

Mémoire

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Faculté : Faculté des Sciences

Diplôme : Master en océanographie, à finalité approfondie

Année académique : 2022-2023

URI/URL : <http://hdl.handle.net/2268.2/18590>

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LIÈGE université
Sciences



Modelling for
Aquatic SysTems

Black Sea and diazotrophs, toward an improvement of modeling the nitrogen cycle

A MASTER THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE MASTER'S DEGREE IN OCEANOGRAPHY

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Acknowledgements:

First, I want to thank Marilaure Gregoire for supervising this project. She always supported me and tried to push me toward better understanding and better results even if I was a complete beginner in modelling. She took the necessary time to talk with me about the objectives and the aim of the current work.

I am thankful to all the MAST's members which welcomed me warmly.

I want to particularly thank Catherine Meulders for the hours that she spent for explaining me the model and the tools that we use in this kind of research. She also spent a lot of time helping me for debugging error code. Without her contribution, this work wouldn't exist.

I also want to show my gratefulness to Luc Vandebulcke who especially helped me on the validation of the model and the understanding of NetCDF files. Like Catherine, without his help, this work wouldn't be there.

Finally, I want to thank the members of the Jury who accepted to take part of this journey.

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Conformément aux règles imposées à la rédaction, ce mémoire ne doit pas dépasser 50 pages, rédigées en Times 12 ou équivalent.

Abstract

[EN] The nitrogen cycle in oxygen depleted environment is a major concern in our world as soon as the oxygen minimum zones are spreading due to global warming and climate changes. The Black Sea basin has a constant stratification in which the conditions are anoxic under 100m all the yearlong. This feature creates an outdoor laboratory for scientists which facilitates the studying of anoxia and where typical events of the world ocean like ventilation, denitrification or nitrogen fixation can be more easily investigated. Thus, we use a 3D model of the Black Sea to investigate the nitrogen cycle, where we have added di-nitrogen fixers (diazotrophs). Previously di-nitrogen was considered to be lost in the water column because it was considered to be inert regarding biology. During this work, we have investigated di-nitrogen fixers and we have found that a non-negligible amount of nitrogen is recycled in the water column which enhance the accuracy of the nitrogen cycle. Indeed, the production of di-nitrogen in the water column was found to be constantly inferior to the di-nitrogen fixation by diazotrophs, which shows their importance. However, we have to investigate more efficiently our knowledge of diazotrophs as soon as their physiology is only poorly understood.

[FR] Le cycle de l'azote dans un environnement anoxique est une préoccupation majeure en océanographie sachant que les zones de minimum d'oxygène s'étendent en raison du réchauffement de la planète et des changements climatiques. Le bassin de la mer Noire présente une stratification constante dans laquelle les conditions sont anoxiques en dessous de 100m tout au long de l'année. Cette caractéristique crée un environnement qui facilite l'étude de l'anoxie et où les événements typiques de l'océan mondial tels que la ventilation, la dénitrification ou la fixation de l'azote peuvent être plus facilement étudiés. Ainsi, nous utilisons un modèle 3D de la mer Noire pour étudier le cycle de l'azote, auquel nous avons ajouté des fixateurs de diazote (diazotrophes). Auparavant, on considérait que le diazote se perdait dans la colonne d'eau parce qu'il était considéré comme inerte du point de vue biologique. Au cours de ce travail, nous avons étudié les fixateurs de diazote et nous avons découvert qu'une quantité non négligeable d'azote est recyclée dans la colonne d'eau, ce qui améliore la précision du cycle de l'azote. En effet, la production de diazote dans la colonne d'eau s'est avérée être constamment inférieure à la fixation de diazote par les diazotrophes, ce qui montre leur importance. Cependant, nous devons approfondir plus efficacement nos connaissances sur les diazotrophes dès lors que leur physiologie n'est que peu comprise.

Introduction

Generalities

The Black Sea is a semi-enclosed basin situated between western Europe and Asia. 24 Countries are surrounding its basin as Russia, Romania, Ukraine, Turkey, Bulgaria, and others. It covers 436 400km² with a maximum depth of 2 212m. It is surrounded by the Mediterranean Sea which is connected to the Black Sea by the Sea of Marmara. It is also surrounded by the Azov Sea through the Kerch Strait. On its northern side, the Sea has a large continental shelf which is approximately 200km wide, with a mean depth of 150m and represents 13% of the area. It is also the place where we find the mouth of the Danube, the Dnestr and the Dnepr rivers which bring a large amount of fresh water and nutrients.

The Black Sea interest for scientists rose at the end of the 20th century because of its eutrophication due to population development. Nowadays, modeling provides a powerful tool to understand physical and biochemical processes. In this master thesis, 3D physical-biochemical coupled model is used to understand the nitrogen dynamics and in particular, the potential role of nitrogen fixers (diazotrophs) in compensating for the loss of fixed nitrogen by denitrification and ANAMMOX (ANAerobic AMMonium OXidation).

Circulation and lateral boundaries

The Black Sea's general circulation is mainly characterized by the presence of a cyclonic boundary current; the rim current (Stanev, 1990). This current results mainly from the balance between the pressure gradient generated by the wind and the Coriolis force (geostrophic balance). The circulation is then modulated by the topography and river discharges (Figure 1). The Rim current is stable throughout the year, with instabilities in summer when the current intensity decreases. These instabilities create eddies and jet that mix coastal and open sea waters. On the northwestern shelf, the circulation exhibits important variability with consequences for the ecosystem. The current shows reversal following seasons which changes sediment and nutrient supply (Gregoire & Beckers, 2004). At low river discharges and strong

northerly winds, the current is cyclonic while it is anticyclonic at low river discharges and low winds patterns (April-May to November).



Figure 1: Main currents and gyres in the black sea. (Toderascu & Rusu, 2013)

The water and salt interactions of the basin can be described as follows.

The Bosphorus strait connects the Black Sea with the Mediterranean Sea via the Marmara Sea. This is the only connection of the Black Sea with other seas. Thus, it is the only way for salty water to enter the sea (Konovalov et al., 2003). On the strait, Jarosz et al., (2011) found that the surface layer has an average salinity of 18.04 PSU while the bottom layer has an average of 35.97 PSU. The upper layer is on average an outflow while the bottom one is more an inflow. Regarding this characteristic, it is known that the Bosphorus inflow creates deep waters which are more concentrated in salt than the Black Sea water which enhances the stratification. It is also a flow which brings nutrients into the cold intermediate layer.

Regarding the river discharges, the Danube represents the main influx of freshwater into the Black Sea. It represents about 75% of the freshwater inputs in the Black Sea (Grégoire et al., 2008). This input of fresh water has a direct consequence in keeping low salinity to the surface water. Indeed, the vertical mixing is restricted. Surface salinity has characteristic values under 17PSU along the western coast while the salinity in the center of the sea is $S \sim 18.0-18.5$ PSU at the surface and ~ 22 in the bottom (Grégoire et al., 2008).

Regarding the nitrogen supply that the rivers represent, estimations were made by (Codispoti & Christensen, 1985). Their results computed a $25\text{Tg N}\cdot\text{yr}^{-1}$.

Why is the black sea a highly stratified basin?

It is well known that the Black Sea is a highly stratified basin where the salinity (over the temperature), mainly drives the stratification of the basin (Senderov et al., 2019).

To explain this situation, it is needed to analyze the past. Before the rising of the ocean level that occurred 8000 years ago, the Black Sea was a fully enclosed freshwater lake (Degens & Ross, 1972). With the rise of the ocean level, the Mediterranean Sea overflowed into the Black Sea and created an input of salt water, which was consequently heavier than freshwater. Therefore, Mediterranean waters sank below the freshwater, which created a strong halocline. (Stanev, 1990) showed that the vertical circulation was ten times above the integrated horizontal circulation. This phenomenon inhibited the deep water mixing and then enhanced the formation of hydrogen sulfide in deep water and since that, Black Sea is considered as the largest sulfidic and anoxic basin in the world.

Moreover, the rivers discharges which were described, are increasing the stratification of the sea. The input of lighter water inhibits the mixing of the surface water in depth, lowering the salinity in surface water. (Grégoire et al., 2008).

Even though the permanent halocline inhibits the mixing of the water at depth, two types of ventilations can be considered in the Black Sea (Stanev et al., 2014).

First, we must introduce what we call the “Cold Intermediate Layer” (CIL). It is a layer of water that is theoretically the coldest in the sea. The CIL is colder (heavier) than the upper layer of water and fresher than the lower layer which sticks it at a given depth. This property gives typical density value for the CIL: Regarding density, it is approximately situated at $\sigma \approx 14.5$ (Fuchsman et al., 2008). The winter cooling of upper and lighter water and the Bosphorus strait intrusion are at the base of the cold intermediate water composition (CIL) which is found between 50 and 100 meters depth (Stanev, 1990); (Stanev et al., 2014). We often consider that the CIL is the lower boundary for the vertical mixing (Fuchsman et al., 2008), its connection with the upper layer depends on the weather (Tolmazin, 1985), the more energy is introduced into the sea by winds, the deeper will be the mixed layer. Because it is formed by cold waters

coming from the surface, the CIL is well oxygenated. Its propagation contributes to the basin ventilation.

The second ventilation mechanism, which is less efficient, is linked to the intrusion of the well oxygenated Mediterranean waters via the Bosphorus strait (around 36PSU (Stanev, 1990)). These waters are enriched in oxygen relative to the water where they are brought, which oxidize intermediate waters. As a consequence, the intrusion of these oxygen-rich water contributes to oxidize the flux of H₂S coming from the anoxic layer.

The Black Sea's biogeochemistry

As a first simplification, the structure of the Black Sea can be divided into two main layers: the oxic layer which extend from the surface up to 100-150m and the anoxic layer that extends to the bottom and is enriched with ammonia and sulfide produced by the degradation of organic matter using sulfate reduction (Murray & Yakushev, 2006). 85% of the Black Sea's volume is sulfidic and anoxic which makes it the largest euxinic basin in the world.

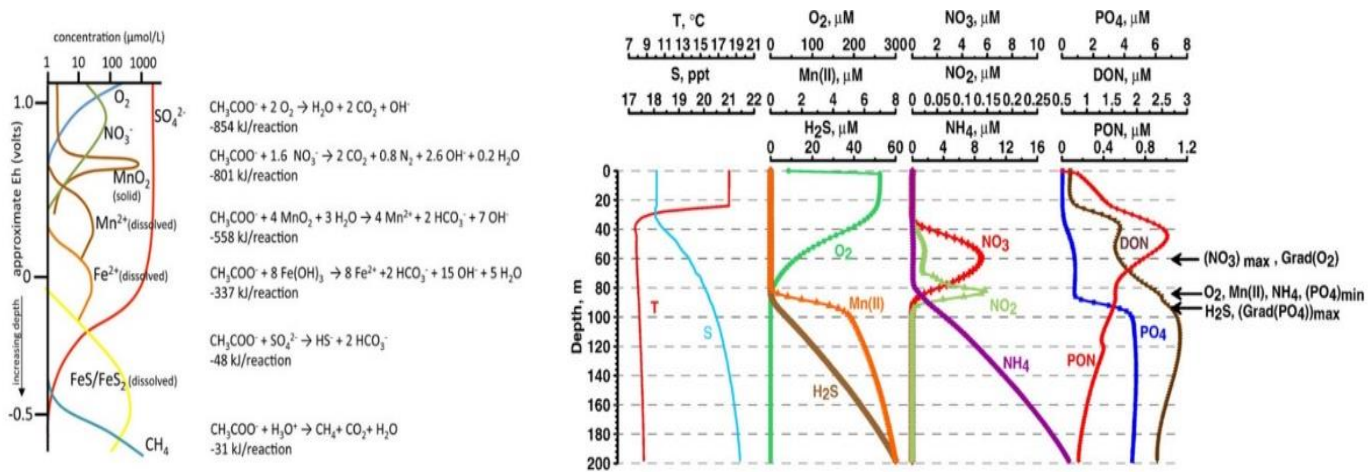


Figure 2: Microbial reactions for oxygen depletion in sediment and in ocean environments. (Yakushev et al., 2007)

Due to the presence of a vertical oxygen gradient, different redox reactions will occur along the depth involving the N, C, S, P, Fe, Mn cycles. The understanding and quantification of these processes are essential for the assessment of the budget of biogeochemical elements in the

Black Sea. Figure 2 compares the microbial redox reactions that are induced in a sediment and in the Black Sea's water column because of oxygen depletion. The difference with the sediment zone is that the depletion of oxygen and the redox zone are distributed over several tens of meters in the Black Sea (~30 m) instead of a few millimeters in the sediment. (Murray & Yakushev, 2006).

Very often, the layer where redox reactions take place is called the "suboxic layer". In the suboxic layer, the oxygen conditions are intermediate between the oxic and anoxic zones, usually between 0-20 μM . It is the depth where the degradation of organic matter occurs via denitrification. This last 10 years, the use of advanced techniques for O_2 measurements (STOX sensors) highlighted that denitrification can be inhibited by O_2 concentrations at nano levels. (Tiano et al., 2014) demonstrate that the observation of O_2 concentrations of a few μmoles in regions where denitrification takes place was an artefact and results from the incapacity of the Winkler technique to measure very low O_2 levels. Thus, (Canfield & Thamdrup, 2009) recommends that the suboxic denomination has to be banished from our vocabulary. The concentration of oxygen is too low to measure it precisely and brought researchers to misunderstanding. Consequently, we assume that the suboxic definition is confusing and does not define clearly distinct reactions. This appellation is therefore outdated.

The vertical profiles of biogeochemical variables are often represented as a function of density rather than depth in the Black Sea to get rid of the spatial and temporal variability associated to physical processes (that are assumed to occur mainly along isopycnals). Like that, variability of the vertical profiles on a density scale is due to biogeochemical processes or diapycnal processes.

Consequently, the Black Sea is well known for its stable water column and for the absence of oxygen at depth. This makes that the Black Sea is often considered as an ideal laboratory where processes typical of the world ocean like ventilation, denitrification, nitrogen fixation can be more easily investigated.

Vertical profile

The vertical profile of O_2 presents a maximum usually at the surface, while in summer it could be more in depths due to the presence of subsurface bloom. Deeper, the O_2 concentration decreases to 0 and we have the formation of an oxycline. Most of the time, oxygen found at 85m ($\sigma = 15.83$) is equal or below to $5\mu\text{M}$. The oxygen depletion goes up to 110m depth ($\sigma = 16.15$), after which it is not possible to find oxygen (figure 3).

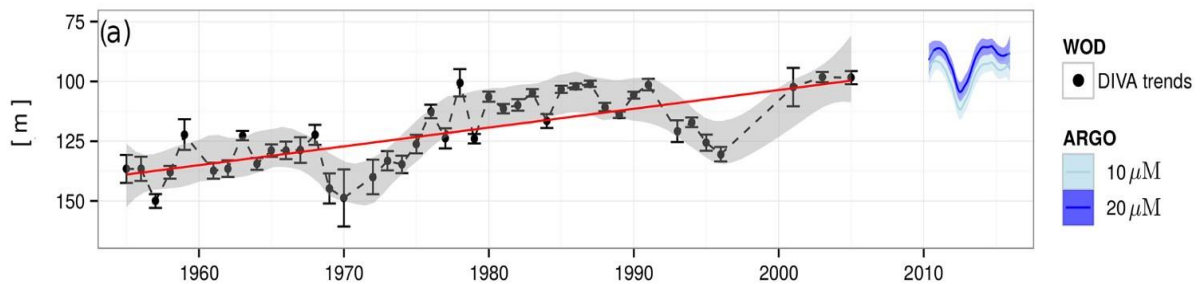


Figure 3: Oxidation/Anoxic layer evolution through time (Capet et al., 2016)

We note that (Capet et al., 2016) showed that with time, the oxycline has a clear tendency to go shallower (figure 3). Knowing that we are computing 2017-2018, it should not be a surprise to observe constant anoxia under 100m.

The nitrate (NO_3^{2-}) has a maximum which is reached at 76m depth ($\sigma = 15.61$) and has a concentration of $5\mu\text{M}$ approximately. We can also note that the nitrite (NO_2^-) in the Black Sea has a concentration that is slightly elevated and does not show a particular peak. Indeed, its concentration varies between 0.02 and $0.042\mu\text{M}$ at 55, 80 and 105 meters. (Lam et al., 2007) only observed nitrite above the detection level at these different depths. Regarding ammonium, we cannot detect it before a concentration in oxygen below $10\mu\text{M}$. In the oxic part, ammonium levels are under 0.08nM (there are local exceptions) and its concentration starts to increase with depth at the end of the nitrate peak. Indeed, when oxygen is present with ammonium, this latter will be oxidized and then transformed into nitrite and nitrate, consequently, we do not observe nitrate and ammonium peak at the same location (Lam et al., 2007) (figure 4).

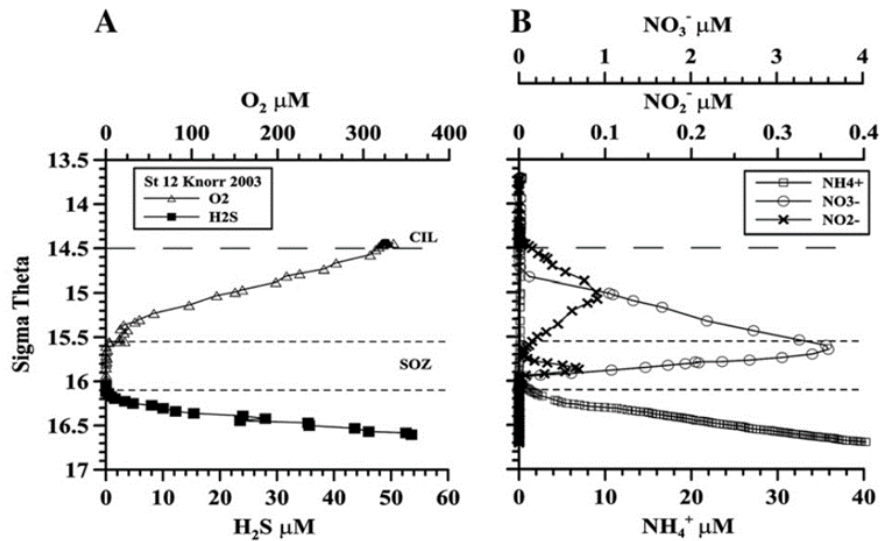


Figure 4: Density profile (sigma-t) in function of nutrient (μM). (Fuchsman et al., 2008)

As we can see in figure 4, the concentration of dihydrogen sulfide only starts to increase after the depletion of oxygen (>16 sigma t). Under the oxygenated layer, the concentration in dihydrogen sulfide never decreases. It reaches approximately $400\mu\text{M}$ at the bottom of the sea (Fuchsman et al., 2008).

As a consequence, the biological characteristics are constantly driven by the biogeochemical condition of the layer in which they belong. Moreover, the lateral loadings of nutrients by the river or through the Bosphorus strait modify consequently the local conditions for life development. Consequently, even if the Black Sea has a similar vertical profile everywhere on a density scale, local variations could also affect the biogeochemical conditions.

Following these characteristics, it is a playground for scientists, regarding oxidoreduction reactions. It is found that microorganisms living in this area are well adapted to these biogeochemical conditions and are capable to catalyze oxidoreductions reactions which are different following the biogeochemical conditions (Murray & Yakushev, 2006).

Anammox, denitrification and nitrification

Two reactions are involved in process of producing di-nitrogen gas: heterotrophic denitrification ($2\text{NO}_3^- + \text{Organic Matter} \rightarrow \text{N}_2$) and anammox ($\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + \text{H}_2\text{O}$) (figure 5). These reactions are found where the oxygen concentration becomes negligible (I.e., $\text{O}_2 < 1\mu\text{M}$). On the opposite, we find the nitrification reaction, which leads to the formation of nitrate in oxic conditions.

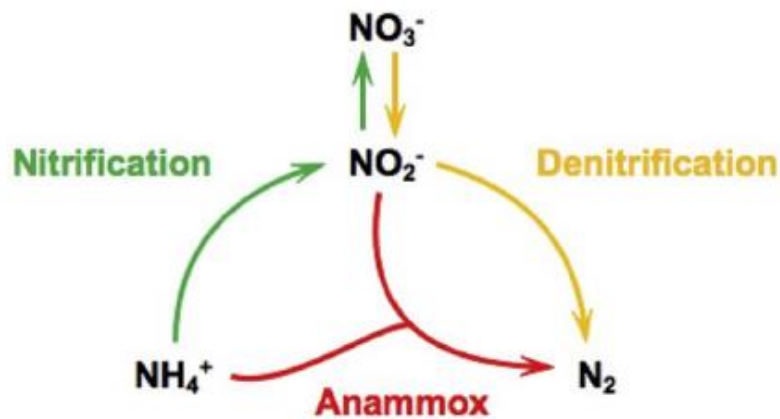


Figure 5: Nitrogen main reactions related to oxygen (Dai et al., 2019)

1) Anammox

Anammox stands for anoxic ammonium oxidation. Anammox is a particular reaction that occurs with ammonium and nitrite. Nitrite has the capacity to oxidize ammonium and the products are water and gaseous nitrogen.

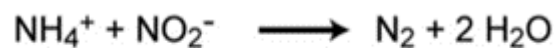


Figure 6: Anammox reaction

However, this reaction occurs only if the environment is anoxic because the anammox reaction is slower compared to the "simple" ammonia oxidation. Due to this characteristic, in oxic conditions, ammonia does not have the time to react with nitrite and simply uses oxygen. It is

the reason why it occurs only in low oxygen environments. This reaction is catalyzed by a monophyletic group of bacteria called: *Planctomyces*.

2) Denitrification

In the other hand, denitrification is the process through which nitrate is converted in dinitrogen (figure 5). Once again, the denitrification reaction occurs in anoxic marine environment. Many researchers pointed out the importance of anammox against denitrification in the Black Sea and all assumed that its rate is in the order of 1% compared to anammox (Konovalov et al., 2008).

3) Nitrification

Finally, nitrification is the process through which ammonium is converted in nitrate. The conditions for the production of nitrate are opposite to the conditions for denitrification and anammox. This reaction typically occurs on the upwelled ammonia through the oxic layer. The presence of oxygen in the medium favor the oxidation of this latter which is transformed in nitrate.

Diazotrophs

Dinitrogen was not always assumed to be fixed. Before the presence of diazotrophs, models considered dinitrogen gas to be inert and fully lost in the water column or in the atmosphere. Since we know that diazotrophs grow into the Black Sea, it would be a significant improvement on the model, especially regarding the nitrogen cycle, to model them. One hypothesis here is that the lost amount of dinitrogen in the Black Sea is too high compared to reality. Indeed, the fixation of dinitrogen through diazotrophs transforms it to ammonia, which brings back nitrogen into the system. This newly transformed nitrogen is therefore not lost in the water column, which obviously reduces the amount of nitrogen that sinks away from the upper layer.

1) Concept and utility of N^*

In classical conditions, nitrogen and phosphorus are supposed to be consumed and released by microorganisms at the same rate, corresponding to the Redfield ratio. To quantify the N_2 uptake by diazotrophs, a method was developed by Gruber & Sarmiento, (1997). Indeed, the computation of N^* and P^* quantify the deviance from their Redfield ratio known as $N/P = 16/1$.

They assume that the reaction of fixing N₂ is a consequence of the deviance in this ratio. It is considered that the di-nitrogen fixation fulfils the lack of nitrate and ammonia fixation which should be 16 times more than the phosphorus uptake. Therefore, we assume here that phosphorus is the limiting nutrient. We assume that this computation gives us a reliable methodology to accurately quantify the amount of N₂ fixed in the water column.

Importance of N cycle

Nitrogen is one of the most important nutrients in marine life. The nitrogen is brought by river discharges, atmospheric deposition and through the Bosphorus strait. It is consumed by the phytoplankton, which represents the primary producers of the Sea. Therefore, we assume that the nitrogen (and its cycle) drives the primary production of the Black Sea which is crucial in modeling purposes.

Moreover, it plays a major role due to its intense consumption by denitrification and Anammox and its production by nitrification. In anoxic conditions, nitrite and nitrate are converted to dinitrogen gas which is an important loss for the fixed nitrogen pool (Fuchsman et al., 2008) while oxic conditions are producing nitrate.

Source/process	Flux, Gg N y ⁻¹	Year of measurement/ estimation	Measured/ estimated	Reference
In-flux				
Atmospheric input	1159±136	2011-2012 (TN)	Meas. and estim.	This study
Fluvial input				
- Danube	362.6	2003-2005 (DIN)	Measured	TDA, 2007; BSC, 2008
	180	1997-2005 (DON)	Estimated	Oguz <i>et al.</i> (2008)
- Dnieper	11.2	1996-2000 (TN)	Measured	UNDP, 2003; BSEP, 1999
- Dniester	22.8	1996-2000 (TN)	Measured	UNDP, 2003; BSEP, 1999
Coastal input	217	1996-2000	Meas. and estim.	UNDP, 2003; BSEP, 1999
Flow to the Black Sea <i>via</i> Bosporus	60	1986-1992 (TN)	Meas. and estim.	Polat and Tugrul, 1995
Biological N ₂ fixation	462	1965-1980	Estimated	McCarthy <i>et al.</i> (2007) after Sorokin, 2002 and Cole, 1998
Out-flux				
Anammox	345*	[2000-2002]	Meas. and estim.	Kuypers <i>et al.</i> (2003)
Denitrification	106 ^a	[1988-1990]	Meas. and est.	^a Ward and Kilpatrick, 1991
	1000 ^b	1991-1996	Estimated	^b Yakushev and Neretin, 1997
Sedimentation	142	1995-1998	Meas. and estim.	Teodoru <i>et al.</i> (2007)
Flow to the Marmara Sea <i>via</i> Bosporus	190	1986-1992 (TN)	Meas. and estim.	Polat and Tugrul, 1995

* - estimated for sea area with depth >80 m; [] - approximate period

Figure 7: Nitrogen budgets in the Black Sea (Sergiy, 2014).

The Black Sea nitrogen Budget

The Black Sea gains bioavailable nitrogen via the inputs from the rivers ($396\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$), the atmosphere ($1159\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$) and the Mediterranean Sea ($60\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$) while the fixation of nitrogen gas ($462\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$) is favored when the losses are through heterotrophic denitrification ($106\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$) and ANAMMOX ($345\text{Gg}\cdot\text{N}\cdot\text{y}^{-1}$) (figure 7).

In this master thesis, we will use a model to quantify the nitrogen budget at basin scale. We will model the rates of nitrogen loss (anammox + denitrification) and the rate of nitrogen fixation. As Codispoti & Christensen, (1985) and Brandes & Devol, (2002) pointed out, the production of di-nitrogen gas by heterotrophic denitrification and ANAMMOX is thought to be the major sink of fixed nitrogen in the whole Black Sea. Indeed, as we can see in figure 6, if we consider the Anammox and the denitrification, the production of N_2 is not negligible. N_2 can be fixed by specific bacteria called "diazotroph" that will produce their organic matter from di-nitrogen. When diazotrophs die or are consumed their organic matter will be mineralized to fixed nitrogen compensating for the loss by denitrification and Anammox.

Because of the lack of information, the rates of Anammox, Denitrification and di-nitrogen are not well-known and needs further research to be precisely computed (most of the rates on figure 7 are estimated and not measured for example). Thus, modeling here is a powerful tool to have an estimation of these rates which are not well known and even measured.

We are thus facing a situation where we suppose that the loss of nitrogen that was described in previous studies could be partly compensated by our new variable; the diazotrophs. This capacity of fixing dinitrogen gas is supposed to balance the denitrification and anammox processes.

Objectives

As we have seen during this introduction, the nitrogen cycle is our interest in this master thesis. To do so, we are not simply computing the budgets of nitrogen, but we have also added a new state variable which is supposed to increase our accuracy regarding the nitrogen cycle. Indeed, in the Black Sea modeling, we often observe that we are losing too much nitrogen in the depth

of the oxycline by denitrification and anammox which is an issue for marine life development. As expected, if we miscompute the loss of dinitrogen (too much loses here), our model would not be able to sustain life as it is the case in real life and our understanding of the nitrogen cycle would be biased.

The addition of diazotrophs should decrease this mistake as soon as they are fixing di-nitrogen. We assume that if the di-nitrogen is fixed, then, it goes back in the nitrogen cycle and could be used again in the upper layer, which should decrease our misconception of nitrogen dynamics. This current question was already asked in modeling, but it is the first time that these processes have been computed on a 3D model for the Black Sea.

Therefore, this master thesis aims to assess the usefulness of the presence of diazotrophs in the model to enhance our precision in the modeling of the nitrogen cycle. Knowing that, our first objective of this master thesis is to compute the amount of di-nitrogen fixed by diazotrophs. This latter will be compared with the real losses of nitrogen in depth, which will let us quantify its importance and the possible modification in the budget of the overall sea.

In long term objectives, we would like to assess the state of equilibrium of the nitrogen in the Black Sea. In other words, the aim would be to make long time simulation to assess the state of equilibrium in the actual global warming system.

Material and method

General description

A coupled hydrodynamical-biogeochemical model is run in 3D in the Black Sea. The hydrodynamic model is Nucleus for European Modeling of the Ocean (NEMO 4.06), and the biogeochemical model is the Biogeochemical Model for Hypoxic and Benthic Influenced areas (BAMHBI). Here below we describe the main characteristics of the coupled model with a focus on the module that I have specifically developed for my master thesis.

The hydrodynamical model

Navier-Stokes Primitive equations:

The very basis of the model is built on the Navier-Stokes equations which describe the fluid mechanics on a rotating system (table 1). Unfortunately, these primitive equations are not resolvable if assumptions are not considered. Following the NEMO BOOK (Gurvan et al., 2017), 6 main assumptions are made to correctly resolve the model.

First, the spherical earth approximation assumes that the local gravity is always perpendicular to the earth's surface. Second, the thin shell approximation conveys the fact that the depth is smaller than the horizontal axis, which often implies slow fluid advection along the vertical. Following, turbulent fluxes are computed in terms of large-scale features which means that the Kolmogorov's cascade depends on the turbulent kinetic energy on larger scales. As a comparison, the more we stir our café au lait the faster and the stronger the mixing will be. Next, density variations are neglected along the water column except for the buoyancy force. After that, the hydrostatic hypothesis implies that the vertical momentum is a balance between the vertical pressure and the buoyancy force. Finally, the fluid is considered incompressible, divergence of the velocity vector is equal to 0.

Considering (i,j,k) , an orthogonal set of three-dimensional vectors where k is the local upward vector while (i,j) are orthogonal horizontal vectors, we can define variables that are computed

along them. The vector velocity is \mathbf{U} , T is the temperature, S the salinity and ρ the in-situ density.

The following set of equations is the one described in the NEMO-BOOK (Gurvan et al., 2017):

$$\frac{\partial \mathbf{U}_h}{\partial t} = - \left[(\nabla \times \mathbf{U}) \times \mathbf{U} + \frac{1}{2} \nabla (\mathbf{U}^2) \right]_h - f \mathbf{k} \times \mathbf{U}_h - \frac{1}{\rho_o} \nabla_h p + \mathbf{D}^{\mathbf{U}} + \mathbf{F}^{\mathbf{U}} \quad 1.1$$

$$\frac{\partial p}{\partial z} = -\rho g \quad 1.2$$

$$\nabla \cdot \mathbf{U} = 0 \quad 1.3$$

$$\frac{\partial T}{\partial t} = -\nabla \cdot (T \mathbf{U}) + D^T + F^T \quad 1.4$$

$$\frac{\partial S}{\partial t} = -\nabla \cdot (S \mathbf{U}) + D^S + F^S \quad 1.5$$

$$\rho = \rho(T, S, p) \quad 1.6$$

Table 1: Navier-Stokes primitive equations. (Gurvan et al., 2017)

In this set of equations, t is the time, z the vertical axis, p the pressure, f is the Coriolis parameter and g the gravitational acceleration. D terms are parametrization for small-scale physics which will be described further. F terms are linked to the surface forcing terms.

Turbulent kinetic energy

Thereafter, an amount of kinetic energy is transferred to turbulent energy and needs to be modelled. This behavior is computed with TKE which is the turbulent closure scheme (TKE stands for Turbulent Kinetic Energy). The given prognostic equation provides a closure model following \bar{e} , the computed turbulent kinetic energy.

$$\frac{\partial \bar{e}}{\partial t} = \frac{K_m}{e_3^2} \left[\left(\frac{\partial u}{\partial k} \right)^2 + \left(\frac{\partial v}{\partial k} \right)^2 \right] - K_\rho N^2 + \frac{1}{e_3} \frac{\partial}{\partial k} \left[\frac{A^{vm}}{e_3} \frac{\partial \bar{e}}{\partial k} \right] - c_\epsilon \frac{\bar{e}^{3/2}}{l_\epsilon} \quad 2.1$$

Table 2: Turbulent Kinetic Energy. (Gurvan et al., 2017)

Regarding the sub grid scale processes, we already mentioned that they are computed with a dependency for the larger scale processes. The formulation of these variables is analogous to the molecular dissipation and diffusion. Different equations are written to compute different motions for vertical and horizontal movements and for the different tracers (U, T, S)

Model grid and resolution

The current version of the physic model is run in operational and reanalysis mode for the Copernicus Marine Service (<https://www.copernicus.eu/fr/node/26412>), which solves the whole Black Sea with a horizontal resolution of 3 km and 31 vertical layers unevenly spaced cartesian z-layers. The vertical levels are positioned to better solve the surface and oxic/anoxic interfaces where biogeochemistry is particularly active due to the presence of light and the availability of nutrients. Therefore, the upper layers need to be more precise, which is the reason why the first one is only 0.5m deep while the bottom layers are larger than 100m. This discretization of the sea provides a simple numerical discretization and visualization, making the Interpretation of the results effortless. It resolves the pressure gradient which is important for the chemical correlation. However, the z-coordinate ignores small bottom slopes which is not a big concern knowing that the biochemical processes stop under 150m.

The biogeochemical model: BAMHBI Generalities

The Biogeochemical Model for Hypoxic and Benthic Influences areas (BAMHBI) describes the marine food web of the sea along 34 state variables going from the lowest trophic levels such as phyto- and zooplankton to the largest gelatinous zooplankton. It also simulates the chemical reactions that are driven by the local conditions (mainly following oxygen concentration which drives redox reactions through oxygen depletion).

Moreover, benthic processes are computed on a specific module comprised in the model. It considers mostly the continental shelf and is not crucial regarding the computation of the nitrogen dynamics, which is the reason why we do not use it in this master thesis.

Since the Black Sea has oxygen depletion which deprived oxic conditions under 150m depth, redox reactions are driven by the local condition and need to have an accurate rate of reactions which matches the environment. Consequently, the most important chemical processes are nitrification/denitrification, annamox and (di)nitrogen fixation. For this purpose, diagenetic processes are modelled at the interface between the oxic and the anoxic layer which couple them. This latter variable is called ODU and encompasses all the reduced molecules.

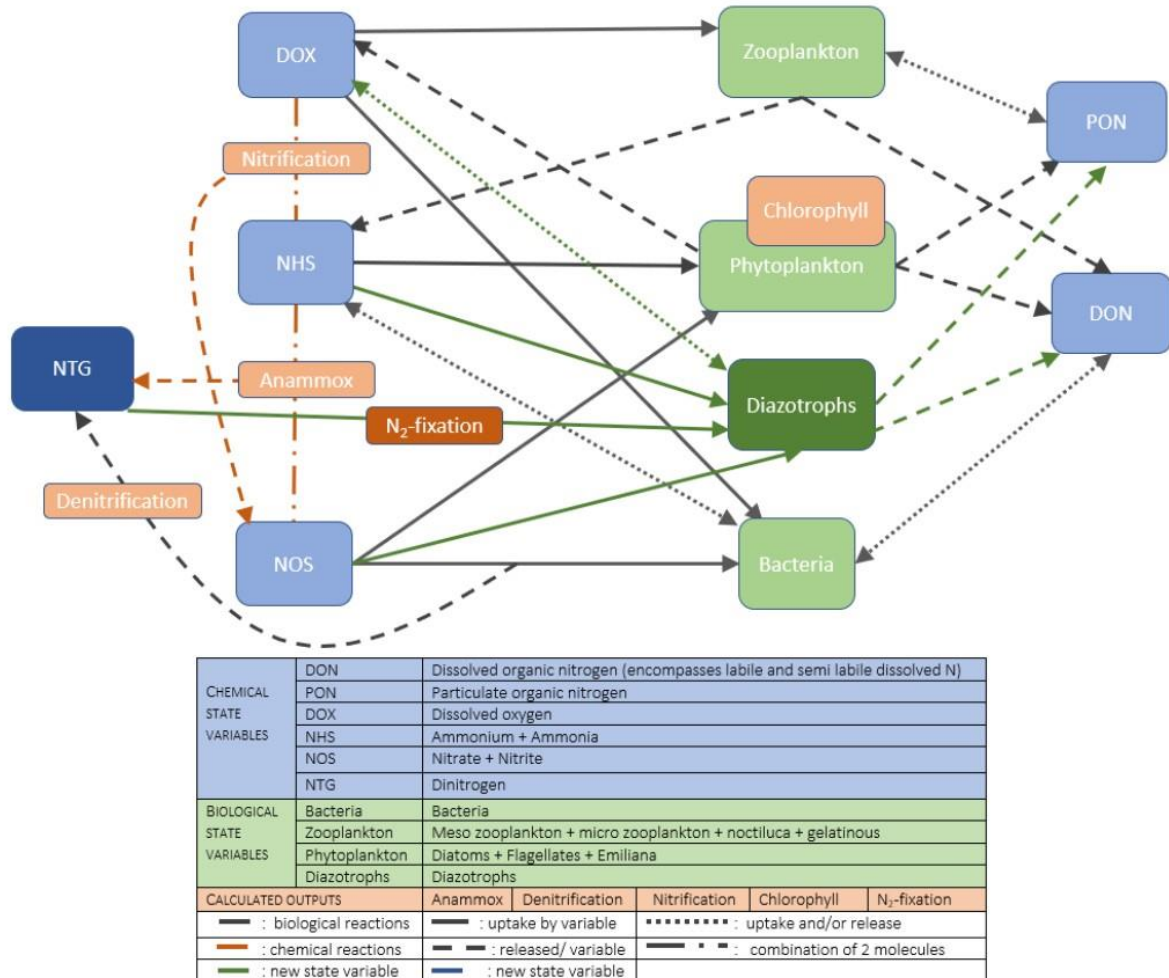


Figure 8: Schematic representation of the model (Meulders & Université de Liège > Master en océanogra., 2019)

Nitrification/Denitrification

More precisely, inorganic nitrogen is compound of two state variables which are NHS (reduced form of the nitrogen = ammonia and aggregated ammonia) and NOS (oxygenated form of the nitrogen = nitrate and aggregated nitrate). Their sources and sinks are shown in figure 8. Both

can be taken up by phytoplankton but the energy requirement to fix this form are different because of their different oxidation state.

Every reaction is following the nitrification rate, which is based on a limiting term (considering the concentration of oxygen at a given (l,j,k) position), on temperature, on ammonia and oxygen availability (at the same (l,j,k) position). Regarding the temperature, it affects the kinetic and is assumed to be computed at 20°C for these reactions.

Anammox

At the end of the 20th century, a phenomenon was discovered in bioreactors that permits oxygenation of ammonium in near anoxic environments. This phenomenon is catalyzed by *Planctomyces* bacteria phylum. The discovery of this reaction brought a second way to transform ammonium (or nitrate) into gaseous nitrogen.

Thus, it was needed to understand this process and search for this reaction in the Black Sea. (Dalsgaard et al., 2003); (Kuypers et al., 2003); (Thamdrup et al., 2006) all found non negligible rates of this reaction in the Black Sea. Unfortunately, only little was known regarding anammox. They didn't know exactly which are the regulators or where it occurs and even at which rates. Two main chemical components drawn the attention of the scientists and are described below.

First, anammox is restricted by oxygen. At the very beginning (Strous et al., 1997) postulated that the reaction was totally inhibited by oxygen, even if the concentration was as low as 1µM. This idea was revised when (Kuypers et al., 2005) found that anammox could occur in the Benguela upwelling system, which is not strictly anoxic (with O₂ concentration up to 9µM of oxygen).

On the other hand, hydrogen sulfide is also active in this reaction. (Jensen et al., 2007) has shown that hydrogen sulfide concentration could inhibit anammox reactions. Indeed, in Denmark, it is possible to find high rates of denitrification while anammox is not measurable because of the presence of hydrogen sulfide. Research and experiments are lacking regarding this point, it is impossible to explain if it is due to competitive or normal inhibition. (Jensen et al., 2008).

Regarding the two latest points, we can support the observation for the anammox process. It is active at low oxygen concentration where the hydrogen sulfide is not detectable. There, the concentration in oxygen must be below $10\mu\text{M}$. (Kuypers et al., 2003) has shown that Planctomyces was present in this zone where they are responsible for the consumption of ammonium.

Diazotrophs

Further, diazotrophs were recently added to the model. Indeed, with the aim of characterizing the nitrogen budget throughout the Black Sea, we cannot neglect the presence of these nitrogenous algae. They are closely related to the nitrogen cycle; they interact with different forms of nitrogen where their particularity is to fix dinitrogen gas under low oxic conditions which was not considered in previous models of the Black Sea. Moreover, our model contains a state variable to represent dinitrogen which is also rare for Black Sea modelling. The denitrification and anammox reactions are feeding this variable while the N_2 uptake led to lose of this latter.

As we mentioned in the introduction, the computation is based on the deviance of the redfield ratio. The di-nitrogen uptake is active when the redfield ratio of N/P goes under 16. Therefore, we compute a diagnostic called “N*” which is described here:

$$\text{N}^* = \text{NOS} - 16 \text{PHO}$$

Following literature, Deutsch et al., (2007) (and many others), decreases in the N/P ratio may reveal significant rates for N_2 fixation. When the inorganic nitrogen is depleted compared to phosphate, we consider in our model that the dinitrogen has to compensate the missing nitrogen uptake, based on phosphate.

Therefore, it would be interesting to study this rate to shed light on a more accurate nitrogen fixation into the water column.

In other models (such as global ocean models), it is common to compute an iron limitation regarding the di-nitrogen fixation, but it is not the case of the Black Sea, which is never missing

iron. Therefore, we will only consider the phosphorus limitation (i.e. (Paulsen et al., 2017); (Sañudo-Wilhelmy et al., 2001)).

These bacteria were first added to a one-dimension bamhbi-nemo Black Sea model and the rates for the dinitrogen uptake were found to be lower than every other study that tried to measure this rate (Meulders & Université de Liège > Master en océanogra., 2019). Indeed, the Black Sea 1D model found that most of the year, diazotroph act like other algae, meaning that they only use N_2 when it is the only nutrient available. However, on a non-negligible part of the year, N_2 is directly fixed by diazotrophs with a maximum rate of $7\mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ and a maximum concentration of $6\text{mmol}\cdot\text{N}\cdot\text{m}^{-3}$ (Meulders & Université de Liège > Master en océanogra., 2019) (Appendix figure A).

Regarding the chemical structure of the di-nitrogen (triple bound between the nitrogen atoms), it is easily understandable that the energy cost to fix it into a microorganism is higher than for nitrate or ammonia. As a result, its respiration will be enhanced to support this physiological behavior. Therefore, its oxygen consumption will be higher, and its carbon production will also be enhanced.

For this purpose, in our model we consider the following equations (table 3)

Along with that, if the quantity of bioavailable nitrate and ammonia is larger than the phosphate uptake, then, the N_2 uptake is cancelled (equations 2.1 & 2.2), and the nitrate and ammonia fixed nitrogen will be equivalent to the phosphorus relative to the redfield ratio (equations 2.8-2.12). The contrary allows the N_2 uptake to fulfill the lack of nitrogen against the phosphorus uptake and is described by the equation called " N_2 uptake" (equation 2.7).

A 0.5 coefficient is used to take the right amount of N. Indeed, if we take the phosphorus mole equivalent for the N_2 , the diazotrophs will uptake two times more nitrogen compared to what is required (compared to the amount of nitrogen uptake if the diazotrophs only use NO_3^- and NH_4^+).

Moreover, literature (i.e., Paulsen et al., 2017) mentions that the sink term for the diazotroph is only natural mortality because the grazing of the zooplankton on these cells is negligible. Therefore, it is not needed to compute for grazing by zooplankton in this case.

if $\text{NH}_s \text{ uptake} + \text{NO}_s \text{ uptake} > \frac{\text{puptake}}{\text{PNRedfield}_{\text{diaz}}}$,	3.1
$\text{N}_2 \text{ uptake} = 0$, otherwise :	3.2
if $\text{NH}_s \text{ uptake} + \text{NO}_s \text{ uptake} < \frac{\text{puptake}}{\text{PNRedfield}_{\text{diaz}}}$,	3.3
$\text{NO}_s \text{ uptake} = \text{NO}_{\text{umax}} * f^T * (1 - \frac{N:C}{(N:C)_{\text{max}}}) \frac{\text{NO}_s}{\text{NO}_s + k_{\text{NO}_s}} \frac{k_{\text{in}}}{k_{\text{in}} + \text{NH}_s} \text{CDZ}$	3.4
$\text{NH}_s \text{ uptake} = \text{NH}_{\text{umax}} * f^T * (1 - \frac{N:C}{(N:C)_{\text{max}}}) \frac{\text{NH}_s}{\text{NH}_s + k_{\text{NH}_s}} \text{CDZ}$	3.5
$\text{PO}_4 \text{ uptake} = \text{P}_{\text{umax}} * f^T * (1 - \frac{P:C}{(P:C)_{\text{max}}}) \frac{\text{P}}{\text{P} + k_{\text{P}}} \text{CDZ}$	3.6
$\text{N}_2 \text{ uptake} = \frac{1}{2} (\frac{\text{puptake}}{\text{PNRedfield}_{\text{diaz}}} - (\text{NH}_s \text{ uptake} + \text{NO}_s \text{ uptake}))$	3.7
for $\text{NH}_s \text{ uptake} + \text{NO}_s \text{ uptake} \geq \frac{\text{puptake}}{\text{PNRedfield}_{\text{diaz}}}$	3.8
$\text{NO}_s \text{ uptake} = \text{NO}_{\text{umax}} f^T * (1 - \frac{N:C}{(N:C)_{\text{max}}}) \frac{\text{NO}_s}{\text{NO}_s + k_{\text{NO}_s}} \frac{k_{\text{in}}}{k_{\text{in}} + \text{NH}_s} \text{CDZ}$	3.9
$\text{NH}_s \text{ uptake} = \text{NH}_{\text{umax}} f^T * (1 - \frac{N:C}{(N:C)_{\text{max}}}) \frac{\text{NH}_s}{\text{NH}_s + k_{\text{NH}_s}} \text{CDZ}$	3.10
$\text{puptake} = \text{P}_{\text{umax}} f^T * (1 - \frac{N:C}{(N:C)_{\text{max}}}) \frac{\text{P}}{\text{P} + k_{\text{P}}} \text{CDZ}$	3.11
$\text{N}_2 \text{ uptake} = 0$	3.12

Table 3: Di-nitrogen uptake computation following Phosphorus and nitrate availability.

Coupled model:

The coupled BAMHBI-NEMO are described in this section. Here we will describe the ODU behavior, the parametrization of diazotrophs and we will provide the boundary conditions that are performed to simulate the Black Sea environment. Moreover, the used simulation of this model will be described (temporality, initial conditions, and parametrization). In addition, we will describe the tools that are used in order to validate the model.

Oxidation modelling in anoxic environment: ODU

Several oceanic components are driven by redox equilibrium (Fe, Mn, H₂S), therefore, they can be oxidant or reductors which confer an important role in the oxic/anoxic environment. In modelling purposes, it is a tough task to model them separately and to associate them with

specific rates of reactions. To avoid this problem, a variable was created which represents all of them and is called ODU. This method was first applied by (Soetaert et al., 1996) and the principle is reused in this model. It is important to note that one mole of carbon produces one mole of ODU while the oxidation of this latter requires one mole of oxygen. This way of modelling is accurate for this master thesis as soon as we are not interested in iron and manganese cycle.

Model implementation

Parameter	Value	Units	Justification
Q10Diaz	1.8	/	Reaction temperature constant
MaxNCrDiazotrophs	0.2	/	Maximum N/C inside diazotrophs
MinNCrDiazotrophs	0.05	/	Minimum N/C inside diazotrophs
MinChINrDiazotrophs	1	/	Maximum Chl/N inside diazotrophs
MaxChINrDiazotrophs	2	/	Minimum Chl/N inside diazotrophs
NosMaxUptakeDiazotrophs	0.000011574	s ⁻¹	Maximum nitrate uptake rate
ksNOsDiazotrophs	2.0	/	Half-sat constant for nitrate uptake
NhsMaxUptakeDiazotrophs	0.000017361	s ⁻¹	Maximum ammonium uptake rate
ksNHsDiazotrophs	1.0	/	Half-sat constant for ammonium uptake
PO4MaxUptakeDiazotrophs	0.000000723	s ⁻¹	Maximum phosphate uptake rate
ksPO4Diazotrophs	0.2	/	Half-sat constant for ammonium uptake
N2MaxUptakeDiazotrophs	0.000000723	s ⁻¹	Maximum di-nitrogen uptake rate
ksN2Diazotrophs	0.2	/	Half-sat constant for di-nitrogen uptake
alphaPIDiazotrophs	0.000003472	s ⁻¹	Half-sat light intensity
MuMaxDiazotrophs	0.000023148	s ⁻¹	Max growth rate
RespirationDiazotrophs	0.000000104	s ⁻¹	Respiration rate
GrowthRespDiazotrophs	0.000001157	s ⁻¹	Growth rate linked to the respiration
MortalityDiazotrophs	0.0000003472	s ⁻¹	Mortality natural rate
Dfix_diaz	0.000001389	s ⁻¹	Extra parameter for diazotrophs respiration

Table 4: Parametrization of diazotrophs

Boundary conditions:

a) Atmospheric forcings:

The atmosphere boundary of our coupled model NEMO is now forced by an atmospheric model named ERA5. This model belongs to ECMWF within the Copernicus Climate Change Service (C3S) considering the land surface and the wave form. It presents a 31km horizontal resolution with 3h and 6h as temporal resolution. It computes the atmospheric forcing (temperature, humidity, cloud coverage...) and is used to model the air-sea exchanges of O₂ and CO₂ (Causio et al., 2021).

b) Lateral boundaries forcing:

Lateral boundaries are essentially the rivers and the Bosphorus strait.

1) Rivers

The actual BS-BIO system includes 6 different rivers. Danube, Dniepr, Dniestr, Rioni, Sakarya, and Kiziliark. The Danube, which is by far the most important river and therefore, the most important freshwater inflow, is the only river which is computed by near real time (NRT) data for its freshwater discharge (Grégoire et al., 2008). Its river mouth is decomposed into 3 different arms as is the data. Regarding the other rivers, nutrients and fluxes load are computed by climatological parameters. These climatological parameters compute inorganic and organic nutrients loading. These latter are obtained with the long time series data given by (Ludwig et al., 2009)

2) Bosphorus strait

The Bosphorus strait is considered as an open boundary which is a common feature in the Black Sea models (Stanev et al., 1997); (Stanev & Beckers, 1999). The Bosphorus Strait is the only open lateral boundary of the Sea and therefore is crucial in the overall budget. For modeling purposes, velocities and salinity are computed to have a conservation along time. To have this result, the temperature has no gradient across the strait (Hersbach et al., 2019).

3) Land sea boundary

Regarding the land-sea boundary, every variable is multiplied by what we call a “mask-array”. This latter is a matrix compound of 0 and 1, when the location corresponds to a wet area, the variable is multiplied by 0 to ensure the fact that none of the variables has a value on land. On the opposite side, the variables are multiplied by one when they are in the water.

Simulation length and time step

The current model will be running for 8 years (2012-2019) but only two of these years will be analyzed. For validation purposes, years known with high chlorophyll concentrations will be analyzed. These years are 2017-2018. For the time step, the model has 576-timesteps in one day but only one value is given.

Initial conditions

For the initial conditions, we use restart which are the conditions that we find at the last time step of the past year. To obtain these conditions at the very beginning of model, we implement the lowest concentrations, and the model is run for years until to reach a state of equilibrium. This state of equilibrium is then the initial state of the model for year number one. In this master thesis, we will then use the initial conditions given by the restart of the end of 2016.

Validation framework:

The 2017 and 2018 years are validated against ARGO. These data were collected and made freely available by the International Argo Program and the national programs that contribute to it. (<https://argo.ucsd.edu>, <https://www.ocean-ops.org>). The Argo Program is part of the Global Ocean Observing System.

Argos data are a powerful tool for forecasting the ocean. Indeed, since their development, we are able to collect data in depth and without dependency on the meteorological conditions

(which is not the case for the satellites data). On the global ocean, we find more than 3000 floats and are capable of measuring several components (I.E. salinity, oxygen, temperature, chlorophyll etc.) down to 2000m depth. It is thus a reliable instrument to validate our model. However, all the Argo floats are not capable of measuring biogeochemical parameters.

The validation script for oxygen and temperature was performed with a python script. The profile's location varies in time and in position as soon as it has to follow the Argo float. Figure 9 display the trajectory followed by the Argo that sampled the oxygen.

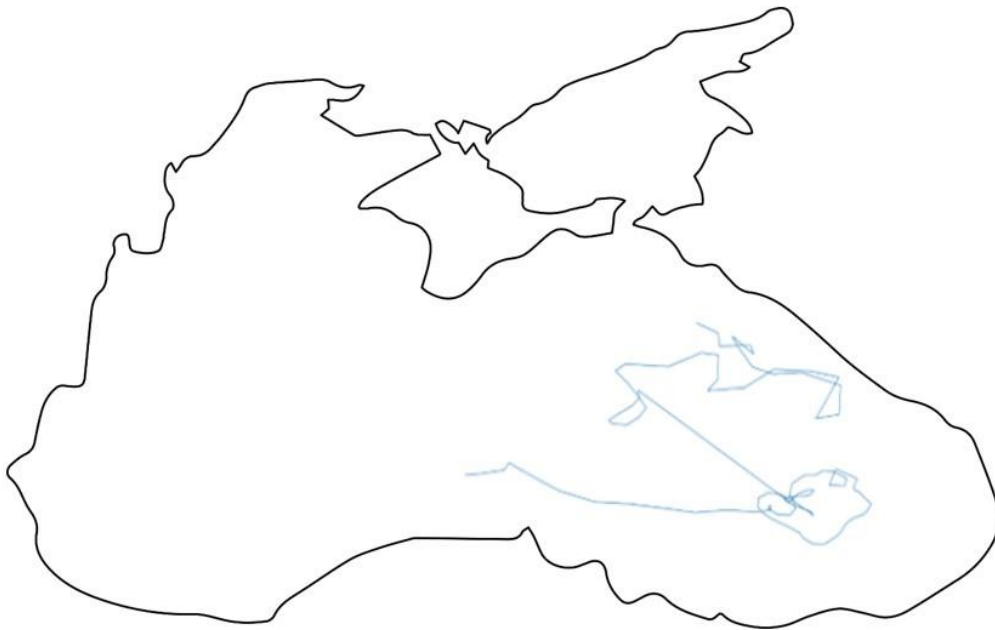


Figure 9: Argo float trajectory for oxygen in the Black Sea

A Julia script was used to compare the chlorophyll profile along the year. The script gives comparison for the profile themselves, the maximum depth, and the maximum concentration of chlorophyll. To do so, every month has a mean value and is compared to the mean value for the ARGO profile. The script gives us statistics (pearson correlation, bias...) which gives us indication on the reliability of the model.

Results

Results are given in this section for two consecutive years: 2017-2018. We first compare model state variables with in-situ data. We use ARGO for temperature, oxygen and chlorophyll and ship data for nutrients.

The vertical profiles of temperature, oxygen and chlorophyll simulated by the model and obtained from Argo are compared. Before starting the comparison, we underline that Argo floats are not directly measuring chlorophyll but instead perform a measure of fluorescence from which the chlorophyll concentration is estimated using empirical equations. These equations are not adequate for oxygen deficient environments as underlined by (Ricour et al., 2021).

Following, we will analyze the main nutrients, such as nitrate and ammonium. The main goal of displaying these nutrients is to compare them with literature to assess if their concentration is in the right order of magnitude.

We are also comparing simulated and data-retrieved rates of dinitrogen fixation, anammox, nitrification and denitrification throughout the year. As we do for the nutrients, we will also compare these rates to the literature.

A revised nitrogen budget of the Black Sea is discussed. In particular we analyze how nitrogen fixation can compensate for fixed nitrogen loss by heterotrophic denitrification and anammox.

Validation

Figure 10 shows the vertical profile (until 100m) of temperature compared to in-situ measurement, figure 11 displays oxygen and also in situ measurement. Figure 12 shows chlorophyll simulated and obtained from Argo floats over 2017-2018.

For temperature and oxygen, the sampling in the model outputs follows the trajectory of the Argo float in order to make the datasets comparable (the trajectory for the temperature and the oxygen are not the same). Oxygen and temperature profiles are plotted against time and

depth, which display the temporal and vertical variability. The trajectories determined by the Argo floats are plotted in the material and method (figure 9)¹.

Regarding chlorophyll, three main features are compared with observations by month. First, a simple profile comparison, plotting the concentration against depth. Second the maximum concentration by month. And finally, the maximum depth at which we find chlorophyll will also be compared to observations.

1) Temperature

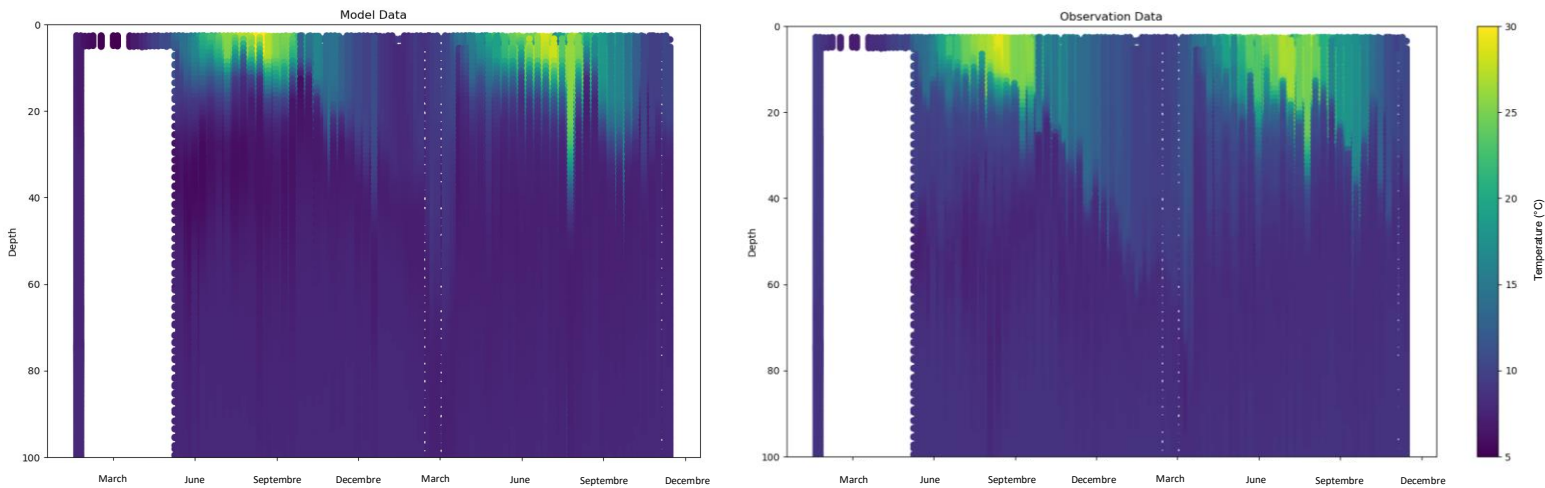


Figure 10: Comparison between the modeled and the ARGO temperature (°C): Left for model right for Argo data over 2017-2018.

Regarding the temperature profile (figure 10), we can first observe that the model and the observation data are similar. Indeed, for one year, we expect to observe a cooling of the surface water during the winter. As consequence, the stratification is lowered, and the mixing is more efficient. It refills the cold intermediate layer before the warming up of the surface water which creates a thermocline. It remains in this state from the beginning of summer until the beginning of the next winter. The breaks of the thermocline bring back to the refill of the cold intermediate layer and the cycle restarts.

¹ The Argo that measured temperature followed another trajectory than the one displayed on figure 9.

The overall behavior of the temperature is well modeled as soon as the main features of the seasonal (warming up of the upper layer in summer) and the vertical variability (presence of the cold intermediate layer and temperature gradient from the surface) are present. Indeed, the warming up of the surface layer and the homogenization of the temperature due to the expansion of the mixed layer is well reproduced in the model even if these phenomena are shallower than in the observed data. However, we observe that the Cold Intermediate Layer is colder and shallower in the model, compared to the observations.

2) Oxygen

As we have seen in the introduction of this master thesis, oxygen is one of the most important variables in the case of anoxic basin as the Black Sea. Its depletion and the location at which we enter in the anoxic layer drives the upper layer dynamics. We then expect to observe concentration of oxygen ranging from $300\mu\text{M}$ on the surface to $>1\mu\text{M}$ at around 100m of depth. As shown (Capet et al., 2016), the anoxic layer is uplifted with time due to global warming. Considering his results, it would not be a surprise to observe anoxia upon 100m depth.

Regarding the oxygen profile (figure 11), we observe that the depletion of oxygen in our model occurs deeper than the observed data, which is a well-known issue for this version of the model. Moreover, we observe a concentration on the surface layer which is larger on the

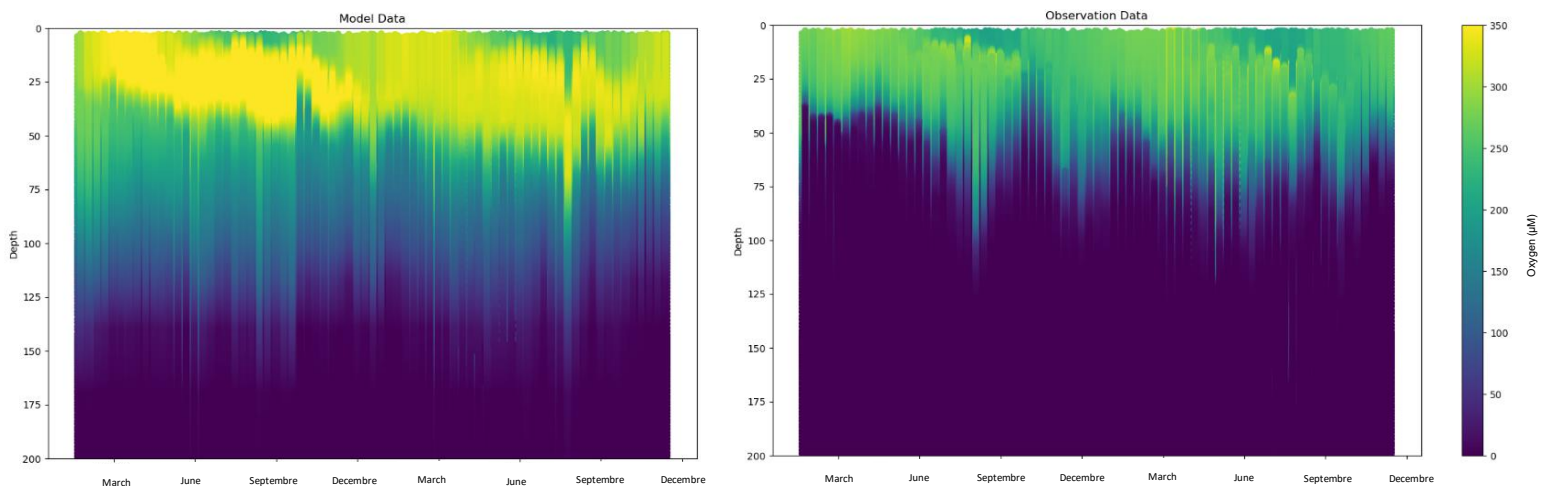


Figure 11: Vertical variations of the Oxygen concentrations (μM) along the Argo floats trajectories (described in Figure 9): left model right Argo data over 2017-2018.

model than on the observed data (ranging until $350\mu\text{M}$ for the data and until $\sim 310\mu\text{M}$ for the observations).

Regarding the seasonal variability of the penetration of oxygen, we can see on the observations that the oxygen could exceptionally penetrate at more than 100m while it is a constant feature for the model.

Moreover, we observe a peak of concentration on the first hundred meters of the column in the model which is not observed by the Argos. This latter is probably linked to the large development of chlorophyll at this depth and to the enhanced stratification that we observe during summer. This feature will be debated further.

3) Chlorophyll

Figure 12 compare the vertical profiles of chlorophyll computed by the model (red lines) and measured by the Argo floats (blue lines). Each graphic corresponds to one month and several profiles are sampled every month.

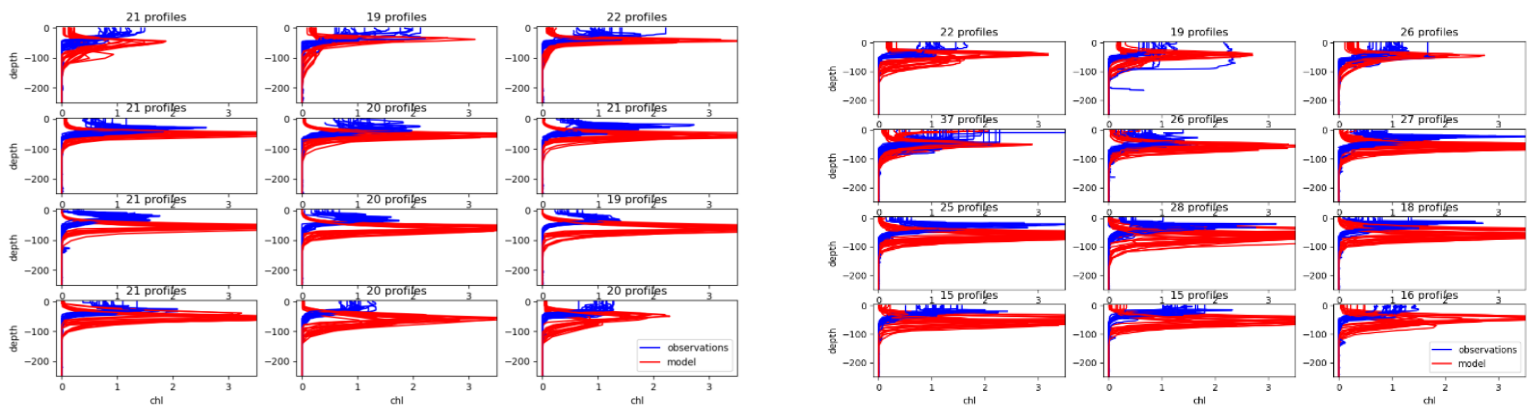


Figure 12: Month by month chlorophyll comparison between the model and Argo floats. Left 2017, Right 2018.

(μM)

Over the year, the Argos on Figure 12 display the vertical chlorophyll profiles. It shows a seasonality variation. Indeed, during the summer, we observe the peak of concentration around 25m depth while the peak of concentration is situated on the top layer the remaining time. Regarding the modeled chlorophyll, we observe that the peak of concentration is never situated on the top layer. Thus, simulated chlorophyll does not undergo the expected seasonality variations in term of depth.

Moreover, in term of concentration, the observed chlorophyll is situated between 1.5 and 3 μ M, where the maximum occurs on early summer (I.E. June 2018). Regarding the model, this concentration often exceed 3 μ M which is too large.

This is a main concern regarding model validation, as soon as the surface chlorophyll is one of the main features of the Black Sea. This will obviously have consequences on the nitrogen budget, knowing that algae are the primary producers and therefore, the first biological species to interact with the nitrogen.

	2017	2018
Pearson correlation between model and Argo	0.13	0.203

Table 5: Pearson correlation between observations and Argo for chlorophyll.

The Pearson correlations are not surprising. Indeed, they are not significantly good, which could be explained by the two reasons that we have pointed out earlier. However, the correlation is larger in 2018. The profiles overlaps more on this year even if the overall correlation remains poorly significant.

Thereafter, regarding the maximum concentration of the chlorophyll for both years, we obtain the following plot:

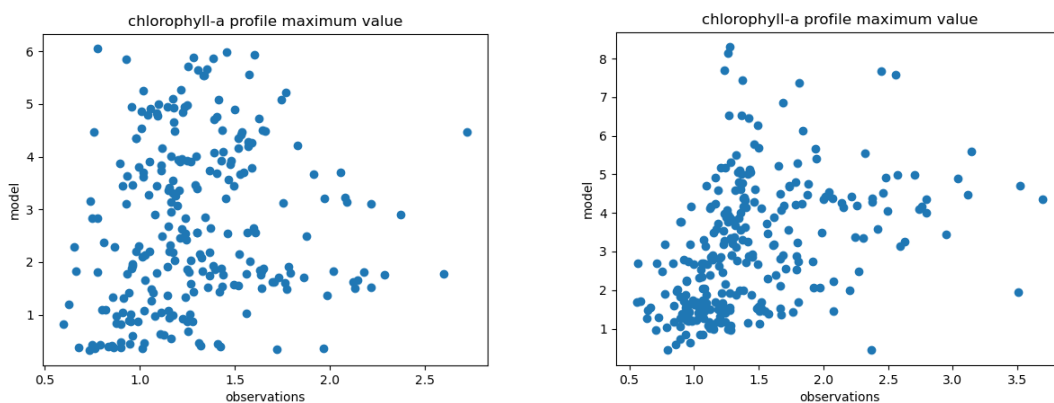


Figure 13: Maximum value of chlorophyll computed by the model compared to the observations made by Argo floats. Left for 2017 & right for 2018.

On this plot, we observe that the maximum value is too elevated (figure 13) and is not surprising. We have already seen in the first part of the validation that the concentration of chlorophyll is often too high compared to the values that are observed. If we take a look on

the axis ranges, we observe that the model axis goes from 0 to 8 μ M while the observation axis only goes up to 3.5 μ M. This conclusion meets our previous consideration regarding our profile validation.

Hence, we observe a positive correlation between the observations and the model:

	2017	2018
Pearson correlation for maximum depth between the model and Argo	0.12	0.415

Table 6: Pearson correlation between observations and Argo for chlorophyll maximum depth.

The correlation is higher on the second year than on the first one. It is not a surprise since we can assume that the observed maximum concentration in 2018 is often closer to the modelled concentration (figure 13). Indeed, for months as June-July and August in 2018, the measured chlorophyll concentration exceed 3 μ M which has consequences on the overall correlation.

Finally, we will analyze the maximum depth at which we find our chlorophyll. Regarding the previous results of the validation, we can expect to have a maximum deep chlorophyll below the expected depth.

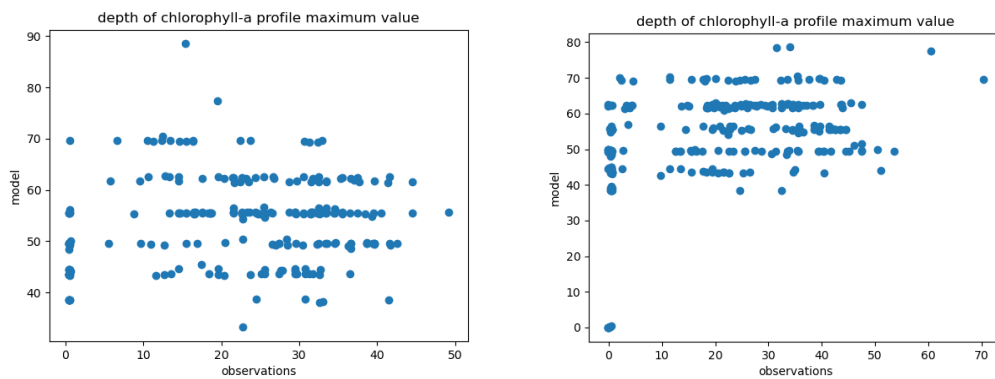


Fig 14: Comparison of the maximum chlorophyll computed by the model with the observation made by Argo floats. Left for 2017 & right for 2018.

	2017	2018
Pearson correlation for maximum chlorophyll between the model and Argo	0.16	0.4

Table 7: Pearson correlation between observations and Argo for chlorophyll maximum concentration.

As expected, our deep maximum for chlorophyll is too deep according to the Argos data (figure 14). As we can observe on the graphics, the scale of the model observation is larger than the one for the Argos observations, showing us once again that the chlorophyll is too deep. Even so, between both years, the error that is made on the depth maximum is the same. Indeed, the chlorophyll depth maximum of the model is always situated between 40 and 80 meters, while it is normally situated between 0 and 50 meters. Once more, the second year remain the greatest regarding the statistics.

Thus, our validation on chlorophyll has two main issues. The first issue is the fact that none of the algae develops on the surface layer, while it should be the layer with the best conditions for primary production. Secondly, the maximum concentration is always higher than the expected concentration. Finally, we can assume that the second year is more in accordance with the observation than the first year.

Main nutrients and seasonal variation

For nitrate and ammonium, the profiles are sampled in the model at the same location than the one followed by the validation of the Oxygen (figure 9). It creates a consistent sampling location which is useful in term of comparability (the same stations are compared on every profile).

In this part of the results, we expect to assess the presence (or the absence) of anomalies linked to modelling errors, and to shed light on these later. In order to do that, we will compare the profiles with literature or with the 1D model.

Nitrate

In our results for nitrate, we expect to observe one peak of nitrate situated upon the oxycline, while the very first meter of the water column should be depleted in nutrients because of the enhanced primary production at this depth (fig 2 & 15B).

Nitrate is the major nutrient of the upper layer of the Black Sea as soon as the ammonia is oxidized when upwelled in the oxic zone. The order of magnitude of the nitrate concentration is in accordance with the expected values which are between 0 and $5\mu\text{M}$ (fig 15B) upon the anoxic layer (I.e Yakushev et al., (2007); Lam et al., (2007)).

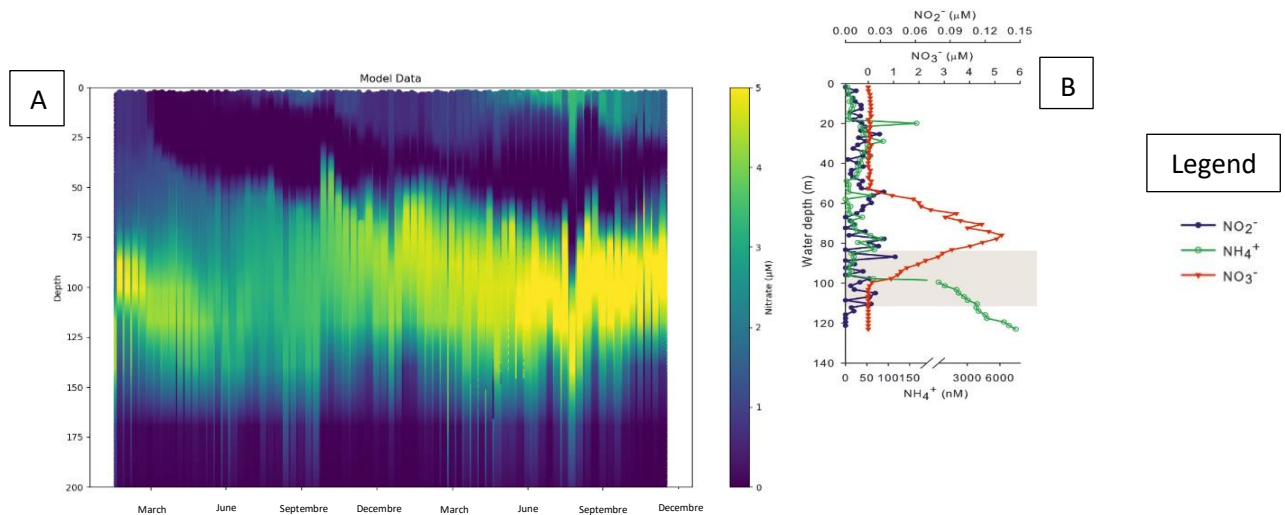


Figure 15: [A]: Nitrate profile computed along time and depth for both years (2017-2018). [B] Vertical profile of nitrogen species according to (Yakushev et al., 2007) (μM)

Unfortunately, the profile that our model provides shows 2 clear peaks of concentration in the first tens of meters of the water column (figure 15A), which is not the case in literature (I.e., Yakushev et al., (2007) & Fuchsman et al., (2008)). Indeed, except for the very beginning of 2017, the surface layer is never depleted in nitrate. Its surface concentration even reach $3\text{-}4\mu\text{M}$ which is a large error, knowing that most of the time, the variable is sampled in the open sea (figure 9).

Therefore, it points out one of the issues of the model. This concern will be debate in the discussion.

On the other hand, the second peak is quite consistent with literature as it is always situated upon the oxycline and in the right order of magnitude (I.e. (Yakushev et al., 2007); (Lam et al., 2007)). This last conclusion is significant as soon as the oxygen is responsible for the oxidation of nitrogen and its transformation in nitrate (figure 5).

Moreover, in our sampled profiles, the concentration of nitrate in the upper layer is higher in 2018 than in 2017 while the depth of the nitrate peak seems to be stable through the years.

Ammonia

Ammonia is also in the right order of magnitude regarding literature (i.e. Yakushev et al., (2007); Lam et al., (2007)). As an example, (Lam et al., 2007) computed a concentration in ammonium reaching 3-6 μ M at 120m depth which is almost the case in our model.

Its concentration starts to increase at the very bottom of the oxic layer which is in accordance with the observations. By a comparison between figure 15 and 16, we can assume that the oxidation of ammonium in nitrate (or the reverse) is well modelled.

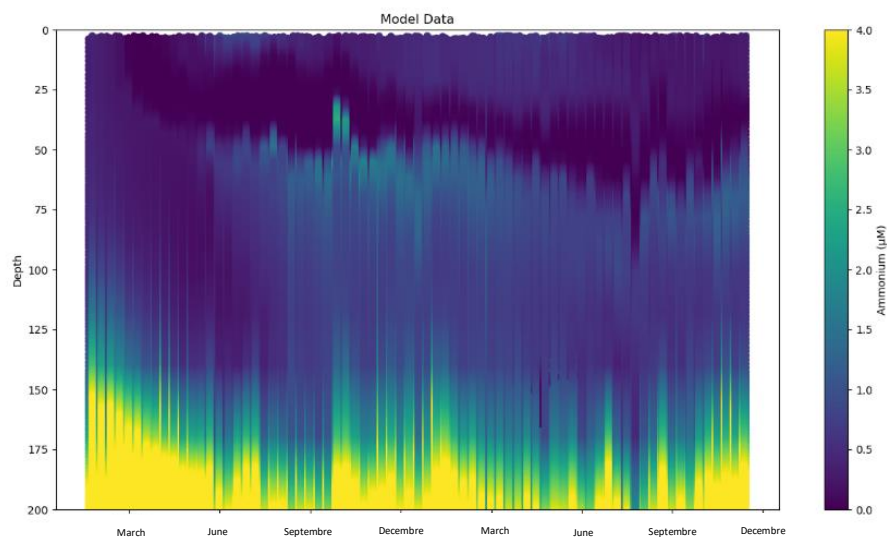


Figure 16: Ammonia concentration over 200m on the Black Sea, for both years (2017-2018) (μ M)

Diazotrophs

As we mentioned earlier, diazotrophs are poorly understood and these results only give a try to understand and locate their development in space and time. Our model simulates diazotrophs all the yearlong and we can observe them at different depths. Their distribution is described here.

To analyze diazotrophs we are first going to inspect the carbon content of these latter (called CDZ in our model) which gives us a proxy of their biomass. Knowing that they don't grow at the same depth depending on the biogeochemical conditions, we will provide their time evolution for the first 200 meters of depth.

As figure 17 shows, our model reveals significant diazotrophs development in surface (which will be debated), and more in depth, where nitrate and ammonium are depleted (around 50m depth).

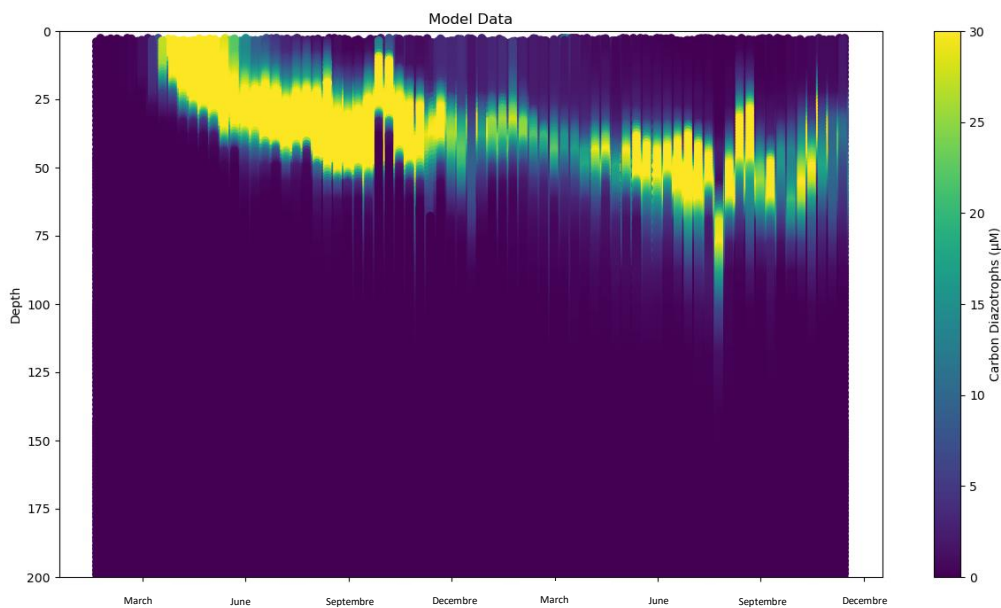


Figure 17: Carbon content of diazotrophs evolution through time and space for both years (2017-2018) (μM)

As we have previously described, the fixation of di-nitrogen by diazotrophs is performed in waters that are deficient in nitrogen compared to phosphorus in a Redfield sense. To compute it we have defined in the section material and method, a formula that computes it.

$$N^* = \text{NOS} - 16 \cdot \text{PHO}$$

The figure 18 display the N^* diagnostic for the 200 first meters of the water column: When the value is equal to 0, it means that the content in nitrate is 16 times the content of phosphate. In negative values, we have a deficiency in inorganic nitrogen compared to phosphorus (as

estimated by Redfield), this situation is expected to favor nitrogen fixers that are able to compensate the nitrogen deficiency by fixing nitrogen gas.

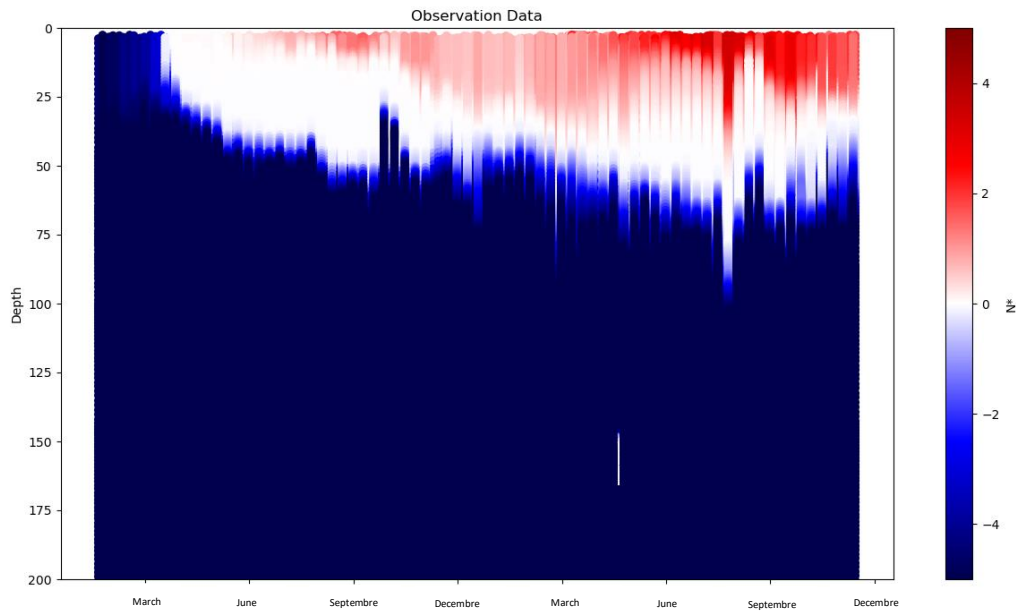


Figure 18: N^* over 200m for both years (2017-2018)

As soon as the N^* is negative or near zero on the surface layer at the very beginning of 2017, it is not surprising to observe a bloom at this period (figure 17 & 18). Regarding the concentration of nutrients on surface during the bloom, nitrate reaches concentration on the order of magnitude of nano molar (I.E., $8,5\text{nM}$ on the 28th of February 2017) while the phosphate concentration is slightly under $1\mu\text{M}$. After this bloom, it is observed that the surface phosphate concentration decreases ($6.4 \times 10^{-5} \mu\text{M}$ on the 15th of March) and never increase again until the end of the simulation (Figure 22). This feature is represented by the figure 18, indeed, the surface layer has a value which is positive, showing that the phosphorus concentration is low (nitrate has concentration around $3\mu\text{M}$ on the surface, figure 15A).

The peak of the bloom is reached on the 88th day of the year (29th March) with $52.8\mu\text{M}$, before decreasing to $1\mu\text{M}$ on the 206th day (25th July). As we have mentioned earlier in the master thesis, diazotrophs cannot develop where the oxygen concentration is too elevated due to the

inhibition of the nitrogenase by the oxygen. Here the oxygen concentration has an average value around $300\mu\text{M}$ (figure 11) which affirm that this bloom is out of sense.

Following figure 17, we can see that the development of diazotrophs deepens with time. After the bloom, the growth of diazotrophs tends to be situated around 40-50m depth. Considering figures 15A & 16, it is the depth where nitrate and ammonia are depleted which is coherent with our computation for this phytoplankton (table 2). However, we observe on the figure 18 that their development is situated where the N^* value is close to 0 and not where the values are largely negative. In our expectation, and due to the large concentration of di-nitrogen in the Black Sea, we would expect to observe large diazotrophs development where the N^* is the lowest. This conclusion is even more surprising as soon as we observe that the phosphorus concentration is on the order of magnitude of $1\mu\text{M}$ at depth (Figure C appendix).

Moreover, we observe that their concentration decreases during the colder month of the year, which can be explained by the replenishment in nutrients of the mixed layer.

As conclusion, by comparison of the figure 15A and 16, 17 & 18, we observe that the development of diazotrophs does not actually develop at a constant depth. Indeed, figure 17 clearly display that the CDZ has tendency to deepens through time. We also note that their development occur where the N^* is close to zero and not where its value is largely negative which is enigmatic knowing how we compute their development (table 3). Nonetheless, we also observe that their concentration has tendency to diminish during winter.

As a comparison, the 1D model simulates a deep bloom (70m depth) for diazotrophs on the second part of the year (between the 200th day and the end of the year) and is the only moment where the carbon concentration of diazotrophs exceed $1\mu\text{M}$ (Meulders & Université de Liège > Master en océanogra., 2019) (Appendix figure A). Comparatively, our model reaches $71\mu\text{M}$ on the 16th of September which is on order of magnitude over.

Anammox, Denitrification and di-nitrogen fixation

In view of establishing the nitrogen budget of the basin, we analyze processes that consume (di-nitrogen fixation by diazotrophs) and consume (anammox, denitrification) di-nitrogen. Figure 19 shows the vertically integrated rates of anammox, denitrification and nitrogen fixation, computed over a region located at 42° degree of latitude and 33.5° degree of longitude. It is part of the open sea, and we consider it to be representative of the Black Sea state.²

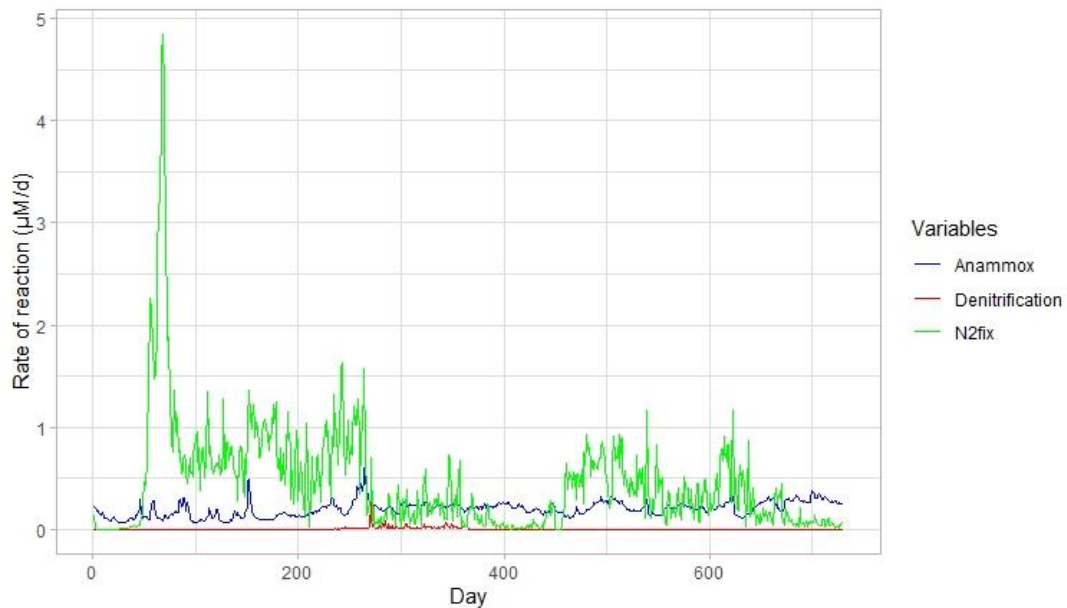


Figure 19: Evolution of anammox, denitrification and nitrogen fixation over 2017-2018. ($\mu\text{M}/\text{d}$)

a) Anammox and denitrification rates

Past studies have shown the importance of Anammox and denitrification in the Black Sea and shed light on the fact that anammox is the reaction that is responsible for a major part of fixed nitrogen removal (96%) (Francis et al., 2007).

This feature is well represented by the model; indeed, the integrated rates of denitrification are most of the time non-detectable (equal to 0), while the integrated rates for anammox are found to be ranging from 0.1 to 1.5 $\mu\text{M}/\text{day}$ all year long. However, we can notice that

² We note that the peak that we observe at the beginning of 2017 is linked to the bloom of diazotrophs, which we do not consider to be relevant regarding the real di-nitrogen fixation.

denitrification is detectable on the last part of the first year but is still widely lower than the anammox.

Moreover, anammox is not a reaction that should occur in the surface layer as soon as this reaction is inhibited by oxygen. Concentrations of O_2 above $1 \mu\text{M}$ can inhibit anammox. Therefore, the depth at which it occurs is mainly linked to the depth of disappearance of oxygen. To assess the mean depth of this reaction, we will sample anammox in the model at the same location that the one followed by the Argo for oxygen and confirm if we can observe a dependency between the rate of reaction and the oxygen.

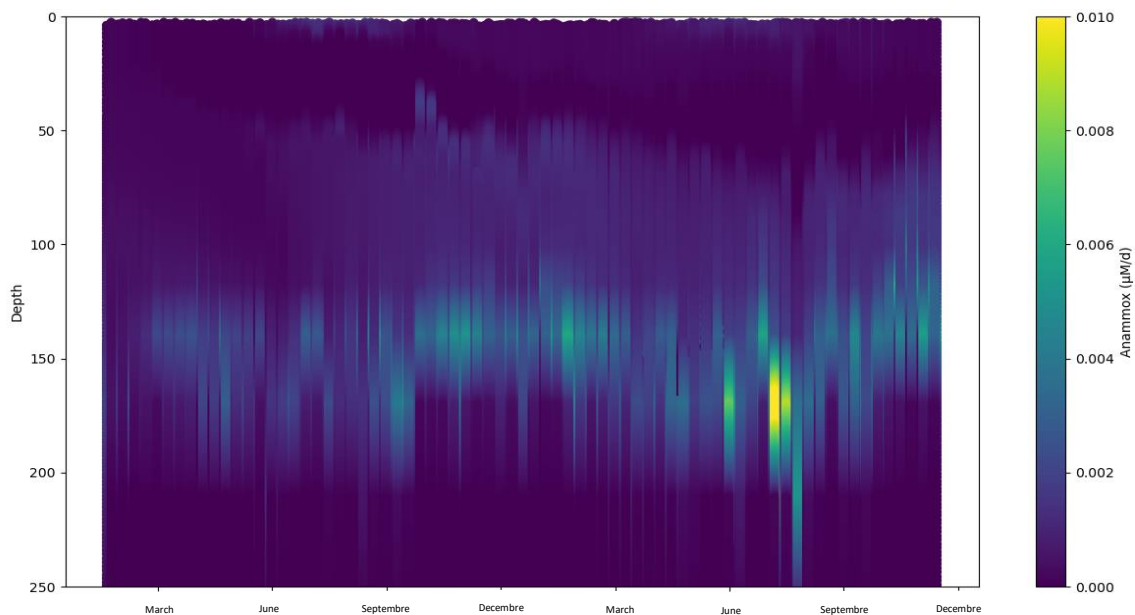


Figure 20: Anammox rates computed along time and depth($\mu\text{M}/\text{d}$) for both years (2017-2018).

Regarding the dependency of anammox reaction with depth, we can assume that it occurs around 130m/150m depth (with variations). We find rates of $0.008 \mu\text{M}/\text{d}$ in depth while in we find rates of $\sim 0.002 \mu\text{M}/\text{d}$ or under in shallower waters. It is consistent with our expectations as soon as this reaction is not supposed to occur in oxic waters. At these locations (Figure 9), we observe oxygen concentration going below the detection level around 130m (figure 11) and never increase again. It is the reason why we observe a maximum of Anammox at those layers. We note that as we mentioned previously, the oxycline is deeper than expected, thus the peak of anammox reaction should be shallower as it is the case for oxygen.

b) Di-nitrogen fixation:

Among researchers, Yakushev et al., (2007) and McCarthy et al., (2007) are among those that measured the rates of fixation for di-nitrogen in the Black Sea. As was mentioned, the rates are in the order of magnitude of 1 to 150 nM/d, without knowing which ones are the most precise (a bunch of rates were measured or estimated, and it is a tough task to assess which is the more precise because of the lack of observations). We can therefore assume that our model is in the right order of magnitude as soon as we model rates of di-nitrogen fixation between 30nM/d and 100nM/d (figure 21).

Moreover, as we can see on figure 19 & 21, we observe that the dinitrogen fixation has lower rates during the colder parts of the year which is a consequence to the replenishment of nutrients. The direct consequence is the lowering of the N/P ratio, which diminish the uptake of di-nitrogen.

In their research, (McCarthy et al., 2007) has found that the depth of the maximum rate of di-nitrogen fixation is around 80m depth. In our model, the maximum rate of dinitrogen fixation is most of the time at 50m depth which is shallower than expected. The figure 21 display the dinitrogen fixation for both years of the simulation.

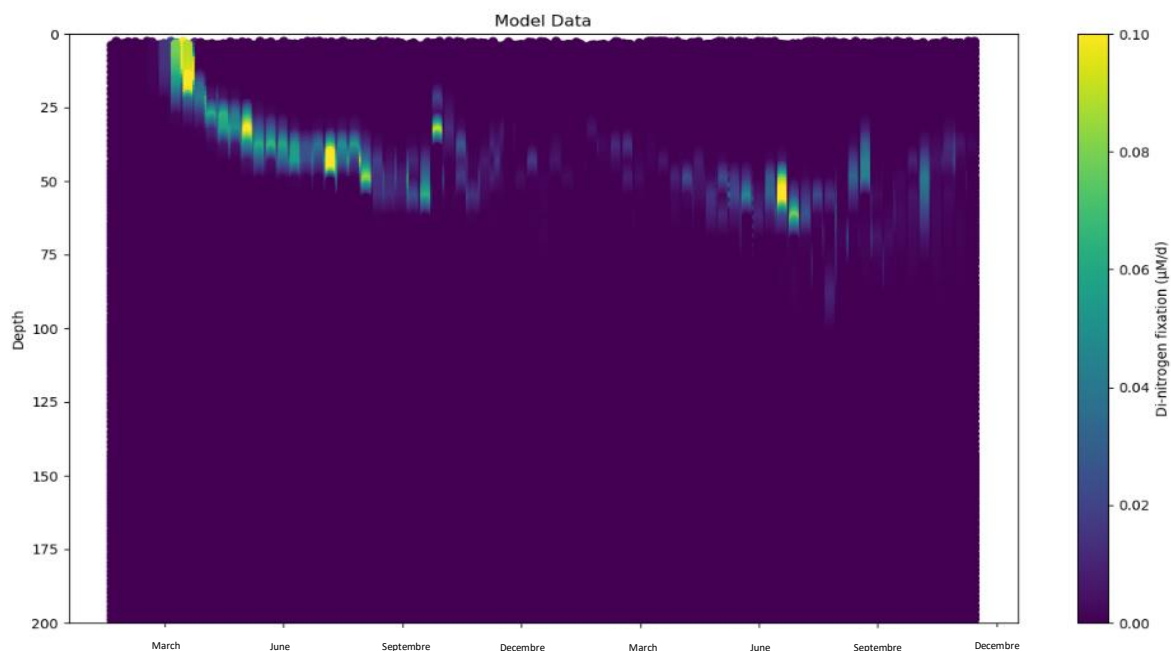


Figure 21: Di-nitrogen fixation in function of time and depth ($\mu\text{M}/\text{d}$) for both years (2017-2018).

c) Conclusion of the nitrogen fixation

As a conclusion, figures 19, 20 & 21 show that the di-nitrogen fixation has a clear seasonal variability while anammox and denitrification does not seem to have a seasonal pattern. For N₂ fixation, we observe lower rates during winter compared with the rest of the year.

During the all simulation, di-nitrogen fixation remains higher than the rate of di-nitrogen production, which bring a strong result; In our model, the production of di-nitrogen by anammox plus denitrification is most of the time lower than the rate of fixation of di-nitrogen gas. Thus, we can confirm that the presence of diazotrophs compensate the production of nitrogen gas. Therefore, we can assume that the diazotrophs in a model of the Black Sea is significantly influencing the nitrogen cycle.

Finally, the conclusion that we brought here give us a more precise idea on the importance of the dinitrogen fixation against its production and was found to be an important feature for the fixation of di-nitrogen.

Discussion

First, it is important to keep in mind that this master thesis remains an attempt for modeling Black Sea with di-nitrogen fixation and diazotrophs. We are aware of the imprecisions and the unknowns to which we are confronted. These latter have to be discussed in order to give to this work a direction. We will discuss the different results, starting with nutrients (mainly oxygen and nitrate), we will link them to the primary production before discussing the diazotrophs behavior and the nitrogen budget.

Oxygen validation

Referencing the Quality Information Document (Grégoire & Vandebulcke, 2023), we can assess that the oxygen computation is considered as “excellent” since the behavior of this latter is well reproduced.

However, the oxygen profile has a well-known issue; the penetration in depth is too large. The presence of too high concentrations of oxygen at depths results from an underestimation of the stratification intensity in the model.

The second is more complex and is a consequence of the non-development of phytoplankton on the surface layer. Indeed, the development of phytoplankton fulfill the environment with oxygen. For confirmation, we observe that during the bloom of diazotroph (figure 17), we observe a replenishment in oxygen for the surface layer (figure 11). As soon as no phytoplankton has a bloom on the surface layer during the simulation, it is not a surprise to observe a lack of oxygen compared to the layer below (around 25m). The reason for the lack of chlorophyll on the surface layer will be discussed in the following section.

Chlorophyll validation

The validation of the Chlorophyll using Argo floats was performed. Referencing the Quality Information Document (Grégoire & Vandebulcke, 2023), we assess that for the first time, the chlorophyll deep maximum is sustained by the model which is a large improvement. Moreover, the presence of near-real time data for the Danube discharges enhance the exactitude of primary production.

However, we have pointed out issues in the result section. First, the chlorophyll concentration on the surface layer is always under the observed values, second, the concentration of the maximum is always too large compared to the measured values, and the deep maximum is too deep, which is probably linked to the first issue.

We can interpret this issue as follow; It seems to be linked to the phosphorus concentration. As we have seen, on the beginning of 2017, the bloom of diazotrophs deplete the upper layer regarding the phosphorus concentration (figure 22).

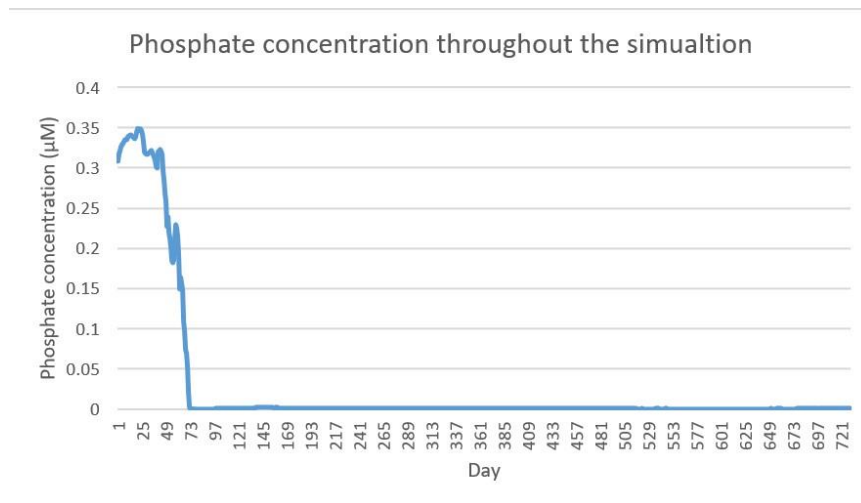


Figure 22: Phosphorus concentration on the surface layer after the first bloom for both years (2017-2018) (μM)

Indeed, the ability of the diazotrophs to grow due to the lowering of N^* (figure 18) has a consequence. The phosphate is consumed until its depletion because the dinitrogen concentration does not limit the algae development (dinitrogen concentration is always in the order of $300\mu\text{M}$). As the figure 22 shows us, the replenishment in phosphate never occurs after the first bloom, and therefore limits the following development of other algae at this layer.

The reason for which the phosphate concentration remains low is unknown. Even the winter mixing of the water column which should re-increase the nutrients concentration does not occur for the phosphate.

To improve that, it would be necessary to improve the initial conditions of the year to abolish the bloom that occurs in surface for the di-nitrogen fixers. Thus, the surface layer would be theoretically fulfilled in phosphorus which could let the other algae growth. Unfortunately, we do not know if the development of other algae (such as diatoms) would lead to the depletion of phosphate. Thus, the problem could also arise from the mixing if the upper layer.

In order to abolish the subsurface bloom of diazotroph, it would be interesting to add a limitation with the oxygen for their development. However, studies reported that some of the diazotrophs species were able to develop in the oxic layer, using physiological feature to limit the oxygen repression on the nitrogenase protein. This will be debate in the last part of the discussion.

Anammox and denitrification

As we have seen in the results, the Anammox profile reach maximum values in the very lower part of the oxic layer (figure 20 & 11) which is in accordance with our expectation and literature. I.E. (Kirkpatrick et al., 2012) and (Fuchsman et al., 2019) measured the importance of the anammox reaction against the denitrification and showed that the Anammox rates are always higher than the denitrification, which is well reproduced by the model. Moreover, (Konovalov et al., 2008) measured anammox rates equal to 7nM/d which is exactly in the same order of magnitude with our model. Indeed, we computed rates ranging from 5 to 10nM/d (figure 20).

However, as we have seen previously, the oxygen of our model tends to be too deep and so is the Anammox. We can expect that with the update of the physics, the anammox reaction would be in accordance with our expectations as soon as the oxygen drives the depth of this reaction.

In its overall behavior, we can assume that anammox is in great agreement with literature.

Diazotrophs and di-nitrogen

In our model, the concentration of dinitrogen is linear with depth and stops at the bottom of the sea. We obtain a concentration in di-nitrogen ranging between 250 and 300 μ M. In comparison with (Fuchsman et al., 2008), our model lack in di-nitrogen. In their observation, they obtain concentration going to 600 μ M and slightly decreasing with depth. Thus, we underestimate the di-nitrogen content of the sea. In terms of computing the di-nitrogen budget, is introduces an error. However, regarding the development of diazotrophs, we assume that N₂ is never limiting their development knowing that the di-nitrogen fixer

(diazotrophs) never has the capacity to deplete its concentration. Therefore, we assume that the diazotrophs development is not impacted by the lower dinitrogen concentration.

Moreover, a preconceived idea was the close coupling between anammox and the development of the diazotrophs. However, we have seen in the results that the development of diazotrophs in our model is situated near 50m depth while the anammox is significantly deeper as soon as it is in the deep part of the oxic layer.

The growth of diazotrophs in our model is allowed with all the nutrients but is the only algae that can uptake dinitrogen when other form of nutrients are lacking. In our model, we have seen that it computes a constant lack of ammonia and nitrate (fig 15 & 16) around 50m depth. Thus, it is not a surprise to see the development of diazotrophs at this depth as soon as it is the only primary producer that has the capacity to growth there. Regarding the N^* computation and the N_2 fixation, we observe that the diazotrophs grow where the N^* is close to 0 and not where the values are the lowest. This enigmatic behavior has to be revised as soon as the phosphate concentration is the largest where diazotrophs are not growing.

In the reality of our model, diazotrophs seems to act as a filler when other algae cannot develop in a given biogeochemical conditions. In literature, author like (Bombar et al., 2016) (and many other) shed light on our incomplete understanding of the di-nitrogen fixation and moreover, on the ecology of diazotrophs. Indeed, we have studied more in depth the behavior of *Trichodesmium*, which were thought to be representative of the di-nitrogen fixer in the sea. This idea led to an incomplete analysis of the diversity of these algae. Nowadays, scientists as (Luo et al., 2012) studied more in depth the *NifH* gene which attest the presence of di-nitrogen fixation. Depending on this gene variation, they explored the diversity of the nitrogenase protein which is assumed to be representative of the diversity of diazotrophs. Thus, in the reality of the Black Sea (and therefore other seas where diazotrophs are present), we find a wide range of diversity simply by analyzing the diversity of the protein itself. (Luo et al., 2012) also assume that diazotrophs can also have multiple *NifH* genes which are regulated depending on the biogeochemical conditions. They assume that to study the di-nitrogen fixation, it is needed to consider this poorly known diversity.

With increasing interest for diazotrophs, studies like the one of (Kennedy & Bishop, 2005) shed light on the fact that even if the oxygen was an inhibitor of the nitrogenase, evolutionary

purposes led the bacteria phylum to be able to fix di-nitrogen in the oxic layer (it is here the case for Azobacter).

Therefore, the idea that we brought in the discussion of the chlorophyll validation, which consist of limiting the diazotrophs with oxygen is not suitable for all diazotrophs species.

This last conclusion inevitably brought us to the fact that for accurate modeling purposes, we need to be more precise regarding the computation of diazotrophs. This could be done in multiple way, but the first objective is to model a better diversity regarding their ability of development, and thus, their physiology.

Conclusion

During this work, we used the current knowledge of di-nitrogen fixer to model them. We assumed that their growth were limited by phosphorus which set the amount of nitrogen that has to be fixed by the diazotrophs. When the amount of nitrate and ammonium is too low to fulfill the requirement given by phosphorus, our diazotrophs are capable of fixing dinitrogen. However, the fixation of di-nitrogen require higher energy in order to break the triple bound between the nitrogen atoms and is the reason why it does not occur in environment with concentrations of nitrate and ammonium higher than the detection level. As consequence, diazotrophs act as a filler when nitrate and ammonia are depleted.

Thereafter, anammox and denitrification were found to be decoupled in space with diazotrophs growth. Indeed, the di-nitrogen uptake occurs around 50m depth while anammox and denitrification are found in the depleted oxic environment, which is deeper.

Moreover, we assessed the accuracy of the rates linked to the di-nitrogen variation (anammox, denitrification & di-nitrogen uptake), which were all found to be in accordance with literature.

Thanks to this model, we have integrated these rates and we have assessed that on average, the production of dinitrogen by anammox (and denitrification) is lower than the amount of di-nitrogen fixed by diazotrophs. We can therefore conclude that diazotrophs are an important feature regarding the nitrogen cycle and more precisely, regarding the loss of di-nitrogen in anoxic environment.

Unfortunately, the oxic repression toward the development of diazotroph remains something that was reported bunch of time in the literature and is not represented by the model. Indeed, the nitrogenase which is the needed protein to fix di-nitrogen is repressed by the presence of oxygen. This last point let us think that diazotrophs cannot simply be a filler in nitrate and ammonium depleted environment and is the reason why it would be interesting to add an oxygen limitation on their growth.

Therefore, the following objectives are aimed for the following of this work.

1) Medium-term objectives

The medium-term objectives has to be focused on the modeling of the diversity of diazotrophs. For this purpose, recent literature of diazotrophs and the literature referenced in the section “diazotrophs and di-nitrogen” will be used in order to get a better understanding of their ecological position and their physiological behavior regarding the dinitrogen. As we have already mentioned, the computation of a limitation by oxygen for their growth is not farfetched. The addition of a new state variable for a new di-nitrogen fixer with another parametrization and limitation could also be a way for modeling diversity.

2) Long term objectives

As long-term objectives, we would assess the state of equilibrium of the nitrogen cycle, considering the new formulation of diazotrophs modeled in the medium-term objectives. If the diazotroph growth and behavior is realistic, we would thus make long term simulations of the model. This latter will be forced by a realistic climatology considering the global warming, and thus all the consequences that it has on the Black Sea (the rise of the oxycline, the lowering of the mixing in winter, the variations of the river discharges etc.). It would show us a realistic evolution of the nitrogen cycle and therefore, would let us forecast the Black Sea.

On the following years, the increased data regarding the Black Sea will be an important tool, which will fade some of the unknowns that we are still facing. The future of the nitrogen cycling in oxygen depleted zone is promising even if it is poorly understood at the moment.

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Appendix:

1) Forms of nitrogen:

NO₃⁻ : This nutrient is a nutrient that is bioavailable. It is the result of the nitrification reaction ($\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}_2\text{O}$). Regarding the first meters of the water column, nitrate is under the detection level, and we observe a maximum threshold close to the anoxic zone, while its concentration rapidly decreases after the peak zone (Fuchsman et al., 2008). Fuchsman et al., (2019) found that the nitrate concentration maximum varies from 4.5 to 7 μM on the upper part of the suboxic zone.

NO₂⁻ : Nitrite is the intermediate nutrient through the nitrification/denitrification reactions. Thus, it is found at lower levels compared to nitrate. We can find two maximum peaks of nitrite concentration, found on the upper side of the nitrate increases and on the bottom side, where the nitrate decreases due to the lack of oxygen. Their concentration varies between 0.05 and 0.19 μM , which is one order of magnitude below the nitrate concentration at its maximum (Fuchsman et al., 2019), which is easily explained by the fact that nitrite is only an intermediate of reaction and thus never stay under nitrite form.

N₂O: (Westley et al., 2006) studied the N₂O dynamics into the Black Sea and assumed that while it is produced by nitrification and denitrification (where it is an intermediate to form N₂), the low oxygen environment has tendency to enhance its production. However, in the Black Sea, it was found that this form of nitrogen does not accumulate. A maximum concentration was measured at 70m ($\sigma_t = 15.3$) for 14.4nM, which is three orders of magnitude below other major nutrients.

NH₄⁺: Ammonia is the favored nitrogen form for fixation due to its redox state. Regarding the metabolism of microorganisms, nitrate must be reduced when it is taken by organisms to fix it in their metabolism. This is not the case for ammonia which is already reduced. However, the concentration of ammonia is found to be below 0.1 μM until the lower part of the near anoxic zone. Indeed, when ammonia is upwelled out of the anoxic zone, the presence of oxygen directly converts it to oxidized form (nitrite and then, nitrate). Thus, in the oxic layer of the Black Sea, the ammonia is not fixed by microorganisms. Its concentration starts to increase in

the lower part of the near anoxic zone and reaches $98\mu\text{M}$ at 200m depth (Fuchsman et al., 2019).

N_2 : N_2 gas is found to be the product of 2 main reactions. The first one is anammox and combine N_2O and NH_4^+ to form N_2 gas. On the other hand, the final product of denitrification is N_2 gas. Studies have shown their relative importance in the Black Sea, it was found that anammox is the most important pathway for N_2 formation (Jensen et al., 2008). As this latest paper mention, anammox has a maximum rate of $11\text{nM } \text{N}_2 \text{ d}^{-1}$ at the interface between nitrate and ammonia while the denitrification is not detectable. However, there are still large uncertainties on the relative importance of these two processes. In this master thesis, we will quantify both using modelling.

2) 1D model results (Meulders & Université de Liège > Master en océanogra., 2019)

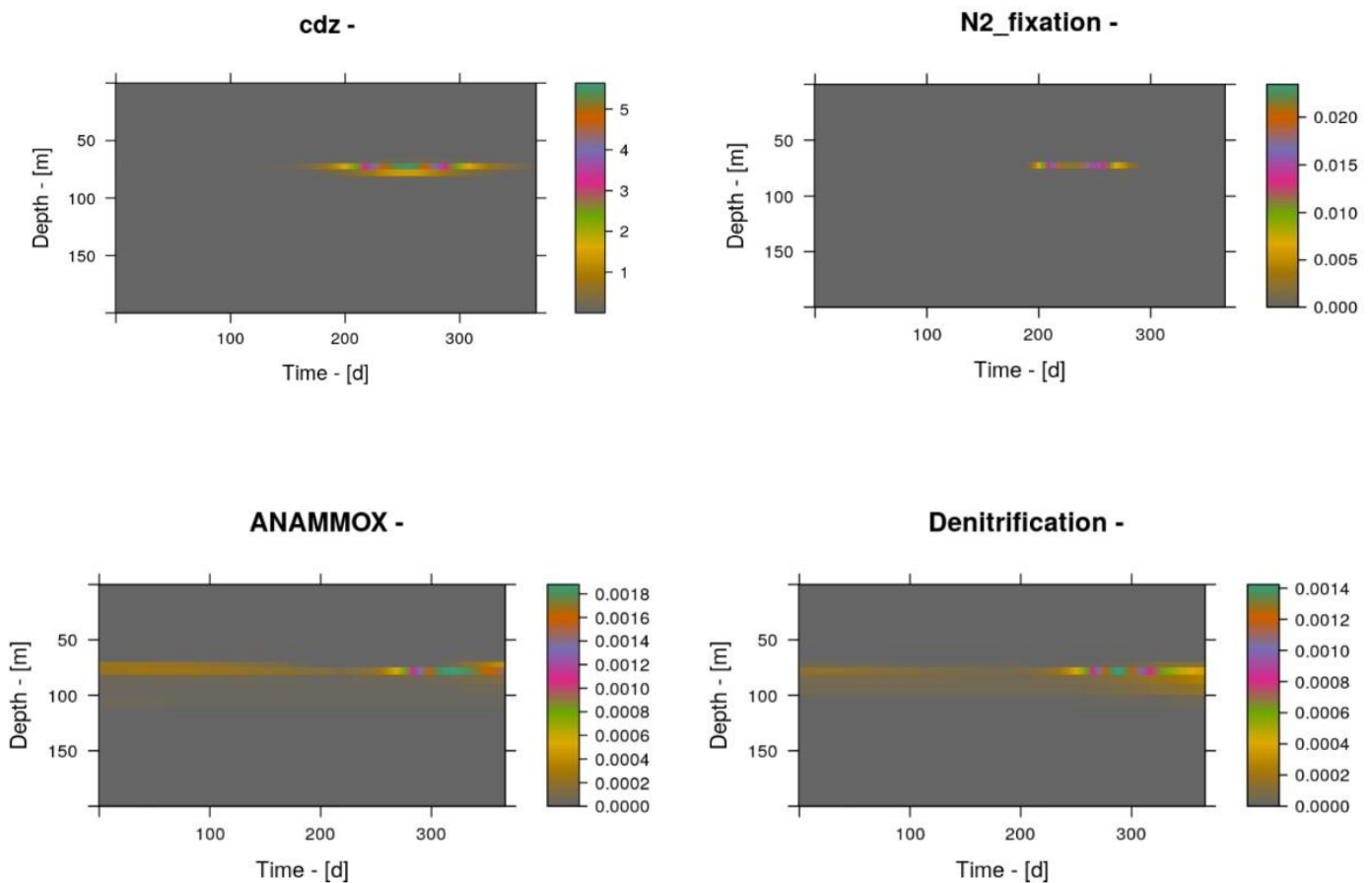


Figure A: Main results for diazotrophs regarding the 1D model (ANAMMOX, Denitrification, CDZ & N_2 fixation) (μM)

3) Diversity of diazotrophs, based on NifH gene (which encode for nitrogenase).

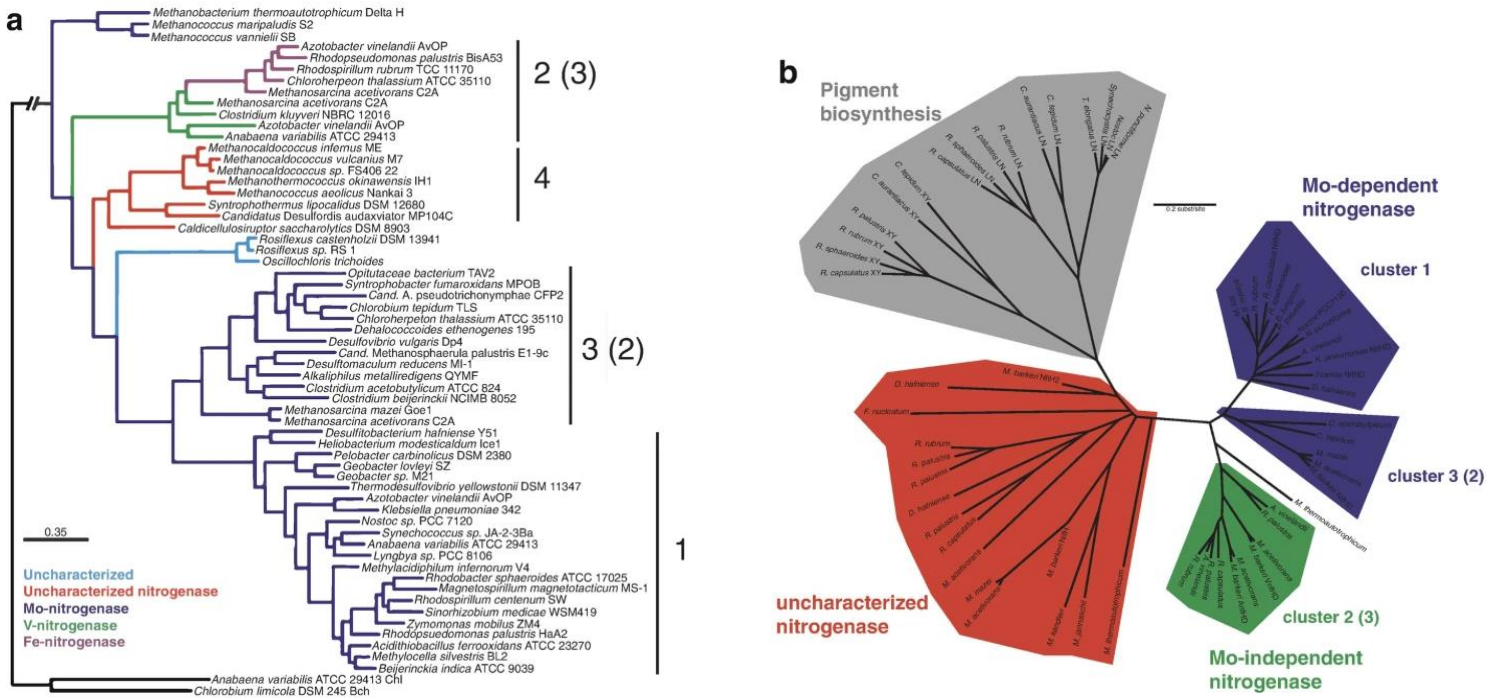


Figure B: Gene tree based on the sequencing of NifH gene

4) Phosphate computed by the model over 200m for both years (2017-2018)

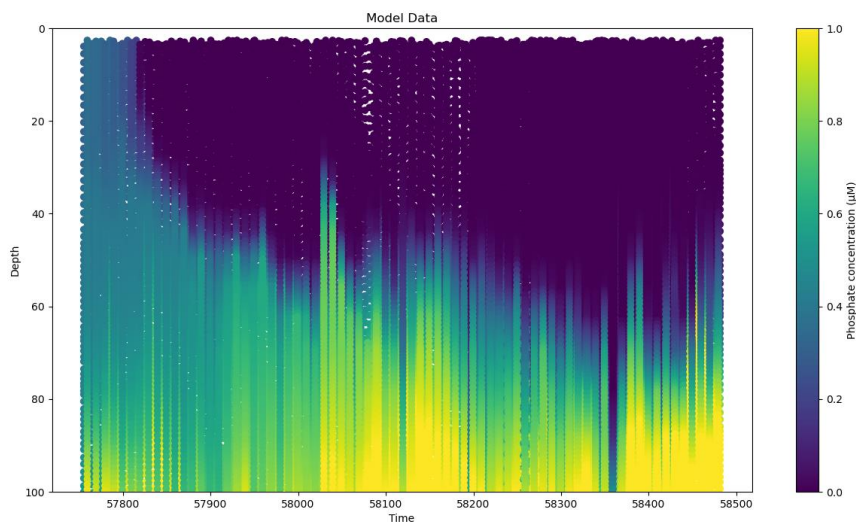


Figure C: Phosphate concentration over 200m for both years (2017-2018) (μM)

