; Biton et al., 2008) and have negligible horizontal transport toward the exterior (Klinker et al., 1976; Manasrah et al., 2006).

- 30 The annual hydrographic cycle exhibits a well-defined seasonality where vertical temperature and salinity distributions are dominantly affected by surface heat fluxes and modified by surface advective fluxes (Carlson et al., 2014). During winter
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(December to March), convective vertical mixing usually extends to depths >300 m (Labiosa et al., 2003), and even reaches the bottom (~700-800 m bottom depth) in some extreme years (Figure 2). From April to September, the water column is thermally stratified, and inflowing warm surface waters from outside the Gulf occupy the layer above the thermocline (Genin and Paldor, 1998; Berman et al., 2000; Biton and Gildor 2011). During fall (October to December), surface cooling and high

5 evaporation rates erode the seasonal stratification and re-establish a well-mixed water column (Berman et al., 2003; Monismith and Genin, 2004). The Gulf experiences net evaporation of approximately 1.6 m yr⁻¹ (Ben-Sasson et al., 2009) due to negligible precipitation and run-off (Wolf-Vecht et al., 1992).

The Gulf is oligotrophic, with surface nitrate (NO₃) and phosphate (PO₄) concentrations usually close to their detection limits during summer stratification (Fuller et al., 2005; Mackey et al., 2009; Meeder et al., 2012). Deep winter mixing supplies

- 10 inorganic nutrients to the surface, and NO₃ and PO₄ reach ~0.1 μ M and ~2 μ M, respectively (Figure 2; Lindell and Post 1995; Lazar et al. 2008). Dust from the desert provides a sufficient atmospheric source of soluble iron (Fe) for microbial growth in the Gulf (Chase et al., 2006; Chen et al., 2007). Phytoplankton spring blooms reach a maximum chlorophyll concentration of ~2 mg Chl-a m⁻³ and their initiation is strongly correlated with the termination of winter cooling of the water column (Zarubin et al., 2017). Interannual variability in the depth of winter convective mixing results in periods of nutrient accumulation in
- 15 deep waters (Figure 2; Wolf-Vecht et al., 1992; Lazar et al., 2008; Carlson et al., 2012), which is re-set during extreme winter mixing events approximately every four years (Silverman and Gildor, 2008). The periodicity of these extreme mixing events has been associated with regional weather patterns that modify the Red Sea water temperatures (Silverman and Gildor, 2008).

3 Methods

We analysed the role of autotrophic and heterotrophic N₂-fixing organisms in determining biogeochemical patterns at an open pelagic site (Station A), located in the northern Gulf of Aqaba, by testing four alternative ecosystem model versions. The ecosystem models are evaluated in terms of their ability to replicate observations of oxygen (O₂), NO₃, PO₄, and chlorophyll. In this section, we first describe the available observations, then the models, and finally the systematic model calibration method.

3.1 Observations

- 25 Meteorological and oceanographic observations are available from the Inter-University Institute (IUI) for Marine Sciences in Eilat, Israel (http://www.iui-eilat.ac.il/Research/ NMPMeteoData.aspx). Meteorological observations are used to calculate surface heat and momentum fluxes for the physical model and incoming light for the biological models. Observed meteorological variables include wind speed, air temperature, air humidity, air pressure, irradiance and cloud cover. This data has been collected continuously and automatically at 10 min intervals by the meteorological instrumental array at the end of
- 30 the IUI pier since 2006.

Monthly CTD and bio-chemical profiles at Station A (29.5° N, 34.9° E) were collected during monthly surveys of the National Monitoring Program (NMP) from 2004 to 2014 (http://www.iui-eilat.ac.il/Research/NMPMeteoData.aspx). CTD profiles are used to nudge temperature and salinity in the physical model (see section 3.2). Bio-chemical profiles, including NO₃, nitrite (NO₂), ammonium (NH₄), PO₄, O₂ and chlorophyll-a (Chl-a), are used for biogeochemical model calibration (years 2006 to

5 2010) and model validation against unassimilated data (years 2011 to 2014). Nutrients were measured using spectrophotometry (QuickChem 8000 flow injection), O₂ was determined by Winkler titrations, and Chl-a concentrations are estimated using fluorometry (Turner Designs 10-AU).

3.2 Model Descriptions

- The ecosystem models are implemented within the General Ocean Turbulence Model (GOTM), a one-dimensional physical model that computes solutions to differential equations for the vertical transport of momentum, salt and heat (Burchard et al., 1999). GOTM is implemented for the 700-m deep station with a vertical resolution of 3 m and forced with hourly meteorological observations from the IUI pier. Temperature and salinity are nudged to observed CTD profiles with a nudging time scale of 30 days. This is done to account for the influence of horizontal advection of heat and salt in the one-dimensional model and ensures a realistic representation of density stratification. The effect of temperature and salinity nudging on the
- 15 results is analysed below (section 4.2.1). As model calibration is computationally expensive, model simulations run only from January 2005 to September 2010. The first year of each simulation is considered model spin-up and excluded from further analysis (climatological meteorological forcing is used for the first year, as this database starts in 2006). NO₃, NH₄, PO₄ and O₂ initial total concentrations match the observed total inventories, using homogenous concentrations throughout the water column of 2.5 mmol m⁻³, 0.05 mmol m⁻³ and 0.15 mmol m⁻³, respectively. Vertical nutrient and oxygen concentrations are
- 20 redistributed within a few months and replicate the observed vertical distributions well starting from October 2005. Non-fixing phytoplankton, zooplankton and detritus are initially set to a homogeneous small value of 0.1 mmol N m⁻³, and also readjust rapidly because the adjustment timescales for these variables are short (Fennel et al., 2006). Diazotrophs initial values are set to lower densities than non-fixing organisms, with a homogenous total value of 0.03 mmol N m⁻³ (i.e., models with multiple diazotrophs maintain the same amount of initial diazotrophic biomass by dividing initial values equally among organisms).
- Four main ecosystem model versions of increasing complexity (referred to as H0, H1, H2 and H3) are treated as alternative hypotheses of how biological processes, especially diazotrophy, control the vertical distribution and temporal variability of dissolved inorganic nutrients and oxygen. H0 is the base model without diazotrophics and follows the model equations described in Fennel et al., (2006, 2013). In general, the model follows Monod kinetics using a fixed N:P ratio (R^{nf}_{N:P} = 16). Sensitivity to the constant N:P ratio is explored in section 4.2.3. We test the H0 model with and without a sediment denitrification flux (model versions H0 and H0', respectively). H0 includes denitrification but no N₂ fixation, as no diazotrophs
- are considered. H0' does include neither denitrification, nor diazotrophic organisms, and thus the underlying assumption of this model version is that there is a balance between inputs from N₂ fixation and losses of fixed nitrogen due to denitrification.

When present, the denitrification flux follows Fennel et al. (2013) with a loss fraction of 6 mol N₂ per mol of organic matter remineralized at the sediment-water interface. This generates an average sediment denitrification flux of 0.25 ± 0.46 mmol N m⁻² d⁻¹, with a maximum value of 3.01 mmol N m⁻² d⁻¹.

H1, H2 and H3 are modified versions of H0, in which different groups of diazotrophic organisms are added sequentially.

- 5 Diazotrophic growth formulations are similar to those of non-fixing phytoplankton, except that they are not limited by nitrogen and have a higher N:P ratio ($R_{N:P}^f = 45$). H1 introduces a generic autotrophic diazotroph; H2 replaces H1's generic diazotroph with two autotrophs representing unicellular and colonial (e.g., *Trichodesmium spp.*) cyanobacteria. The unicellular group overall follows the same formulation as the generic diazotrophs, except that no aggregation term is included. We simplify unicellular diazotroph behaviour in this manner because this group represents free-living picoplanktonic cells that typically do
- 10 not form large colonies although they can aggregate (Bonnet et al. 2016). Instead, we assume this group is grazed by zooplankton at similar rates as non-fixing phytoplankton. This difference between colonial and unicellular groups is consistent with studies suggesting that colonies represent an evolutionary adaptation to decrease grazing pressure (Nielsen, 2006). Aside from their size, *Trichodesmium spp.* colonies may be less palatable and harder to digest due to toxins (Kerbrat et al. 2010, 2011). Grazing is not a major fate of this group (O'Neil and Roman, 1994).
- 15 The last model version, H3, adds a heterotrophic group to the model structure of H2. This functional group is not limited by light and grows by consuming both dissolved inorganic and organic forms of nutrients. An intermediate version H3' is used as a control, where the heterotrophic organisms do not fix nitrogen and are limited by the availability of nitrogen in inorganic forms and from small detritus. Model H3 eliminates the nitrogen limitation and the heterotrophic group becomes a heterotrophic diazotroph group. In a subsequent set of four experiments (H3a, H3b, H3c, and H3d), we remove complexity
- 20 from H3. The heterotrophic group remains, but we sequentially remove the autotrophic groups one-at-a-time: first the colonial cyanobacteria (H3a), then the unicellular cyanobacteria (H3b), then the generic autotrophic diazotroph (H3c), and finally we remove all autotrophic diazotrophs (H3d). A summary of all model versions is given in Table 1 and a description of state variables and full model equations in the Supplement.

We acknowledge that some of these model assumptions are still simplifications of diazotroph behaviour. For example,

25 *Trichodesmium spp.*, as well as other autotrophic and diazotrophic organisms, may also take up dissolved organic matter to support growth (Benavides et al., 2017). Nevertheless, model assumptions and formulations used here are in line with the most commonly accepted understanding of the dominant controls on microbial and diazotrophic growth.

3.3 Model Parameters

3.3.1 Parameter Optimization Method

- 30 Parameter optimization refers to the minimization of misfit between model and observations by adjusting model parameters. We applied the method first to systematically calibrate the most sensitive parameters of H0 (see supplement), and then to
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independently re-calibrate parameters in H1 to H3 after the introduction of diazotrophs. We used an evolutionary algorithm, where changes in the parameter values follow a set of rules inspired by the process of natural selection (Houck et al., 1995; Kuhn et al., 2015). The algorithm starts with a randomly generated "population" of 30 parameter sets (\vec{p}) , which are iteratively modified over a number of generations. During each generation of the population, the cost $I(\vec{p})$ of the model with parameter set \vec{p} is calculated as:

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$$J(\vec{p}) = \frac{1}{V} \sum_{\nu=1}^{V} \frac{w_{\nu}}{N} \sum_{i=1}^{N} \left(\hat{y}_{\nu,i} - y_{\nu,i} \right)^2, \tag{1}$$

where \hat{y} represents a model estimate and y the corresponding observation. N is the number of observations included for each variable v. Here the number of variables V is 5 (nitrate + nitrite, ammonium, phosphate, chlorophyll-a, and oxygen measured as profiles at Station A between 2006 and 2010). Model-data misfits are weighted by the factor $w_v = 1/\sigma_v$, i.e, the inverse

standard deviation of each variable. Half of the parameter sets with the lowest J value "survive" to the next generation. The 10 other half of the population is regenerated from new parameter sets obtained by recombination of two random "parent" sets drawn from the better performing half (i.e., the "survivors" of the previous generation). Parameters also "mutate", i.e. random noise is added, for additional variability in the parameter space. An allowable range of values is set for each parameter based on the literature (Table 2).

3.3.1 Optimized Parameters 15

The parameter optimization method has limitations. Most importantly, the optimization cannot estimate with confidence parameters that are unconstrained by the observations (Fennel et al. 2001; Schartau and Oschlies, 2003; Ward et al., 2010). To avoid this, a subset of H0's most sensitive parameters was selected for optimization through a preliminary sensitivity analysis. Optimized parameters for H0 are identified in Table 2 along with the optimal values. The optimization was replicated 10 times

over 100 generations using the algorithm described in section 3.3.1. Non-optimized parameters are fixed at their a priori 20 estimates based on Fennel et al. (2006, 2013).

For each model version with diazotrophs (H1, H2 and H3), some of the parameters already optimized for H0 required recalibration to properly accommodate the changes in system dynamics. Re-calibrated parameters for each model version are presented in Table 3. No re-calibration was performed for model versions (H0' and H3a-d), as they are aimed to test the relative importance of individual model components.

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3.3.2 Diazotroph Parameters

Since none of the parameters directly related to the diazotroph groups are constrained by the available observations, they were predefined for H1, H2 and H3, based on the observational and modelling literature (Table 3). Previous modelling studies have used maximum growth rates of generic N₂ fixers ranging from 0.4 d^{-1} (Moore, et al., 2004) to 1.25 d^{-1} (Ward et al., 2013).

When model diazotrophs are assumed to represent *Trichodesmium spp.* values range between 0.17 d⁻¹ (Hood et al., 2001) to 30

0.3 d⁻¹ (Fennel et al., 2002). From the observational literature, *Cyanothece* (unicellular cyanobacteria) and *Trichodesmium spp*. cultured under various combinations of Fe and light availability exhibit maximum rates around $0.3 \pm 0.05 d^{-1}$ (Capone et al., 1997; Berman-Frank et al., 2001; Hutchins et al., 2007). Growth rates can be higher (up to 0.5 d⁻¹) at high CO₂ and high light availability (Kranz et al., 2010; Hong et al., 2017). We chose a common reference maximum growth rate of 0.25 d⁻¹ for all

5 photosynthetic diazotrophs, such that differences between the model versions result only from the different assumptions about the losses of each group (e.g., predation of unicellular cyanobacteria vs. sinking of large aggregates). Based on growth rates measured for cultured heterotrophic bacteria, we chose a value of 0.2 d⁻¹ for the heterotrophic diazotrophs (Pomeroy and Wiebe, 2001). Observational and modelling studies were also considered to set the photosynthetic initial slope of photosynthetic diazotrophs (Geider et al., 1997; Moore et al., 2004; Hutchins et al., 2007). Other parameters are based on Fennel et al. (2002).

10 4 Results

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4.1 Observed NO₃ and PO₄ Patterns

To provide context for the evaluation of our model simulations, we first describe the observed interannual and seasonal variability of NO₃ and PO₄ for the complete time series (2004 to 2014) at Station A (Figure 2). From May to January vertical distributions of NO₃ and PO₄ show depletion of nutrients in the euphotic zone and a nutricline between 100 and 200 m. From February to April, nutrient concentrations increase near the surface and decrease in deep waters (>200 m) as result of vertical

- mixing. Multi-year periods of accumulation of nutrients in deep waters were observed from: i) the beginning of the series to the end of 2006, ii) after the winter of 2008 until February 2012, and iii) after the winter of 2013 until the end of the series. These periods are bookended by winters with extremely deep mixing events in 2007, 2008, 2012 and 2013 during which nutrient concentrations are nearly homogenized in the entire water column. Two prolonged periods of these vertically
- 20 homogenous conditions were observed in 2007 and 2008, lasting two to three months. Our model calibration simulations are from 2006 to 2010, allowing us to include two years with deep winter mixing (2007 and 2008) and two years with moderate winter mixing (2009 and 2010).

4.2 Model Results

4.2.1 Sensitivity to Physical Nudging

25 Model runs, with and without temperature and salinity nudging towards observations, demonstrate that nudging has a negligible effect below 200 m, indicating that horizontal advection does not modify the lower part of the water column in a significant way (Supplement). Above 200 m, nudging corrected model errors in the representation of vertical mixing and surface forcing. The average magnitude of the differences due to nudging in the top 200 m is 0.20±0.45 °C and 0.5±0.16 kg

m⁻³. Since these effects are small and limited to the surface, we conclude that neither nudging nor the neglect of horizontal advection affects our conclusions significantly.

4.2.2 Effects of N₂ Fixation on DIP and DIN

- Figure 3 shows simulated NO₃ and PO₄ concentrations from models H0', H0, H1, H2, H3' and H3, along with the
 corresponding measurements. Observed NO₃ and PO₄ concentrations exhibit a marked increase in deep water after the strong winter mixing of 2008. Weaker winter mixing after 2008 results in deep-nutrient accumulation, which is more pronounced for NO₃ than PO₄. Model H0' reproduces some deep-nitrate accumulation, but underestimates NO₃ concentrations in comparison to observations. Model H0 strongly underestimates inorganic nitrogen below the nutricline. Model H1, where N₂ fixation was introduced via a generic autotroph, generates only small changes in the vertical distribution of nutrients. In model H2 the
 representation of NO₃ below the nutricline is slightly improved; however, underestimation of mid-water NO₃ is still noticeable.
- Model H3 significantly improves the representation of deep NO₃ accumulation. All model versions represent similar vertical distributions of PO₄ and underestimate its deep-water concentrations by the end of the series.

These model differences are also summarized in Figure 4, which shows the simulated and observed NO₃ and PO₄ inventories in surface and deep waters. According to the observations deep NO₃ accumulates between 2007 and 2010 at a rate of $0.59 \pm$

- 15 0.08 mmol m⁻² d⁻¹, whereas deep PO₄ accumulates at 0.015 \pm 0.009 mmol m⁻² d⁻¹. During this accumulation period approximately 36 mmol NO₃ per mmol PO₄ appear in deep waters. All five model versions simulate similar magnitudes and temporal variability of PO₄, but NO₃, in particular below 100 m, diverges over time among the models. H0 has the largest deviations from the other models, simulating approximately constant deep NO₃ after 2007. H0', the version without denitrification, produces a rate of increase in deep NO₃ similar to that of model version H2. H3 has the highest accumulation
- 20 rate of deep NO₃, matching the observed slope the best.

Of the additional model versions based on H3 (H3a, H3b, H3c and H3d), results from H3a (heterotrophic and colonial diazotrophs) come closest to H3, while the model without autotrophic diazotrops has the narrowest and most unrealistic range of nutrient concentrations (not shown).

4.2.3 Sensitivity to Planktonic Stoichiometry

To investigate whether the vertical distribution of dissolved inorganic nutrients was affected by our assumption of fixed N:P phytoplankton and diazotroph ratios, we explored the sensitivity of NO₃ and PO₄ to changes in the non-fixing phytoplankton N:P ratio ($R_{N:P}^{nf}$ = 16) and the diazotrophs N:P ratio ($R_{N:P}^{f}$ = 45). In this analysis we used model version H1, which includes a single non-fixing group and a single N₂ fixing group and varied the ratios one-at-a-time. The range of values for $R_{N:P}^{nf}$ varied

from 10 to 28 and the range for $R_{N:P}^{f}$ from 19 to 51. Figure 5 shows examples of the results obtained by increasing and decreasing each ratio.

Changes in the N:P ratios had negligible effects on the vertical distribution of NO₃, but strongly affected PO₄ distribution. In general, lower than Redfield $R_{N:P}^{f}$ of diazotrophs increases PO₄ below 300 m, most strongly at depth. This occurs possibly

5 because more phosphorus returns to the dissolved pool per unit nitrogen trough excretion and remineralization. In contrast, a decrease of more than half the $R_{N:P}^{nf}$ of non-diazotrophs produces only a minor decline in deep PO₄, while increases in the ratio did not have a significant effect. The decline in deep PO₄ occurs because, in the absence of nitrogen limitation, diazotrophs can utilize additional phosphorus.

4.2.4 Effect of N₂ Fixation on Chlorophyll and O₂

- 10 Figure 6 shows simulated and observed chlorophyll and dissolved oxygen values. The seasonal variability of total chlorophyll concentrations is reproduced well by all models, with higher chlorophyll between November and April. During these months, simulated chlorophyll concentrations are homogeneous down to 200 m. In 2007 and 2008, chlorophyll concentrations of ~0.13 mg m⁻³ are observed in the measurements reaching as deep as 500 m. This feature is also captured well by our models, as is the location of the deep chlorophyll maximum (DCM) at ~80 m between March and October. However, there are some
- 15 discrepancies between model results and observations. The models overestimate spring bloom peak concentrations in 2007 and predict peak timing two months earlier than observed in 2008. Model H0 tends to underestimate chlorophyll concentrations from the surface to the DCM during summer months. As chlorophyll concentrations are extremely low during this time of the year, these model-data differences are on the order of 0.05 to 0.1 mg m⁻³. These discrepancies during summer months are corrected in the models with N₂ fixation.
- 20 Simulated oxygen concentrations exhibit larger differences between models and observations, in particular below the mixed layer, where air-sea fluxes do not directly affect oxygen concentrations. Model versions without diazotrophs (H0 and H0') show similar deep-oxygen variability, with a small underestimation of oxygen during the winter of 2007, and a small overestimation after the winter of 2008. Model re-calibration for the model versions with diazotrophs results in changes in deep oxygen. H1, the model with generic diazotrophs, exhibits the largest model-observation misfits. As in the case of deep NO₃, the best deep-oxygen representation is obtained with H3.

4.2.5 Validation against Independent Observations

Observations from 2010 to 2014 (outside the optimization period) are used to independently validate the models. The rootmean-square errors (RMSEs) in Table 4 show that, in terms of chlorophyll, PO₄ and surface O₂, all models behave similarly and achieve similar agreement for assimilated and independent observations. As demonstrated in the previous sections, the

- 30 model versions mainly diverge in their behaviour with respect to NO_3 , with some differences in O_2 concentrations. Between 0
 - 10

and 100 m, H3 has the largest RMSEs for NO₃, but below 100 m it has the lowest values, particularly against unassimilated NO₃. H3 also has the lowest RMSEs for surface and deep oxygen (Table 4).

Figure 7 shows observed and simulated NO₃ inventories in 0 - 100 m and below 100 m outside the assimilation period. Compared against the other model versions, H3 increasingly overestimates surface NO₃ over time. However, the deep NO₃

5 inventory is best represented by H3. By the end of the observed time series, between 2013 and 2014, H3 starts to also overestimate deep NO₃.

4.2.6 Primary Production and N₂ Fixation Rates

We now compare the simulated rates of primary production with those reported for the Gulf of Aqaba by Rahav et al. (2015) and Iluz et al. (2009) (Figure 8a) and the simulated rates of N_2 fixation with those measured by Rahav et al. (2015) and Foster

10 et al., (2009) (Figure 8b). Following Rahav et al. (2015), we show the rates at the DCM and their averages above and below the DCM. The depth-resolved in situ primary production rates reported by Iluz et al. (2009) were also averaged in the same way for comparison. Where necessary, observations of primary production in carbon units were converted to the model's nitrogen units using the Redfield ratio.

Simulated primary production above the DCM ranges from 0.02 to 0.85 mmol N m⁻³ d⁻¹ and exhibits an annual cycle with peaks of productivity in October and April. A prolonged period of low primary production extends from April to September in most model versions. Model versions H3b and H3d maintain rates twice as large as the rest of the models during the summer/fall period. Aside from H3b and H3d, differences between models are small and simulated rates agree with those measured by Iluz et al. (2009) and Rahav et al. (2015).

Above the DCM, models H1, H2, H3 and H3a show a well-defined N2 fixation peak during summer months (i.e., after the

20 peak in primary production). Maximum rates in these models range from 0.001 to 0.1 mmol N m⁻³ d⁻¹, which agrees with the observed rates by Foster et al. (2009) and Rahav et al. (2015). In general, simulated N₂ fixation rates are low during winter and spring. Similar temporal patterns and differences between model versions occur at the DCM and below. Peaks in N₂ fixation at these depth levels occur after the surface peak, and have a shorter duration and smaller amplitude. Deep N₂ fixation rates estimated by models without heterotrophic diazotrophs do not match the observed rates by Rahav et al. (2015).

25 5 Discussion

5.1 Is N₂ Fixation Relevant in the Gulf of Aqaba?

In this study we implemented and optimized a series of models with different assumptions about N_2 fixation in the Gulf of Aqaba. The models range from one neglecting N_2 fixation to another assuming that, in addition to two autotrophic diazotroph groups, N_2 fixation can occur in the entire water column (i.e., independent of light availability). While the models are very similar in their abilities to replicate chlorophyll and PO₄, model H3 performed the best in reproducing the observed pattern of

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deep-NO₃ accumulation and O₂. Overall, all models that consider N₂ fixation accumulate nitrogen at different rates, as they

enrich the nitrogen content of detritus, which is then remineralized at depth over time.

The best model performance was obtained with two groups of autotrophic organisms and a group of heterotrophic organisms (H3). A model without explicit N_2 fixation, but in the absence of sediment denitrification, also increases the accumulation of deep NO₃ in a similar fashion as version H2 but not sufficiently high to match the observations. This suggests that N_2 fixation

5 in the area must exceed denitrification rates. In the models with denitrification, the average sediment denitrification flux is $0.25 \pm 0.46 \text{ mmol N m}^{-2} \text{ d}^{-1}$, with a maximum value of 3.01 mmol N m⁻² d⁻¹. These values at are the lower end of a global compilation of sediment denitrification rates by Fennel et al. (2009), which have a mean of 2.2 mmol N m⁻² d⁻¹ and maximum values exceeding 10 mmol N m⁻² d⁻¹.

Observations from the Gulf of Aqaba exhibit an excess of nitrogen that contrasts to exterior waters from the Arabian Sea and Indian Ocean, which are considered net nitrogen sink regions (Gruber and Sarmiento, 1997). There are too few reported values of dissolved inorganic nitrogen-to-phosphorus ratios for the Red Sea region from Bab-el-Mandeb to the Strait of Tiran to provide a complete idea of its spatial distribution; however, the limited available information supports our conclusions. Naqvi et al. (1986) found a significant difference in N:P ratios between surface incoming and sub-surface outflowing waters at Bab-

el-Mandeb, concluding that N₂ fixation was a process required to explain these anomalies in the nitrogen budget. Higher

- 15 nitrogen concentrations have been observed in the Red Sea in comparison to the Arabian Sea and Indian Ocean, where a strong deficit of nitrogen develops as losses due to denitrification exceed the input of newly fixed nitrogen (Burkill et al., 1993; Naqvi, 1994; Gruber and Sarmiento, 1997; Morrison et al., 1998, 1999). Close to the entrance of the Persian Gulf, average excess phosphate has been estimated to be around 5 mmol m⁻³ at all depths and seasons reported, with maximum excess values on the order of 8 mmol m⁻³ (Gruber and Sarmiento, 1997). Thus, it has been hypothesized that limited deep-water exchange at Bab-
- 20 el-Mandeb allows waters of the Red Sea outside of the Gulf of Aqaba to acquire different characteristics from inflowing Arabian Sea waters (Naqvi et al., 1986). Our model results support this hypothesis and suggest that N₂ fixation is key for the formation of the distinct biochemical characteristics in the Gulf of Aqaba. Considering the regional context, our models suggest that, despite low rates, N₂ fixation is necessary to explain the nitrogen vertical distribution in the Gulf of Aqaba, and the interannual accumulation of deep nitrate during years with weak convection.
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5.2 How does N₂ fixation Contribute to Primary Production?

In this section we discuss the contribution of N_2 fixation to primary production in the Gulf of Aqaba, and our quantitative estimates of N_2 fixation with respect to global rates (Figures 8-9). Our estimates of surface primary productivity agree with those reported by Iluz et al. (2009) for March-April of 2008. However, our models overestimate surface primary productivity

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values in 2010 when compared to those reported by Rahav et al. (2015). On average, our best-performing model version yields an annual primary production rate of 304±56.9 g C m⁻² yr⁻¹ (H3). This rates is higher than previously published annual averages,

which range from 80 g C m⁻² y⁻¹ (Levanon-Spanier et al., 1979) to 170 g C m⁻² y⁻¹ (Iluz, 1991), whereas more recent unpublished primary production estimates at IUI range between 141 and 197 g C m⁻² y⁻¹ (pers. comm. Y. Shaked).

The ratio of new to total primary production (f-ratio) in our experiments ranges from 15% to 80%. Maximum f-ratios are estimated in January and February due to significant contributions from deep NO₃, whereas f-ratios are at their minimum

5 during stratified conditions (June – August). Our best-performing model version, H3, estimates a summer minimum f-ratio 0.22. The average f-ratio for all scenarios is 0.47. This agrees with published estimates for the Gulf of Aqaba of 0.5 during the stratified period as determined from a nitrate-diffusion model (Badran et al., 2005).

Total annual N₂ fixation rates from our best-performing model versions (H3 and H3a) are similar to high estimates reported for other regions (Capone and Carpenter, 1982; Michaels et al., 1996; Lee et al., 2002), while those obtained in the other model

10 experiments are within the range of values reported for the Gulf of Aqaba. The intensity of winter mixing has a minor effect on N₂ fixation rates; the largest effect occurred in H3a where N₂ fixation increased by 15% after deep winter mixing. Based on our best-performing model version (H3), we estimate that 10% to 14% of the total primary production is supported by N₂ fixation.

5.3 Are Heterotrophic N₂ Fixers Important?

- 15 In contrast to previous models (e.g., Hood et al., 2001; Fennel et al., 2002; Monteiro et al., 2010; Moore et al., 2004), our model version H3 relaxes the assumption of light dependence for diazotrophy, through the inclusion of heterotrophic diazotrophs in addition to two groups of autotrophic diazotrophs. This model improves the representation of NO₃ and O₂ at depth (Figures 3, 6). Changes in deep NO₃ can be explained through the enrichment of detritus, while changes in O₂ may reflect the additional sink of O₂ at depth due to the heterotrophic group. All model versions with heterotrophic organisms also
- 20 match observed estimates of N₂ fixation in deep waters of the Gulf of Aqaba, and without them N₂ fixation rates below the DCM are underestimated (Figure 8). Heterotrophic N₂ fixation also impacts total N₂ fixation (Figure 9).

There is growing evidence of non-cyanobacterial N_2 fixation in aphotic waters (Benavides et al., 2017; Moisander et al., 2017). For instance, N_2 fixation rates in mesopelagic and abyssopelagic waters down to 2000 m (Fernandez et al., 2011; Bonnet et al., 2013; Loescher et al., 2014) have been attributed to non-cyanobacterial organisms, including proteobacteria (Turk-Kubo et al.,

- 25 2014). These *nif*H-expressing heterotrophic phylotypes can be as abundant as unicellular cyanobacterial groups and dominate the deep and dark zones of the water column (Church et al. 2005; Langlois et al., 2005; Riemann et al., 2010). Genetic evidence and rate estimates from the Gulf of Aqaba suggest that *nif*H expressing heterotrophic proteobacteria α and γ may explain the correlation of bacterial productivity rates with N₂ fixation rates (Rahav et al., 2013; 2015). Aside from the Gulf of Aqaba, aphotic N₂ fixation and *nif*H gene expression have also been reported in the Baltic Sea (Farnelid et al., 2013), Arabian Sea
 30 (Jayakumar et al., 2012) and Mediterranean Sea (Rahav et al., 2013).
 - Despite the importance of heterotrophic diazotrophs in our model, the simulated colonial diazotroph blooms are responsible

for the highest N2 fixation rates, so they are a necessary model aspect to achieve resemblance with the observations. This is in

line with evidence of extensive blooms of *Trichodesmium spp*. being responsible for the high N_2 fixation rates observed in the Arabian Sea and Red Sea (Capone et al., 1998; Post et al., 2002; Foster et al., 2009). In the northern Gulf of Aqaba, colonies and free trichomes of *Trichodesmium spp*. are found throughout the year down to 100 m depth (Post et al., 2002). Ephemeral blooms of *T. erythraeum* and *T. thiebautii* have been documented near the coast of Eilat (Post et al., 2002, Gordon et al., 1994;

5 Kimor and Golandsky, 1977). However, massive blooms are rare in the Gulf of Aqaba (Foster et al., 2009; Mackey et al., 2007, pers. Communication Berman-Frank) and the model probably overestimates the contribution of *Trichodesmium spp*.'s annual blooming to total N₂ fixation rates, as seen in the much larger surface N₂ fixation rates generated by H2 and H3. As new observational information is collected, further model refinements may be necessary to better reflect the actual contribution of different diazotrophic groups in the Gulf of Aqaba.

10 5.4 Limitations and Uncertainties

The one-dimensional nature of our physical setting, which neglects the contribution of horizontal advection to the vertical structure of simulated tracers, can be considered a limitation of this study. This simplification is, however, necessary to perform model calibration and test multiple model structures at a manageable computational expense. We applied temperature and salinity nudging to ensure accurate representation of the vertical density structure. Comparison of the simulated vertical

- 15 structure with and without nudging shows that this correction has negligible effects on deep waters, where the effects of N₂ fixation are the most relevant. This is consistent with the existing literature about circulation of the Gulf of Aqaba, which describes how geomorphology and bathymetry limit water exchange between the Gulf of Aqaba and the Red Sea to the upper 300 m (Wolf-Vecht et al., 1992; Biton and Gildor, 2011). It is, therefore, unlikely that horizontal transport could explain the observed accumulation of deep NO₃. Nevertheless, transport of nitrogen-enriched sub-surface waters from the Gulf of Aqaba
- 20 towards the exterior may dampen the long-term accumulation of nitrogen (Figure 7).

There are other sources of nitrogen that were not explored in the present study that we discuss here briefly. For instance, we did not include contributions to N_2 fixation by diatom-diazotroph associations, which are significant in other regions. While diatom-diazotroph associations have been detected in the Gulf of Aqaba, they are not as abundant as unicellular diazotrophs, *Trichodesmium* and proteobacteria (Kimor et al. 1992, Foster et al., 2009, pers. Comm Berman-Frank). In general, due to the

25 oligotrophic characteristics of the region, small phytoplankton species (<8 µm) contribute more than 90% of the chlorophylla standing stock (Lindell & Post 1995, Yahel et al. 1998). Dinoflagellates and diatoms together correspond to less than 5% of the phytoplankton biomass, except during ephemeral diatom blooms during spring when they can account for nearly 50% of the total biomass (Al-Najjar et al., 2006).

Another source of nitrogen which could significantly affect this region is atmospheric deposition, as the Gulf receives 30 considerable dust input from the surrounding deserts. Recently, it has been shown that atmospheric dust input does not correlate with chlorophyll variability in surface waters of the Gulf of Aqaba (Torfstein and Kienast, 2018). A previous study suggested that atmospheric deposition of nitrogen could support over 10% of surface primary production in the region, based on measurements of local aerosol composition and a dust deposition model (Chen et al., 2007). However, this estimate had a relatively large uncertainty due to errors associated with the deposition flux calculation and the temporal variability in dust flux (Chen et al., 2007). Moreover, very low nitrogen concentrations and N:P ratios lower than Redfield from the surface down to 80 m were observed during the same time period (Foster et al., 2009). Therefore, the role of atmospheric nitrogen inputs romains uncertain

5 remains uncertain.

In contrast to the improvement in NO₃ distributions with the addition of heterotrophic diazotrophs, all other model versions exhibit similar underestimation of deep total PO₄. This suggests that their structure lacks a process affecting this nutrient. Several processes can independently affect PO₄. Prior knowledge suggests that seasonal advection of nitrogen depleted surface waters may contribute to changes in PO₄, while having minimum impact on NO₃ concentrations. Other relevant missing

- 10 processes include variable plankton stoichiometry and PO₄ remineralization. Our sensitivity analysis highlighted that varying the planktonic N:P ratios has an evident effect on PO₄ but does not affect NO₃ distributions significantly. A related effect was noticed when comparing fixed constant vs. variable plankton nutrient uptake stoichiometry in a model of the central Baltic Sea: seasonal particulate organic C:N ratios were very similar, but C:P diverged (Kreuss et al., 2015). Moreover, preferential PO₄ remineralization may more directly increase deep-PO₄ concentrations as organic matter is decomposed in the bottom
- 15 layers. The inclusion of this process in a model of the North Atlantic Ocean improved the representations of biogeochemical characteristics of the area and increased the N₂ fixation rates obtained by the model (Monteiro and Follows, 2012). Variable nitrogen allocation within diazotrophs also improved the performance of a model representing annual cycles in the North Atlantic sub-tropical gyre (Fernández-Castro et al., 2016). Further investigation of this process is recommended. Finally, an intrinsic caveat that should accompany all biological models is the uncertainty associated with parameter values
- 20 (Denman, 2003). We reduced this uncertainty by using systematic parameter optimization for a subset of parameters, yet others were kept fixed at a priori assumed values. In the case of N:P ratios, a simple sensitivity analysis suggests that they do not affect conclusions with respect to inorganic dissolved nitrogen but modify phosphorus concentrations. In the case of parameters related to diazotrophs, these are largely unconstrained by the observations we used, which are typically available in long-term data sets (i.e., Chl-a, NO₃, PO₄, O₂). We emphasize that results in this study are experimental, testing the effects of assumptions
- 25 about diazotroph behaviour rather than modifying such behaviour by subjectively tuning parameter values.

6 Conclusions

We implemented and optimized biogeochemical models that represent a range of different assumptions about diazotrophy in a 700 m-deep pelagic station from the northern Gulf of Aqaba. Our model results demonstrate the importance of N_2 fixation in replicating the observed water-column-integrated nitrogen and oxygen inventories. The model without N_2 fixation is unable

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to replicate the observed vertical structure of inorganic nitrogen. The models that include diazotrophs significantly modify this variable by increasing the fraction of remineralized nitrogen from organic matter decomposition. The effect of N_2 fixation on O_2 distributions depends on the type of nitrogen fixer. N_2 fixation by autotrophic-photosynthetic organisms increases oxygen

concentrations, while heterotrophic organisms decrease deep-oxygen due to increased respiration and organic matter decomposition. The observed vertical structure of NO₃ and oxygen is reproduced best with a model that includes heterotrophic, and colonial and unicellular autotropic diazotrophs suggesting that heterotrophic N₂ fixation is necessary to explain the observed excess nitrogen at this location. N₂ fixation assumptions do not affect PO₄ concentrations significantly, but they are

- 5 affected by assumptions about N:P ratios of organic matter. The N₂ fixation rates simulated by this model are similar to the highest observational estimates from the Gulf of Aqaba. Aphotic N₂ fixation is simulated to occur at lower rates than maximum autotrophic N₂ fixation yet occurs continuously over a large portion of the water column. This suggests that heterotrophic diazotrophs set a background rate of N₂ fixation in the ocean that should be considered further in global estimates and biogeochemical models.
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Figure 1: Map of study area showing the location of monitoring stations and geographic references.



Figure 2: Observed vertical nitrate and phosphate distributions at Station A (Gulf of Aqaba) from 2004 to 2014. Tick marks are placed on the April 1st of every year.



Figure 3: Observed (coloured circles) and simulated (background) NO3 and PO4 using model versions H0 (no nitrogen fixers), H1 (generic autotrophic fixer), H2 (unicellular and colonial autotrophic fixers), H3' (non-fixing heterotrophic, and unicellular and colonial autotrophic fixers). The spin-up period is not shown.



Figure 4: Observed and simulated vertically integrated NO3 and PO4 between 0 – 100 m and between 100 – 600 m using model versions H0 (no nitrogen fixers), H0' (no sediment denitrification – no fixers), H1 (generic autotrophic fixer), H2 (unicellular and colonial autotrophic fixers), H3' (non-fixing heterotrophic, and unicellular and colonial autotrophic fixers), H3 (heterotrophic, and unicellular and colonial autotrophic fixers). Tick marks are placed on the April 1st of every year.



Figure 5: Sensitivity experiments modifying the fixed N:P ratios in non-fixing organisms $(R_{N:P}^{nf})$ and N₂ fixing organisms $(R_{N:P}^{f})$. Observed (coloured circles) and simulated (background) NO₃ and PO₄ using model versions H1 (generic autotrophic fixer). The spin-up period is not shown.



Figure 6: Observed (coloured circles) and simulated (background) Chl-a and O_2 using model versions H0' (no sediment denitrification – no fixers), H0 (no nitrogen fixers), H1 (generic autotrophic fixer), H2 (unicellular and colonial autotrophic fixers), H3' (unicellular and colonial autotrophic fixers and heterotrophic non-fixer), H3 (non-fixing heterotrophic, and unicellular and colonial autotrophic fixers), and H3 (heterotrophic, and unicellular and colonial autotrophic fixers). Vertical scale in the Chl-a subplots is logarithmic to exaggerate the surface. The spin-up period is not shown.



Figure 7: Observed (circles) and simulated (lines) total nitrate in the surface and deep waters at Station A during the model validation period from 2010 to 2014. Tick marks are placed on the April 1st of every year.



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Figure 8: Comparison of previously reported in situ measurements and model results of primary production (a) and N₂ fixation rates (b), averaged at three depth levels. Depth levels are from the surface to the Deep Chlorophyll Maximum, at the DCM and below it. (c) DCM estimated from observed Chl-a profiles at Station A. 109, F09 and R15 refer to Iluz et al., (2009), Foster et al., (2009), and Rahav et al., (2015), respectively. Control versions H0' and H3' are not shown.



Figure 9: Simulated new, regenerated, and total primary production (a) and N_2 fixation rates (b) obtained by the different simulations (see Table 1 for key to different simulations). A summary of previous estimates of N_2 fixation rates in observational and model studies is included in (b). Redfield C:N ratio is used to transform the model results nitrogen units to carbon units. Control versions H0' and H3' are not shown.

Model Version	Characteristics / Diazotrophs Groups	Denitrification	Generic (A)	Unicellular (A)	Colonial (A)	Heterotrophic (H)
Н0'	Ν	-	-	-	-	-
HO	Ν	1	-	-	-	-
H1	А	1	1	-	-	-
H2	А	1	-	1	1	-
Н3'	ΑH	1		1	✓	Non-fixing
Н3	ΑH	1	-	1	✓	1
H3a	ΑH	1	-	-	✓	1
H3b	ΑH	1	-	1	-	1
H3c	ΑH	1	1	-	-	1
H3d	Н	1	-	-	-	1
	Diazotrophs characterist	ics:				
	Inorganic phosphorus upta	ıke	1	1	1	1
	Organic phosphorus uptak	e	-	-	-	1
	Light growth limitation		1	1	✓	-
	Temperature dependent n rate	naximum growth	1	1	1	✓
	Minimum temperature li (20°C)	imit for growth	-	-	1	-
	Predation		-	1	-	-

Table 1: Summary of model version characteristics and assumptions about diazotroph groups. N = no diazotrophs; A = autotrophic diazotrophs. Checkmarks (\checkmark) represent presence of a model characteristic / functional diazotrophic group in the model; dashes represent their absence.

Parameters	Value	Range	Description	Units	References
μ_{Phy}^0	0.76*	0.1 – 3	Referencephytoplanktonmaximum growth rate at $T = 0^{\circ}C$	d ⁻¹	a, b, c
$k_{Phy}^{NO_3}$	0.05	0.01 - 0.5	Phytoplankton NO ₃ uptake half- saturation	mmol m ⁻³	d, e
$k_{Phy}^{NH_4}$	0.1*	0.01 - 0.5	Phytoplankton NH4 uptake half- saturation	mmol m ⁻³	d, e
k_{Phy}^{DIP}	0.004*	0.001 - 0.5	Phytoplankton DIP uptake half- saturation	mmol m ⁻³	a, f, g
α_{Phy}	0.1*	0.01 - 0.125	Phytoplankton, initial slope of photosynthetic response	molC gChl ⁻¹ (W m^{-2}) ⁻¹ d ⁻¹	d, h
m_{Phy}	0.1	0.01 - 0.2	Phytoplankton mortality rate	d ⁻¹	d
g ^{max} g _{Phy}	1.16*	0.1 - 4	Zooplankton maximum grazing rate	d-1	b, i
k_{Zoo}^{Phy}	0.5*	0.01 - 0.5	Square zooplankton grazing half- saturation	(mmol m ⁻³) ²	d, e
l_{BM}	0.011*	0.01 - 0.15	Zooplankton base metabolic rate	d ⁻¹	d
l_E	0.1	0.05 - 0.35	Zooplankton excretion rate	d ⁻¹	d
m_Z	0.35*	0.02 - 0.35	Zooplankton mortality rate	d-1	d
τ	0.1	0.01 - 25	Small detritus aggregation rate	d ⁻¹	d, e
$ heta_{Phy}^{max}$	0.142*	0.015 - 0.15	Maximum chlorophyll to carbon ratio	mg Chl (mg C) ⁻¹	h
β	0.74*	0.25 - 0.75	Zooplankton assimilation efficiency	non-dim.	j, k
r _{DOM}	0.2	0.05 - 0.5	DOM remineralization rate	d ⁻¹	1

 Table 2: Parameters used in the base biogeochemical model (H0), including minimum and maximum parameters ranges based on

 the literature. Parameters values followed by * were obtained by the optimization.

r_D	0.01	0.005 - 0.15	Detritus remineralization rate	d ⁻¹	l, m
n _{max}	0.3*	0.01 - 0.35	Nitrification rate	d ⁻¹	d, e
k _I	0.1	0.01 – 0.5	Half-saturation radiation for nitrification inhibition	Wm ⁻²	d
I _{th}	0.0095	0.005 - 0.01	Radiation threshold for nitrification inhibition	Wm ⁻²	d
W _{Phy}	0.1	0.01 – 1	Vertical sinking velocity for non- fixing phytoplankton	md ⁻¹	n
W _{DL}	-4.44*	0.01 – 25	Vertical sinking velocity for large detritus	md ⁻¹	d

a. Fennel et al. (2002) b. Fahnenstiel et al. (1995) c. Veldhuis et al. (2005) d. Fennel et al. (2006) e. (Lima and Doney (2004) f. Ward et al. (2013) g. Moore, et al.(2002) h. Geider et al. (1997) i. Gifford et al. (1995) j. Landry et al. (1984) k. Tande and Slagstad (1985) l. Amon and Benner (1996) m. Enríquez et al. (1993) n. Smayda and Bienfang (1983)

Table 3: Diazotrophs parameters and re-calibrated non-fixing phytoplankton parameters for each model version. H0 = no N2 fixers; H1 = generic autotrophic diazotrophs; H2 = unicellular and colonial cyanobacteria; H3 = heterotrophs, unicellular and colonial cyanobacteria. Superscripts in the parameter descriptions denote the corresponding literature references used to define the generic diazotroph parameter values within a realistic range. In order to tease apart the effect of different diazotrophs niches or behaviour, unicellular, colonial and heterotrophic diazotrophs maintain the same parameter values as the generic organism. An exception is the slightly lower reference growth rate used for heterotrophic diazotrophs.

Model version:	H0	H1	Н2	Н3	Units	Description
μ^0_{Phy}	0.76	2.20	1.5	1.5	d-1	Reference phytoplankton maximum growth rate at $T = 0^{\circ}C$
$ heta_{Phy}^{max}$	0.022	0.076	0.076	0.05	mg Chl (mg C) ⁻¹	Maximum chlorophyll to carbon ratio – non fixing phytoplankton
$k_{Phy}^{NH_4}$	0.076	0.076	0.076	0.076	mmol m ⁻³	Phytoplankton NH4 uptake half-saturation
k_{Phy}^{DIP}	0.001	0.015	0.015	0.015	mmol m ⁻³	Phytoplankton DIP uptake half-saturation
m_{Phy}	0.1	0.06	0.06	0.06	d ⁻¹	Phytoplankton mortality rate
g_{Phy}^{max}	1.16	4.0	1.95	1.95	d ⁻¹	Zooplankton maximum grazing rate
β	0.36	0.7	0.7	0.7	non-dim.	Zooplankton assimilation efficiency
$\mu^0_{G_F}$	-	0.25	-	-	d-1	Reference generic diazotrophs maximum growth rate at $T = 0^{\circ}C^{a \cdot j}$
$k_{G_F}^{DIP}$	-	0.001	-	-	mmol m ⁻³	Generic diazotrophs DIP uptake half- saturation ^d
$ heta_F^{max}$	-	0.053	-	-	mg Chl (mg C) ⁻¹	Maximum chlorophyll to carbon ratio – generic diazotrophs ¹
α_{G_F}	-	0.01	-	-	molC gChl ⁻¹ (W m^{-2}) ⁻¹ d ⁻¹	Generic diazotrophs, initial slope of photosynthetic response ^{a, d, g, k}
m_{G_F}	-	0.18	-	-	d ⁻¹	Generic diazotrophs mortality rate ^d
l_{G_F}	-	0.05	-	-	d ⁻¹	Generic diazotrophs respiration rated

Model version:	H0	H1	Н2	Н3	Units	Description
$\mu^0_{U_F}$	-	-	0.25	0.25	d-1	Reference unicellular cyanobacteria maximum growth rate at $T = 0^{\circ}C$
$k_{U_F}^{DIP}$	-	-	0.004	0.004	mmol m ⁻³	Unicellular cyanobacteria DIP uptake half- saturation
$ heta_{U_F}^{max}$	-	-	0.053	0.053	mg Chl (mg C) ⁻¹	Maximum chlorophyll to carbon ratio – unicellular cyanobacteria
α_{U_F}	-	-	0.05	0.05	molC gChl ⁻¹ (W m^{-2}) ⁻¹ d ⁻¹	Unicellular cyanobacteria, initial slope of photosynthetic response
m_{U_F}	-	-	0.20	0.2	d-1	Unicellular cyanobacteria mortality rate
l_{U_F}	-	-	0.05	0.05	d ⁻¹	Unicellular cyanobacteria respiration rate
$g_{U_F}^{max}$	-	-	0.2	0.2	d-1	Zooplankton maximum grazing rate on unicellular cyanobacteria
$k_{Zoo}^{U_F}$	-	-	0.001	0.001	$(mmol m^{-3})^2$	Square zooplankton grazing half-saturation on unicellular cyanobacteria
$\mu^0_{C_F}$	-	-	0.25	0.25	d ⁻¹	Reference colonial cyanobacteria maximum growth rate at $T = 0^{\circ}C$
$k_{C_F}^{DIP}$	-	-	0.004	0.004	mmol m ⁻³	Colonial cyanobacteria DIP uptake half- saturation
$ heta_{C_F}^{max}$	-	-	0.053	0.053	mg Chl (mg C) ⁻¹	Maximum chlorophyll to carbon ratio – colonial cyanobacteria
α_{C_F}	-	-	0.05	0.05	molC gChl ⁻¹ (W m^{-2}) ⁻¹ d ⁻¹	Colonial cyanobacteria, initial slope of photosynthetic response
m_{C_F}	-	-	0.18	0.05	d ⁻¹	Colonial cyanobacteria mortality rate
l_{CF}	-	-	0.18	0.05	d-1	Colonial cyanobacteria respiration rate

Model version:	HO	H1	H2	Н3	Units	Description
$\mu^0_{H_F}$	-	-	-	0.2	d ⁻¹	Reference heterotrophs maximum growth rate at $T = 0^{\circ}C^{m}$
$k_{H_F}^{DIP}$	-	-	-	0.001	mmol m ⁻³	Heterotrophs DIP uptake half-saturation
$k_{H_F}^{DS}$	-	-	-	0.001	mmol m ⁻³	Heterotrophs organic phosphorus uptake half- saturation
m_{H_F}	-	-	-	0.2	d ⁻¹	Heterotrophs mortality rate
l_{H_F}	-	-	-	0.05	d ⁻¹	Heterotrophs respiration rate

a. Moore, et al., (2004); b. Ward et al., (2013); c. Hood et al., (2001); d. Fennel et al., (2002); e. Capone et al., (1997); f. Berman-Frank et al., (2001); g. Hutchins et al., (2007); h. Kranz et al., (2010); i. Hong et al., (2017); k. Geider et al., (1997);
l. Fennel et al., (2006); m. Pomeroy and Wiebe, (2001)

5 Table 4 Root-mean-square-errors between observations and corresponding simulated variables. Observations between 2005 and 2010 were used during model calibrations (i.e., assimilated). Observations between 2011 and 2014 are used for independent model validations (non-assimilated). Control versions H0' and H3' are not shown.

0-100 m									
	2005 - 2	2010 (assim	ilated)		2011 - 20	2011 – 2014 (non-assimilated)			
	NO ₃	PO ₄	CHL	O ₂	NO ₃	PO ₄	CHL	O ₂	
H0	0.71	0.04	0.15	7.57	0.60	0.04	0.16	6.39	
H1	0.77	0.04	0.14	6.99	0.66	0.04	0.15	7.08	
H2	0.78	0.04	0.14	6.96	0.75	0.04	0.14	6.66	
Н3	1.04	0.05	0.14	7.35	1.50	0.05	0.13	6.22	
H3a	1.04	0.06	0.12	7.10	1.41	0.09	0.14	6.47	
H3b	1.91	0.05	0.14	7.94	2.15	0.05	0.16	8.13	
H3c	1.01	0.06	0.12	7.05	1.06	0.08	0.14	6.55	
H3d	1.60	0.05	0.19	7.91	1.78	0.05	0.19	8.21	
100 – 600 m									
	NO ₃	PO ₄	CHL	O 2	NO ₃	PO ₄	CHL	O 2	
H0	1.53	0.05	0.08	15.54	2.26	0.06	0.06	17.34	
H1	1.43	0.05	0.07	15.09	2.02	0.05	0.06	21.70	
H2	1.29	0.05	0.07	14.17	1.56	0.04	0.06	17.57	
Н3	1.05	0.05	0.07	13.28	0.89	0.05	0.06	10.03	
H3a	1.12	0.05	0.07	13.42	0.93	0.07	0.06	11.98	
H3b	1.26	0.10	0.07	18.29	1.51	0.14	0.06	29.99	
H3c	1.14	0.05	0.07	14.37	1.18	0.05	0.06	16.16	
H3d	1.41	0.11	0.14	18.39	1.85	0.14	0.13	30.38	