The suspended small-particles layer in the oxygen-poor Black Sea: a proxy for delineating the effective N_2-yielding section

Rafael Rasse[1], Hervé Claustre[1], and Antoine Poteau[1]

[1]Sorbonne Université and CNRS, Laboratoire d'Océanographie de Villefranche (LOV) UMR7093, Institut de la Mer de Villefranche (IMEV), 06230, Villefranche-sur-Mer, France.

Correspondence to: rafael.rasse@obs-vlfr.fr; rjrasse@gmail.com

Abstract. The shallower oxygen-poor water masses of the ocean confine a majority of the microbial communities that can produce up to 90% of oceanic N_2. This effective N_2-yielding section encloses a suspended small-particle layer, inferred from particle backscattering (b_{bp}) measurements. It is thus hypothesized that this layer (hereafter, the b_{bp}*-layer*) is linked to microbial communities involved in N_2-yielding such as nitrate-reducing SAR11 as well as sulphur-oxidizing, anammox and denitrifyng bacteria — a hypothesis yet to be evaluated. Here, data collected by three BGC-Argo floats deployed in the Black Sea are used to investigate the origin of this b_{bp}*-layer*. To this end, we evaluate how the key drivers of N_2-yielding bacteria dynamics impact on the vertical distribution of b_{bp} and the thickness of the b_{bp}*-layer*. In conjunction with published data on N_2 excess, our results suggest that the b_{bp}*-layer* is at least partially composed of the bacteria driving N_2 yielding for three main reasons: (1) strong correlations are recorded between b_{bp} and nitrate; (2) the top location of the b_{bp}*-layer* is driven by the ventilation of oxygen-rich subsurface waters, while its thickness is modulated by the amount of nitrate available to produce N_2; (3) the maxima of both b_{bp} and N_2 excess coincide at the same isopycnals where bacteria involved in N_2 yielding coexist. We thus advance that b_{bp} and O_2 can be exploited as a combined proxy to delineate the N_2-yielding section of the Black Sea. This proxy can potentially contribute to refining delineation of the effective N_2-yielding section of oxygen-deficient zones via data from the growing BGC-Argo float network.

1 Introduction

Oxygen-poor water masses (O_2 < 3 µM) host the microbial communities that produce between 20-40% of oceanic N_2 mainly via heterotrophic denitrification and anaerobic oxidation of ammonium (Gruber and Sarmiento, 1997; Devries et al. 2013; Ward 2013). The shallower oxygen-poor water masses (~50-200 m) are the most effective N_2-producing section because this is where the microbial communities that condition the process mainly develop and generate up to 90% of the N_2 (Ward et al., 2009; Dalsgaard et al., 2012; Babin et al., 2014). These microbial communities include nitrate-reducing SAR11, and anammox, denitrifying, and sulphur-oxidizing bacteria (e.g. Canfield et al., 2010; Ulloa et al., 2012; Ward 2013; Tsementzi et al., 2016; Callbeck et al., 2018). It is thus important to unravel the biogeochemical parameters that trigger the accumulation of such bacteria in the ocean's oxygen-poor water masses. This information is crucial for understanding and quantifying how bacterial biomass and related N_2 yielding bacteria can respond to the ongoing expansion of oceanic regions with low oxygen (Keeling and Garcia, 2002; Stramma et al., 2008; Helm et al., 2011; Schmidtko et al., 2017). Ultimately, greater accuracy in this domain can contribute to improving mechanistic predictions on how such expansion affects the oceans' role in driving the Earth's climate by sequestering atmospheric carbon dioxide (e.g. Oschlies et al., 2018).

In oxygen-poor water masses, the biogeochemical factors that can affect the abundance of denitrifying and anammox bacteria are the levels of O_2, organic matter (OM), nitrate (NO_3^-), ammonium (NH_4^+), and hydrogen sulfide (H_2S) (Murray et al., 1995;

Ward et al., 2008; Dalsgaard et al., 2014; Bristow et al., 2016). Therefore, to elucidate what triggers the confinement of such bacteria, we need to investigate how the above biogeochemical factors drive their vertical distribution, with high temporal and vertical resolution. To this end, we should develop multidisciplinary approaches that allow us to permanently monitor the full range of biogeochemical variables of interest in oxygen-poor water masses.

Optical proxies of tiny particles can be applied as an alternative approach to assess the vertical distribution of N_2-yielding microbial communities in oxygen-poor water masses (Naqvi et al., 1993). For instance, nitrate-reducing SAR11, and anammox, denitrifying, and sulphur-oxidizing bacteria are found as free-living bacteria (0.2-2 µm), and can be associated with small suspended (> 2-30 µm), and large sinking (> 30 µm) particles (Fuchsman et al., 2011, 2012a, 2017; Ganesh et al., 2014, 2015). Therefore, particle backscattering (b_{bp}), a proxy for particles in the ~0.2-20 µm size range (Stramski et al., 1999, 2004; Organelli et al., 2018), can serve to detect the presence of these free-living bacteria and those associated with small suspended particles.

Time series of b_{bp} acquired by biogeochemical Argo (BGC-Argo) floats highlight the presence of a permanent layer of suspended small particles in shallower oxygen-poor water masses (*b_{bp}-layer*) (Whitmire et al., 2009; Wojtasiewicz et al., 2018). It has been hypothesized that this *b_{bp}-layer* is linked to N_2-yielding microbial communities such as nitrate-reducing SAR11, and denitrifying, anammox, and sulphur-oxidizing bacteria. However, this hypothesis has not yet been clearly demonstrated. To address this, the first step is to evaluate: (1) potential correlations between the biogeochemical factors that control the presence of the *b_{bp}-layer* and such arrays of bacteria (O_2, NO_3^-, OM, H_2S; Murray et al., 1995; Ward et al., 2008; Fuchsman et al., 2011; Ulloa et al., 2012; Dalsgaard et al., 2014; Bristow et al., 2016), and (2) the possible relationship between the *b_{bp}-layer* and N_2 produced by microbial communities.

This first step is thus essential for identifying the origin of the *b_{bp}-layer* and, ultimately, determining if BGC-Argo observations of b_{bp} can be implemented to delineate the oxygen-poor water masses where such bacteria are confined. The Black Sea appears as a suitable area for probing into the origin of the *b_{bp}-layer* in low-oxygen waters in this way. It is indeed a semi-enclosed basin with permanently low O_2 levels where N_2 production and related nitrate-reducing SAR11, and denitrifying and anammox bacteria are mainly confined within a well-defined oxygen-poor zone (Kuypers et al., 2003; Konovalov et al., 2005; Kirkpatrick et al., 2012). In addition, a permanent *b_{bp}-layer* is a typical characteristic of this region, which is linked to such microbial communities and inorganic particles (Stanev et al., 2017, 2018, see details in section 2.0).

The goal of our study is therefore to investigate the origin of the *b_{bp}-layer* in the oxygen-poor waters of the Black Sea using data collected by BGC-Argo floats. More specifically, we aim to evaluate, within the oxygen-poor zone, how: (1) two of the main factors (O_2 and NO_3^-) that drive the dynamics of denitrifying and anammox bacteria, impact on the location and thickness of the *b_{bp}-layer*, (2) NO_3^- controls the vertical distribution of b_{bp} within this layer, (3) temperature drives the formation of the *b_{bp}-layer* and consumption rates of NO_3^-, and (4) particle content inferred from b_{bp} and N_2 produced by microbial communities can be at least qualitatively correlated. Ultimately, our findings allow us to infer that b_{bp} can potentially be used to detect the presence of the microbial communities that drive N_2 production in oxygen-poor water masses – *including nitrate-reducing SAR11, and sulphur- oxidizing, denitrifying and anammox bacteria.*

2.0. Background-nature of the small particles contributing to the *b_{bp}-layer* and their links with N_2 yielding

The oxygen-poor water masses of the Black Sea are characterized by a permanent layer of suspended small particles constituted of organic and inorganic particles (Murray et al., 1995; Kuypers et al., 2003; Konovalov et al., 2005; Kirkpatrick et al., 2012). In the oxygen-poor ($O_2 < 3$ µM) section with detectable NO_3^-, and undetectable H_2S levels, organic particles are mainly linked to microbial communities involved in the production of N_2, and these include nitrate-reducing SAR11, and anammox, denitrifying, and sulphur-oxidizing bacteria (Kuypers et al., 2003; Lam et al., 2007; Yakushev et al., 2007; Fuchsman et al., 2011; Kirkpatrick et al., 2012). The first group listed, SAR11, provides NO_2^- for N_2 yielding, and makes the largest contribution (20-60%) to N_2 yielding bacteria biomass (Fuchsman et al., 2011, 2017; Tsementzi et al., 2016). Meanwhile, the second and third groups of bacteria make a smaller contribution to microbial biomass (~10%; e.g. Fuchsman et al., 2011, 2017) but *dominate* N_2 yielding via anammox ($NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O$) and heterotrophic denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$) (Murray et al., 2005; Kirkpatrick et al., 2012; Devries et al., 2013;Ward, 2013). The last group can potentially produce N_2 via autotrophic denitrification (e.g. $3H_2S + 4NO_3^- + 6OH^- \rightarrow 3SO_4^{2-} + 2N_2 + 6H_2O$; Sorokin, 2002; Konovalov et al., 2003; Yakushev et al., 2007). Finally, *Epsilonproteobacteria* are the major chemoautotrophic bacteria that form organic particles in the sulfidic zone (e.g. oxygen-poor section with detectable sulphide levels (> 0.3 µM) but undetectable NO_3^-; Coban-Yildiz et al., 2006; Yilmaz et al., 2006; Grote et al., 2008; Canfield and Thamdrup, 2009; Glaubitz et al., 2010; Ediger et al., 2019). However, they can also be involved in the production of N_2 and linked formation of organic particles in the oxygen-poor section with detectable levels of sulphide and NO_3^- (see Figure 1, e.g. *Epsilonproteobacteria* Sulfurimonas acting as an autotrophic denitrifier; Glaubitz et al., 2010; Fuchsman et al., 2012b; Kirkpatrick et al., 2018).

The inorganic component is mainly due to sinking particles of manganese oxides (Mn, III, IV) that are formed due to the oxidation of dissolved Mn (II, III) pumped from the sulfidic zone (e.g. $2Mn^{2+}(l) + O_2 + 2H_2O \rightarrow 2MnO_2(s) + 4H^+$; Konovalov et al., 2003; Clement et al., 2009; Dellwig et al., 2010). Ultimately, sinking particles of manganese oxides are dissolved back to Mn (II, III), mainly via chemosynthetic bacteria that drive sulphur reduction (e.g. $HS^- + MnO_2(s) + 3H^+ \rightarrow S^0 + Mn^{2+}(l) + 2H_2O$; Jorgensen et al., 1991; Konovalov et al., 2003; Johnson, 2006;Yakushev et al., 2007; Fuschman et al., 2011; Stanev et al., 2018). Overall, these arrays of bacteria mediate the reactions described above by using electron acceptors according to the theoretical "electron tower" (e.g., $O_2 \rightarrow NO_3^- \rightarrow Mn(IV) \rightarrow Fe(III) \rightarrow SO_4^{2-}$; Stumm and Morgan, 1970; Murray et al., 1995; Canfield and Thamdrup, 2009). Therefore, the vertical distributions of NO_3^- , N_2 excess, and content of small particles are driven by the reactions that occur in the chemical zones of oxygen-poor water masses (e.g. nitrogenous and manganous zones, which correspond to the sections where NO_3^- and Mn(IV), respectively, are predominantly used as electron acceptors; Murray et al., 1995; Konovalov et al., 2003; Yakushev et al., 2007; Canfield and Thamdrup, 2009; see also sections 4.2 and 4.3).

3 Methods

3.1 Bio-optical and physicochemical data measured by BGC-Argo floats

We used data collected by three BGC-Argo floats that profiled at a temporal resolution of 5-10 days in the first 1000 m depth of the Black Sea from December 2013 to July 2019 (Figure 1). These floats — allocated the World Meteorological Organization (WMO) numbers 6900807, 6901866, and 7900591 — collected 239, 301, and 518 vertical profiles, respectively. BGC-Argo float 6901866 was equipped with four sensors: (1) a SBE-41 CP conductivity-T-depth sensor (Sea-Bird Scientific), (2) an Aanderaa 4330 optode (serial number:1411; O_2 range: 0-1000 μM, with an accuracy of 1.5%), (3) a WETLabs ECO Triplet Puck, and (4) a Satlantic Submersible Ultraviolet Nitrate Analyzer (SUNA). These sensors measured upward profiles of: (1) temperature (T), conductivity, and depth, (2) dissolved oxygen (O_2), (3) chlorophyll fluorescence, total optical backscattering (particles + pure seawater) at 700 nm and fluorescence by Colored Dissolved Organic Matter, and (4) nitrate (NO_3^-; detection limit of ~0.5 μM with T/salinity correction processing) and bisulfide (HS^-, detection limit of ~0.5 μM; Stanev et al., 2018). Floats 6900807 and 7900591 were equipped with only the first three sensors.

Raw data of fluorescence and total backscattering were converted into Chlorophyll concentration (*chl*) and particle backscattering (b_{bp}) following standard protocols, respectively (Schmechtig et al., 2014, 2015). Spike signals in vertical profiles of *chl* and b_{bp} and due to particle aggregates were removed by using a median filter with a window size of three data points (Briggs et al., 2011). NO_3^-, HS^- and O_2 data were processed following BGC-Argo protocols (Bittig and Körtzinger, 2015; Johnson et al., 2018; Thierry et al., 2018). Sampling regions covered by the three floats encompassed most of the Black Sea area (Figure 1, and Appendix A). However, we only used data collected during periods without a clear injection of small particles derived from the productive layer and Bosporus plume (e.g. advection of water masses, Stanev et al., 2017). This restriction allowed us to focus on the *in-situ* 1D processes driving local formation of the b_{bp}-*layer*, with minimal interference from any possible external sources of small particles.

We only describe the time series of data collected by float 6901866 because this was the only float carrying a NO_3^-/HS^- sensor. Data acquired by floats 6900807 and 7900591 are described in Appendix A, and nevertheless used as complementary data to those of float 6901866 to corroborate: (1) qualitative correlations between O_2 levels and the location of the b_{bp}-*layer*, and (2) consistency in the location of the b_{bp} maximum within the b_{bp}-*layer*.

3.2 Defining the oxygen-poor zone, mixed layer depth, and productive layer

We used O_2 and NO_3^- to respectively define the top and bottom isopycnals of the oxygen-poor zone where denitrifying and anammox bacteria are expected to be found. To set the top isopycnal, we applied an O_2 threshold of ~3 µM because denitrifying and anammox bacteria seem to tolerate O_2 concentrations beneath this threshold (Jensen et al., 2008; Dalsgaard et al., 2014; Babbin et al., 2014). The bottom isopycnal was defined as the deepest isopycnal at which NO_3^- was detected by the SUNA sensor (0.23 ± 0.32 µM). NO_3^- was used to set this isopycnal because heterotrophic denitrification and subsequent reactions cannot occur without NO_3^- (Lam et al., 2009; Bristow et al., 2017). HS^- was not used to delimit the bottom of this zone because the maximum concentration of HS^- that denitrifying and anammox bacteria tolerate is not well established (Murray et al., 1995; Kirkpatrick et al., 2012; see also section 4.1).

Mixed layer depth (MLD) was computed as the depth at which density differed from 0.03 kg m $^{-3}$ with respect to the density recorded at 1m depth (de Boyer Montégut et al., 2004). We used *chl* to define the productive layer where living phytoplankton were present and producing particulate organic carbon. The base of this layer was set as the depth at which *chl* decreased below 0.25 mg m^{-3}. This depth was used only as a reference to highlight the periods when surface-derived small particles were clearly injected into the oxygen-poor zone.

3.3 Complementary cruise data on N_2 excess and NO_3^-

Published data on N_2:Ar ratios and NO_3^- collected at the southwest of the Black Sea in March 2005 (Fuchsman et al., 2008, 2019) were exploited to complement discussion of our results. N_2 produced by anaerobic microbial communities (N_2 excess, µM) was estimated from N_2:Ar ratios and argon concentrations at atmospheric saturation (Hamme and Emerson, 2004). N_2 excess data were used to: (1) describe the oxygen-poor zone where N_2 is expected to be predominantly produced, and (2) highlight qualitative correlations between N_2 excess, the location of the b_{bp}-*layer*, and vertical distribution of small particles within the b_{bp}-*layer*.

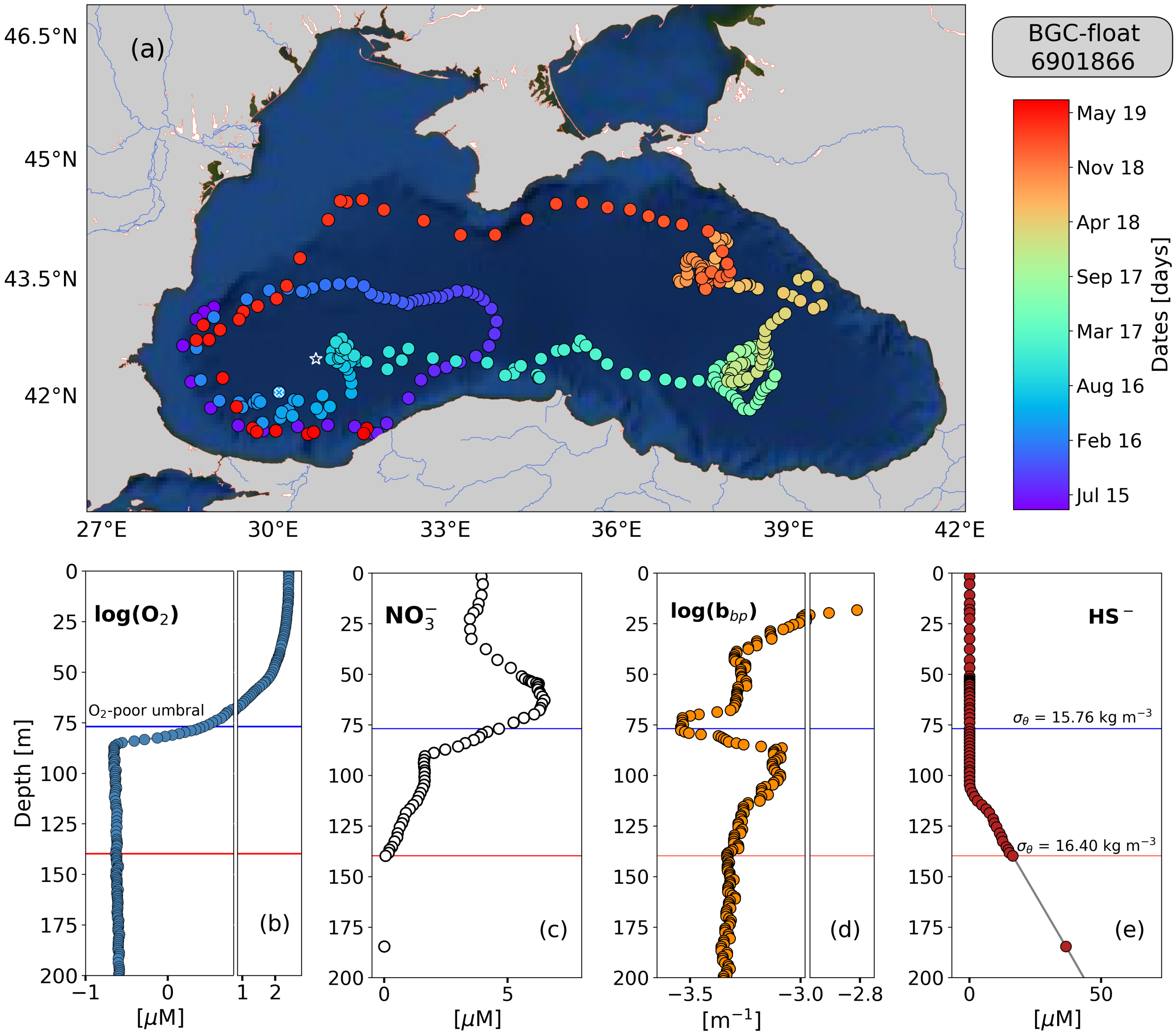

Figure 1: (a) Sampling locations of float 6901866 between May 2015 and July 2019. Colored circles indicate the date (color bar) for a given profile. The white star in (a) marks the sampling site of the cruise (March 2005). The white *x* in (a) highlights the float location on 6th April 2016. Float profiles of (b) log(O_2), (c) NO_3^-, (d) log(b_{bp}), and (e) HS^- collected on 24th November 2018.

4 Results and discussion

4.1 Description of the oxygen-poor zone

The top and bottom of the oxygen-poor zone are located around the isopycnals (mean ± standard deviation) 15.79 ± 0.23 kg m^{-3} and 16.30 ± 0.09 kg m^{-3}, respectively. The two isopycnals therefore delimit the oxygen-poor water masses where nitrate-reducing SAR11, and denitrifying, anammox, and sulphur-oxidizing bacteria are expected to be found (zone hereafter called the $OP_{D\text{-}A}$, Figure 2; Kuypers et al., 2003; Lam et al., 2007; Yakushev et al., 2007; Fuschman et al., 2011; Kirkpatrick et al., 2012). The top location of the $OP_{D\text{-}A}$ shows large spatial-temporal variability ranging between 80-180 m (or σ_θ between 15.5-15.9 kg m^{-3}, Figure 2). Similarly, the $OP_{D\text{-}A}$ thickness varies between 30-80 m, which corresponds to a σ_θ separation of ~ 0.50 kg m^{-3}. The bottom of the $OP_{D\text{-}A}$ is slightly sulfidic (HS^- = 11.4 ± 3.53 µM, n = 86) and deeper than suggested (e.g. σ_θ = 16.20

kg m^{-3}, and $H_2S \leq 10$ nM, Murray et al., 1995). However, our results coincide with the slightly sulfidic conditions of the deepest isopycnal at which anammox bacteria can be still recorded ($\sigma_\theta = 16.30$ kg m^{-3}, and $H_2S \geq 10$ µM; Kirkpatrick et al., 2012).

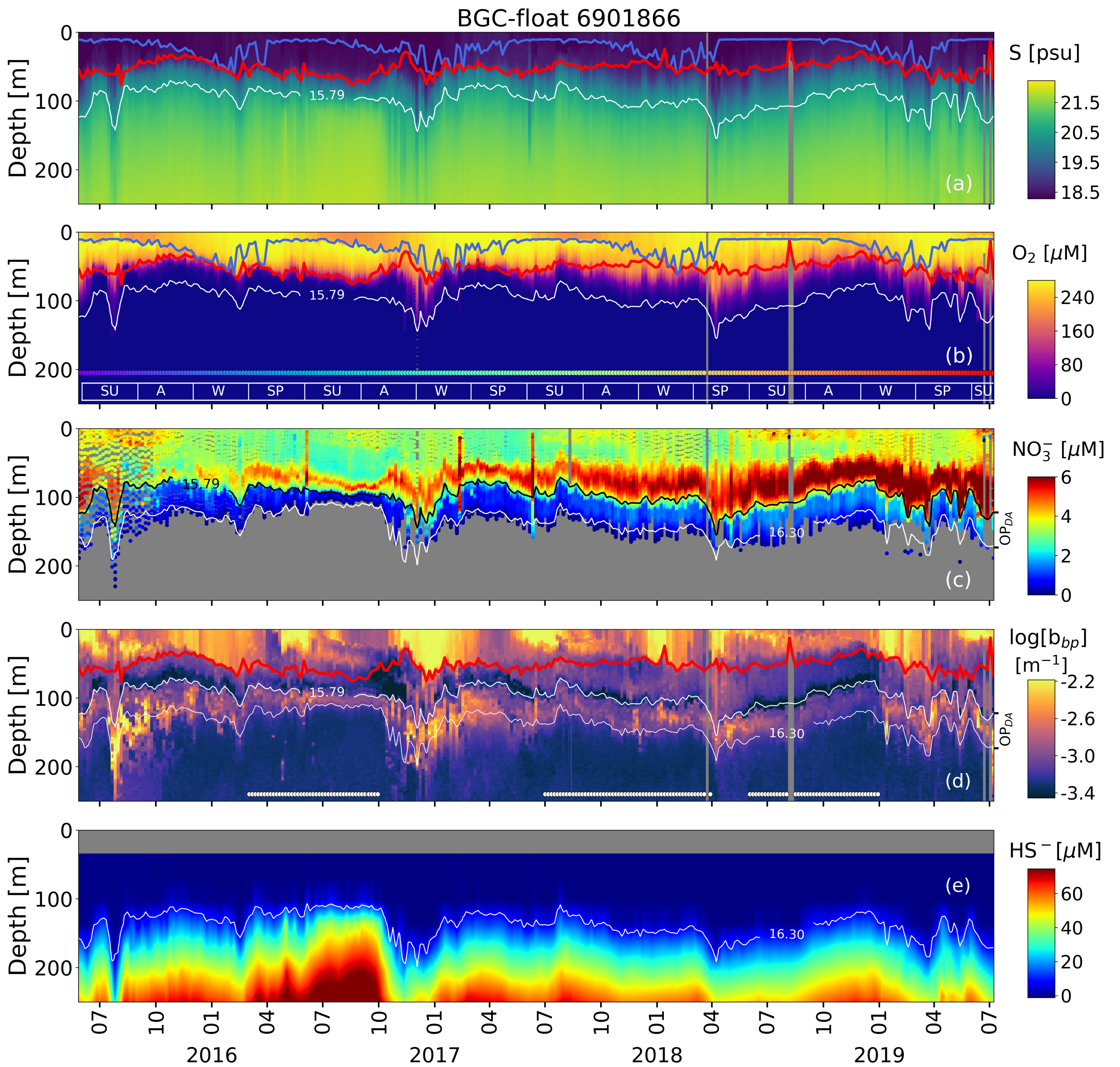

Figure 2: Time series of: (a) Salinity (S), (b) O_2, (c) NO_3^-, (d) log(b_{bp}), and (e) HS^-. The blue lines in (a) and (b) indicate the mixed layer depth. The red lines in (a), (b) and (d) show the base of the productive region. The isopycnals 15.79 kg m^{-3} and 16.30 kg m^{-3} describe the top and bottom of the oxygen-poor zone (*OP_{D-A}*), respectively. SU, A, W, and SP stand for summer, autumn, winter, and spring, respectively. The colored horizontal line in (b) indicates the sampling site for a given date (Figure 1). The horizontal white lines in (d) are the profiles used to: (1) delimit the *OP_{D-A}*, and (2) compute correlations between b_{bp}, NO_3^-, and T within the *OP_{D-A}*.

4.2 NO_3^-, O_2, and MnO_2 as key drivers of the thickness and location of the suspended small-particle layer

The permanent b_{bp}-*layer* is always confined within the two isopycnals that delimit the *OP_{D-A}* (Figure 2). It follows that the thickness and top location of this layer demonstrate the same spatial and temporal variability as the one described for the *OP_{D-}*

$_{A}$ (Figure 2 and Appendix A). This correlation indicates that variations in the thickness and top location of the b_{bp}-*layer* are partially driven, respectively, by: (1) the amount of NO_3^- available to produce N_2 inside the $OP_{D\text{-}A}$ via the set of bacteria communities involved, and (2) downward ventilation of oxygen-rich subsurface waters (Figure 2 and Appendix A).

NO_3^- and O_2 are two of the key factors that modulate the presence of: (1) denitrifying and anammox bacteria working in conjunction with nitrate-reducing SAR11 (Fuschman et al., 2011; Ulloa et al., 2012; Tsementezi et al., 2016; Bristow et al., 2017), and probably with chemoautotrophic ammonia-oxidizing bacteria (in this case, only with anammox, e.g. γAOB; Ward and Kilpatrick, 1991; Lam et al., 2007), and (2) sulphur-oxidizing bacteria (e.g. SUP05 and potentially *Epsilonproteobacteria* Sulfurimonas; Canfield et al., 2010; Glaubitz et al., 2010; Fuschman et al., 2011, 2012b; Ulloa et al., 2012;Kirkpatrick et al., 2018). Therefore, the results described above highlight that at least a fraction of the b_{bp}-*layer* should be due to this array of bacteria. This notion is supported by three main observations. Firstly, the top location of the b_{bp}-*layer* is driven by the intrusion of subsurface water masses (S ≤ 20.36 ± 0.18 psu) with O_2 concentrations above the levels tolerated by denitrifying and anammox bacteria ($O_2 \geq 3$ μM, Jensen et al., 2008; Babbin et al., 2014; Figure 2). As a result, in regions where O_2 is ventilated to deeper water masses, the top location of the b_{bp}-*layer* is also deeper. The contrary is observed when O_2 ventilation is shallower (Figure 2 and Appendix A). Secondly, nitrate-reducing SAR11, and denitrifying, anammox, and sulphur-oxidizing bacteria reside between the isopycnals 15.60-16.30 kg m^{-3} (Fuchsman et al., 2011; 2012a; Kirkpatrick et al., 2012), while the b_{bp}-*layer* is formed between isopycnals ~15.79-16.30 kg m^{-3}. We can thus infer coexistence of such bacteria between the coincident isopycnals where the b_{bp}-*layer* is generated. Thirdly, NO_3^- declines from around isopycnal 15.79 kg m^{-3} to the isopycnal 16.30 kg m^{-3} due to the expected N_2 production via the microbial communities involved (Figures 2-3, and Kirkpatrick et al., 2012).

The ventilation of subsurface O_2 is also key in driving the depth at which MnO_2 is formed ($O_2 \leq 3$-5 μM; Clement et al., 2009), and can thus contribute to setting the characteristics of the b_{bp}-*layer* via its subsequent accumulation and dissolution (Konovalov et al., 2003; Clement et al., 2009; Dellwig et al., 2010). Thus, in regions where subsurface O_2 (e.g. $O_2 \geq 3$-5 μM, and S ≤ 20.36 ± 0.18 psu) is ventilated to deeper water masses, both the formation of MnO_2 and top location of the b_{bp}-*layer* can be expected to be deeper, and vice versa (Figure 2). Finally, the dissolution of MnO_2 should also influence the thickness of the b_{bp}-*layer* because it occurs just beneath the maxima of the optical particles inside this *layer* (Konovalov et al., 2006; see the explanation in section 4.3).

Overall, the qualitative evidence presented above points out that particles of MnO_2 as well as nitrate-reducing SAR11, and denitrifying, anammox, and sulphur-oxidizing bacteria, appear to define the characteristics of the b_{bp}-*layer* (Johnson, 2006; Konovalov et al., 2003; Fuchsman et al., 2011, 2012b; Stanev et al., 2018). This observation leads us to argue, in the next section, that the b_{bp}-*layer* is partially composed of the main group of microbial communities involved in N_2 yielding, as well as of MnO_2.

4.3 Role of the removal rate of NO_3^-, MnO_2, and temperature in the vertical distribution of small particles

We propose that the removal rate of NO_3^- is a key driver of the vertical distribution of small particles and N_2 excess within the $OP_{D\text{-}A}$. This is because the vertical profiles of small particles and of N_2 excess are qualitatively similar, and both profiles are clearly related to the rate at which NO_3^- is removed from the $OP_{D\text{-}A}$ (Figures 3-4). For instance, maxima of N_2 excess and b_{bp} coincide around the isopycnal 16.11 ± 0.11 kg m^{-3} (Figure 3; Konovalov et al., 2005; Fuchsman et al., 2008, 2019). At this isopycnal, the mean concentration of NO_3^- is 1.19 ± 0.53 µM. We thus propose that this NO_3^- threshold value splits the $OP_{D\text{-}A}$ in two sub-zones with distinctive biogeochemical conditions (e.g. nitrogenous and manganous zones; Canfield and Thamdrup, 2009). Ultimately, these two different sets of conditions drive the rates at which NO_3^- and small particles are removed and formed within the $OP_{D\text{-}A}$, respectively (Figure 3, and explanation below).

The first sub-zone is thus located between the top of the $OP_{D\text{-}A}$ (σ_θ = 15. 79 kg m^{-3}) and around the isopycnal 16.11 kg m^{-3}. Here, removal rates of NO_3^- (-0.16 ± 0.10 μM m^{-1}, Figure 4) are likely to be boosted by: (1) high content of organic matter (dissolved organic carbon = 122 ± 9 μM, Margolin et al., 2016) and NO_3^- (≥ 1.19 ± 0.53 μM), and (2) O_2 levels staying between a range that maintain the yielding of N_2 (0.24 ± 0.04 μM ≥ O_2 ≤ 2.8± 0.14 μM, n = 100, the means of the minima and maxima of O_2, respectively, in the first sub-zone) and promote the formation of MnO_2 (e.g. maximum of Mn(II) oxidation is at O_2 levels ~0.2 μM; Clement et al., 2009). Consequently, the formation of biogenic and inorganic small particles (and related N_2 excess) increases from the top of the $OP_{D\text{-}A}$ to around the isopycnal 16.11 kg m^{-3} (Figure 3). This hypothesis is: (1) in part confirmed by significant and negative power-law correlations between the suspended small-particle content and NO_3^- in this sub-zone (Figure 3), and (2) in agreement with the progressive accumulation of MnO_2 from around isopycnal 15.8 kg m^{-3} to the isopycnal 16.10 kg m^{-3} (e.g. Konovalov et al., 2006).

The second sub-zone is located between isopycnal 16.11 kg m^{-3} and the bottom of the $OP_{D\text{-}A}$ (σ_θ = 16.30 kg m^{-3}, Figure 3). Here, NO_3^- is low (≤ 1.19 ± 0.53 μM) and O_2 is relatively constant (0.23 ± 0.02 μM, n= 2284, mean of O_2 calculated in the second sub-zone for all profiles), or lower than the minimum of O_2 recorded by this sensor (0.22 ± 0.02 μM, n = 89). These constant (or lower) levels of O_2 roughly correspond to those at which anammox and heterotrophic denitrification are inhibited by ~50% (0.21 μM, and 0.81 μM, respectively; Dalsgaard et al., 2014). In addition, low levels of NO_3^- necessarily promotes the microbial use of Mn(IV) as an electron acceptor, ultimately dissolving the particles of MnO_2 into Mn(II) (e.g. manganous zone; Konovalov et al., 2006; Yakushev et al., 2007; Canfield and Thamdrup, 2009). As a result, this sub-zone exhibits a decline in removal rates of NO_3^- (-0.04 ± 0.01 μM m^{-1}, Figure 4) along with inhibited formation of biogenic small particles and dissolution of MnO_2. Ultimately, both the content of small particles and related N_2 excess decrease from around isopycnal 16.11 kg m^{-3} to the bottom of the $OP_{D\text{-}A}$ (Figure 3). These results are in agreement with: (1) significant and positive exponential correlations computed between the small-particle content inferred from b_{bp} and NO_3^- within this sub-zone (Figure 3), and (2) the overlap of nitrogenous and manganous zones in this sub-zone because the content of MnO_2 particles and dissolved Mn(II) concurrently declines and increases just beneath the isopycnal 16.11 kg m^{-3}, respectively (e.g. Murray et al., 1995; Konovalov et al., 2003, 2005, 2006; Yakushev et al., 2007; Canfield and Thamdrup, 2009).

Strong-positive linear correlations are also recorded between b_{bp} and T in the first sub-zone of the $OP_{D\text{-}A}$ (Figure 4). This is likely to indicate that the formation of small particles is sensitive to very tiny increments in T (0.003 ± 0.001 °C m^{-1}, n = 133). We thus infer a tendency for the decline rates of NO_3^- and related production of N_2 to increase with T. This hypothesis is at least partially supported by the significant correlation between NO_3^- decline rates and T increase rates in this sub-zone (Figure 4). Within the second sub-zone, T continues increasing while b_{bp} decreases, likely due to inhibition of the formation of small particles for the reasons described above (Figure 4). These observations suggest that the production of small particles is likely to have first- and second-order covariations, with NO_3^- and T, respectively — a likelihood backed up by a lack of correlation between NO_3^- decline rates and T increase rates in this sub-zone (Figure 4). Finally, more information is needed to investigate the physical and/or biogeochemical processes driving the correlation between the increase rates of T, and declines rates of NO_3^- in the first sub-zone. This is however out of the scope of our study.

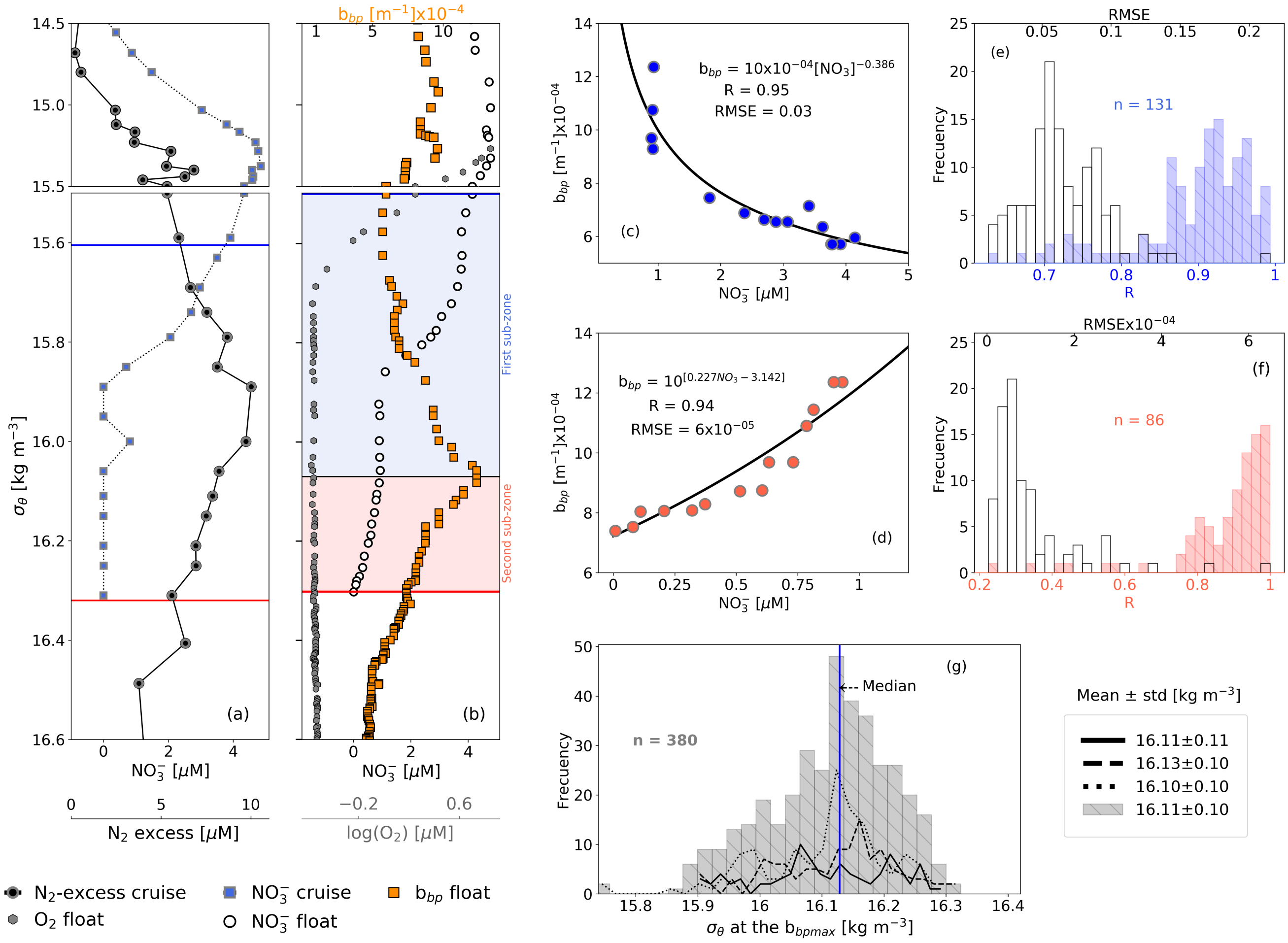

Figure 3: (a) Cruise profiles of NO_3^-, and N_2 excess, collected in March 2005 (Fuchsman et al., 2019). (b) Float profiles of NO_3^-, b_{bp}, and log(O_2) measured on 6th April 2016. Profiles in (a) and (b) were conducted at the northwest of the basin (see Figure 1). The top and bottom of the *$OP_{D\text{-}A}$* are described in (a) and (b) as horizontal blue and red lines, respectively. The b_{bp} maximum is the horizontal black line in (b). The first and second sub-zone of the *$OP_{D\text{-}A}$* are respectively highlighted in (b) as blue and red squares. NO_3^- vs b_{bp} in (c) the first, and (d) the second sub-zone, of the float profile in (b). The number of data points visualized in (c) is lower than in (b) for the first sub-zone because b_{bp} and NO_3^- are not always recorded at the same depths. (e) Frequency distributions of correlation coefficients (R, blue bars), and root mean square errors (RMSE, white bars) for NO_3^- vs b_{bp} in the first sub-zone. (f) Same as (e) but for the second sub-zone. (g) Frequency distributions of the isopycnals at which b_{bp} maxima are found within the *$OP_{D\text{-}A}$*. Dotted, dashed, and solid black lines in (g) are data collected by floats 7900591, 6901866, and 6900807, respectively. Gray bars include all data.

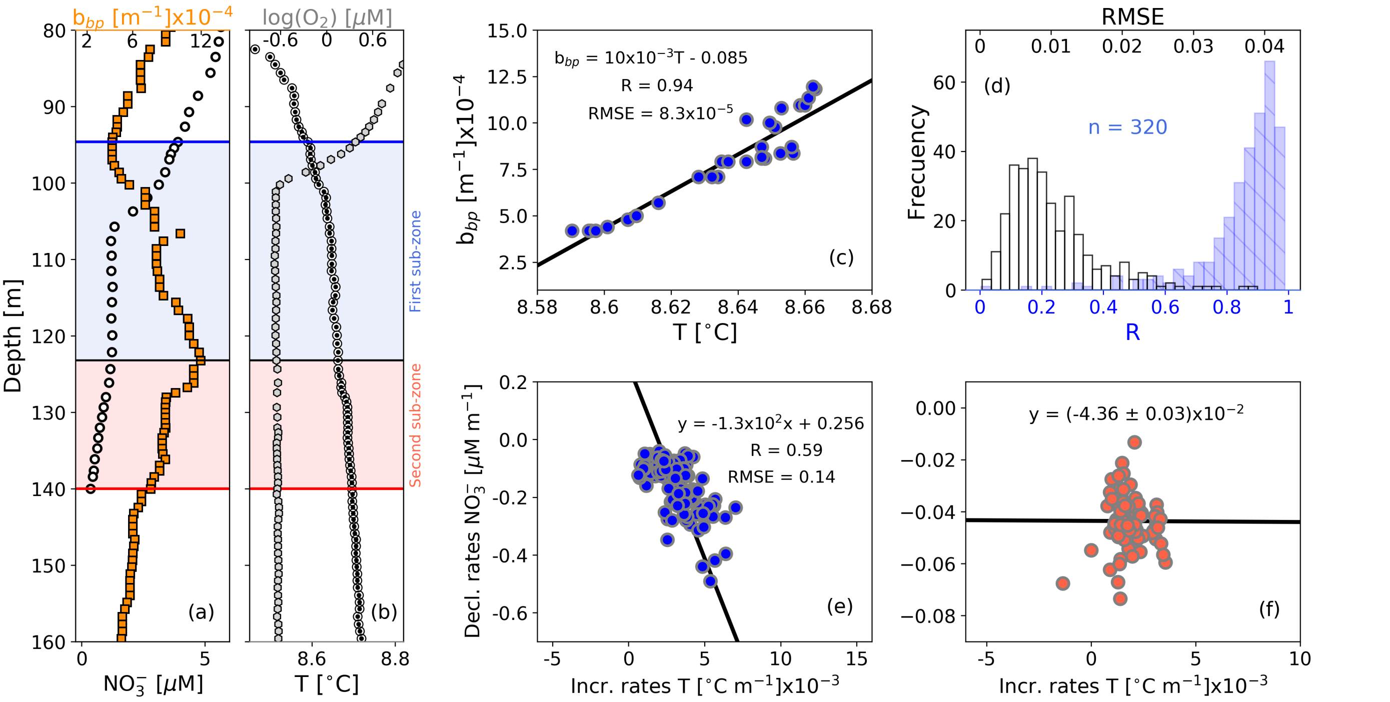

Figure 4: Float profiles of (a) NO_3^-, and b_{bp}, and (b) T and log(O_2) collected on 10th September 2017. Horizontal blue and red lines in (a) and (b) are the top and bottom of the $OP_{D\text{-}A}$. The b_{bp} maximum is indicated in (a) and (b) as horizontal black lines. The first and second sub-zones of the $OP_{D\text{-}A}$ are respectively highlighted in (a) and (b) as blue and red squares. (c) b_{bp} vs T for the first sub-zone of the profile in (b). (d) Frequency distributions of correlation coefficients (R, blue bars), and root mean square errors (RMSE, white bars), for b_{bp} vs T in the first sub-zone, including data collected by the three floats. Decrease rates of NO_3^- vs increase rates of T in (e) the first and (f) the second sub-zone.

To summarize, BGC-Argo float data combined with a proxy of N_2 production suggest that in regions without the Bosporus plume influence, the b_{bp}*-layer* systematically tracks and delineates the *effective* N_2-yielding section independently of: (1) the biogeochemical mechanisms driving N_2 yielding, and (2) the contribution that MnO_2 and other microorganisms can be expected to make to the formation of the b_{bp}*-layer* (e.g. Lam et al., 2007; Fuchsman et al., 2011; 2012a; Kirkpatrick et al., 2018). It is thus finally inferred that this b_{bp}*-layer* is *at least partially* composed of the predominant anaerobic microbial communities involved in the production of N_2, such as *nitrate-reducing SAR11, and anammox, denitrifying, and sulphur-oxidizing* bacteria. These results also suggest that N_2 production rates can be highly variable in the Black Sea because the characteristics of the b_{bp}*-layer* show large spatial-temporal variations driven by changes in NO_3^- and O_2 (Figures 2 and 4). Finally, we propose that b_{bp} and O_2 can be exploited as a combined proxy for defining the N_2-producing section of the oxygen-poor Black Sea. We consider that this combined proxy can delineate the top and base of this section, by applying an O_2 threshold of 3.0 µM, and the bottom isopycnal of the b_{bp}*-layer*, respectively. This section should thus be linked to free-living bacteria (0.2-2 µm), and those associated with small suspended particles (> 2-20 µm), as well as to small inorganic particles (0.2-20 µm).

4.4 New perspectives for studying N_2 losses in ODZs

The conclusions and inferences of this study, especially those related to the origin and drivers of the b_{bp}*-layer*, primarily apply to the Black Sea. However, these findings may also have a wider application. In particular, the shallower water masses of oxygen-deficient zones (ODZs) are similarly characterized by the formation of a layer of suspended small particles that can be optically detected by b_{bp} and the attenuation coefficients of particles (Spinrad et al., 1989; Naqvi et al., 1993; Whitmire et al., 2009). This layer is mainly linked to N_2-yielding microbial communities because: (1) its location coincides with the maxima of N_2 excess, microbial metabolic activity, and nitrite (NO_2^-, the intermediate product of denitrification-anammox that is mainly accumulated in the N_2-yielding section, Spinrad et al., 1989; Naqvi et al., 1991, 1993; Devon et al., 2006; Chang et al., 2010, 2012; Ulloa et al., 2012; Wojtasiewicz et al., 2018), and (2) MnO_2 is not accumulated as in the Black Sea (Martin and Knauer, 1984; Johnson et al., 1996; Lewis and Luther, 2000). Therefore, our findings suggest that highly resolved vertical profiles of b_{bp} and O_2 can potentially be used as a combined proxy to define the *effective* N_2-production section of ODZs. Such definition can be key to better-constrained global estimates of N_2 loss rates because it can allow us to: (1) accurately predict the oxygen-poor water volume where around 90% of N_2 is produced in the ODZ core (Babin et al., 2014), and (2) evaluate how the location and thickness of the N_2-yielding section vary due to changes in the biogeochemical factors that modulate anammox and heterotrophy denitrification.

Global estimates of N_2 losses differ by 2-3 fold between studies (e.g. 50-150 Tg N yr^{-1}, Codispoti et al., 2001; Bianchi et al., 2012, 2018; DeVries et al., 2012; Wang et al., 2019). These discrepancies are caused in part by inaccurate estimations of the oxygen-poor volume of the N_2-production section. Other sources of uncertainties arise from the methods applied to estimate the amount of POC that fuels N_2 production. For instance, POC fluxes and their subsequent attenuation rates are not well resolved because they are computed respectively from satellite-based primary-production algorithms and generic power-law functions (Bianchi et al., 2012, 2018; DeVries et al., 2012). POC-flux estimates based on these algorithms visibly exclude: (1) POC supplied by zooplankton migration (Kiko et al., 2017; Tutasi and Escribano, 2020), (2) substantial events of POC export decoupled from primary production (Karl et al., 2012), and (3) the role of small particles derived from the physical and biological fragmentation of larger ones (Karl et al., 1988; Briggs et al., 2020), which are more efficiently remineralized by bacteria in ODZs (Cavan et al., 2017). In addition, these estimates do not take into consideration the inhibition effect that O_2 intrusions may have on N_2-yield rates (Whitmire et al., 2009; Ulloa et al., 2012; Dalsgaard et al., 2014; Peters et al., 2016; Margolskee et al., 2019).

Overall, mechanistic predictions of N_2 losses misrepresent the strong dynamics of the biogeochemical and physical processes that regulate them. Consequently, it is still debated whether the oceanic nitrogen cycle is in balance or not (Codispoti, 2007; Gruber and Galloway, 2008; DeVries et al., 2012; Jayakumar et al., 2017; Bianchi et al., 2018; Wang et al., 2019). The subsiding uncertainty points to a compelling need for alternative methods that allow accurate refinement of oceanic estimations of N_2 losses.

Our study supports the proposition that robotic observations of b_{bp} and O_2 can be used to better delineate the N_2-yielding section at the appropriate spatial (e.g. vertical and regional) and temporal (e.g. event, seasonal, interannual) resolutions. In addition, POC fluxes and N_2 can be simultaneously quantified using the same float technology (BGC-Argo, Bishop et al., 2009; Dall'Olmo and Mork, 2014; Reed et al., 2018; Boyd et al., 2019; Estapa et al., 2019; Rasse and Dall'Olmo, 2019). These robotic measurements can contribute to refining global estimates of N_2 losses by better constraining both the oxygen-poor section where N_2 is produced, and POC fluxes that fuel its loss. Ultimately, O_2 intrusions into the N_2-yielding section can potentially be quantified by BGC-Argo floats to assess their regulatory effect on N_2 losses.

Conclusions

Our results along with those from previous studies suggest that the b_{bp}*-layer* of the oxygen-poor Black Sea is at least partially composed of nitrate-reducing SAR11, and anammox, denitrifying, and sulphur-oxidizing bacteria. The location and thickness of this layer show strong spatial-temporal variability, mainly driven by the ventilation of oxygen-rich subsurface waters, and nitrate available to generate N_2, respectively. Such variations in the characteristics of the b_{bp}-*layer* highlight that N_2-production rates can be highly variable in the Black Sea. We therefore propose that high resolution measurements of O_2 and b_{bp} can potentially be exploited as a combined proxy to delineate the *effective* N_2-yielding section of ODZs. This proposition is in part supported by evidence that the b_{bp}-*layer* and a majority of N_2-yielding microbial communities are both confined in the shallower oxygen-poor water masses of ODZs. We however recommend investigation into the key biogeochemical drivers of the b_{bp}-*layer* for each ODZ. This information will be critical for validating the applicability of the b_{bp}-*layer* in assessing spatial-temporal changes in N_2 production.

Finally, it is evident that BGC-Argo float observations can acquire essential proxies of N_2 production and associated drivers at appropriate spatial and temporal resolutions. The development of observation-modeling synergies therefore holds the potential to deliver an unprecedented view of N_2-yielding drivers if robotic observations become an integrated part of model validation. Ultimately, this approach could prove essential for reducing present uncertainties in the oceanic N_2 budget.

Appendix A: Supplementary Figures

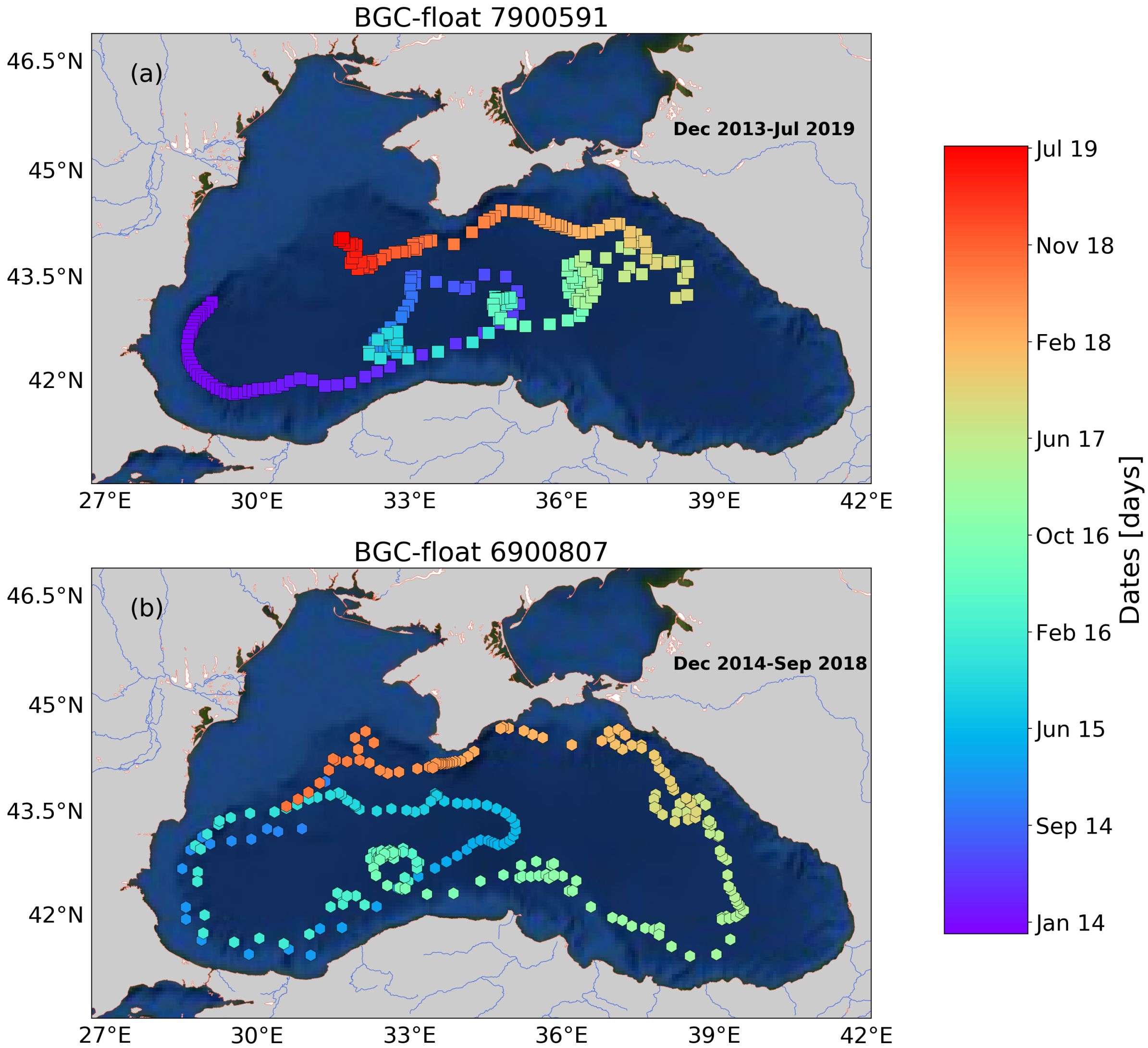

Figure A1: Sampling locations of floats (a) 7900591 and (b) 6900807 between December 2013 and July 2019. Colored squares and hexagons indicate the date (colorbar) for a given profile of floats 6900807 and 7900591, respectively.

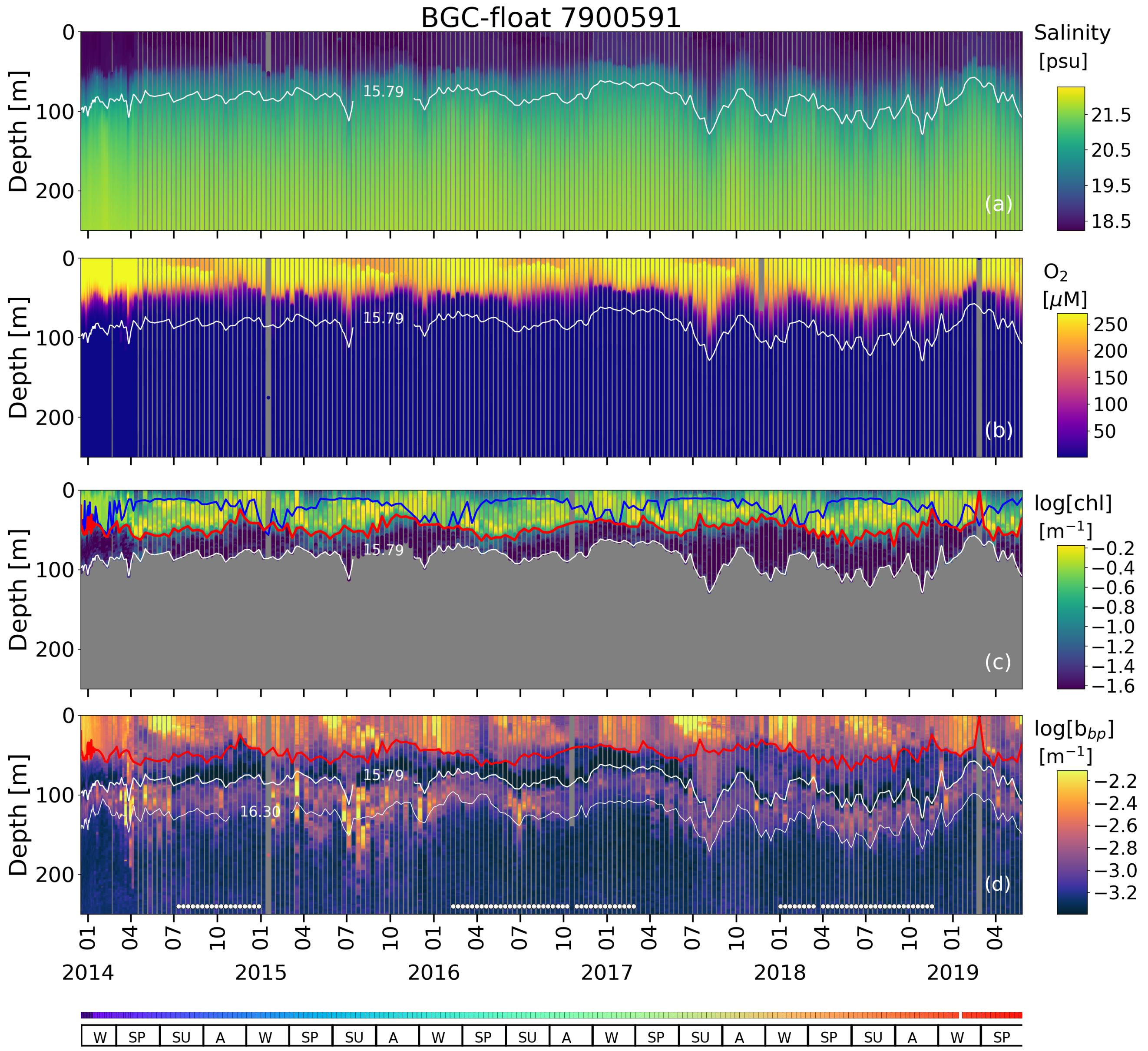

Figure A2: Time series of (a) S, (b) O_2, (c) log(*chl*), and (d) log(b_{bp}) for float 7900591. The blue line in (c) indicates the mixed layer depth. The red lines in (c) and (d) show the base of the productive region. The isopycnals 15.79 kg m^{-3} and 16.30 kg m^{-3} describe the top and bottom of the oxygen-poor zone (*OP_{D-A}*), respectively. SU, A, W, and SP stand for summer, autumn, winter, and spring, respectively. The colored horizontal line at the bottom indicates the sampling site for a given date (Figure S1). The horizontal white lines in (d) are the profiles used to: (1) delimit the *OP_{D-A}*, and (2) find the isopycnals at which b_{bp} is maximum in the *OP_{D-A}*. *chl* is set to zero in the *OP_{D-A}* due to fluorescence contamination (Stanev et al., 2017).

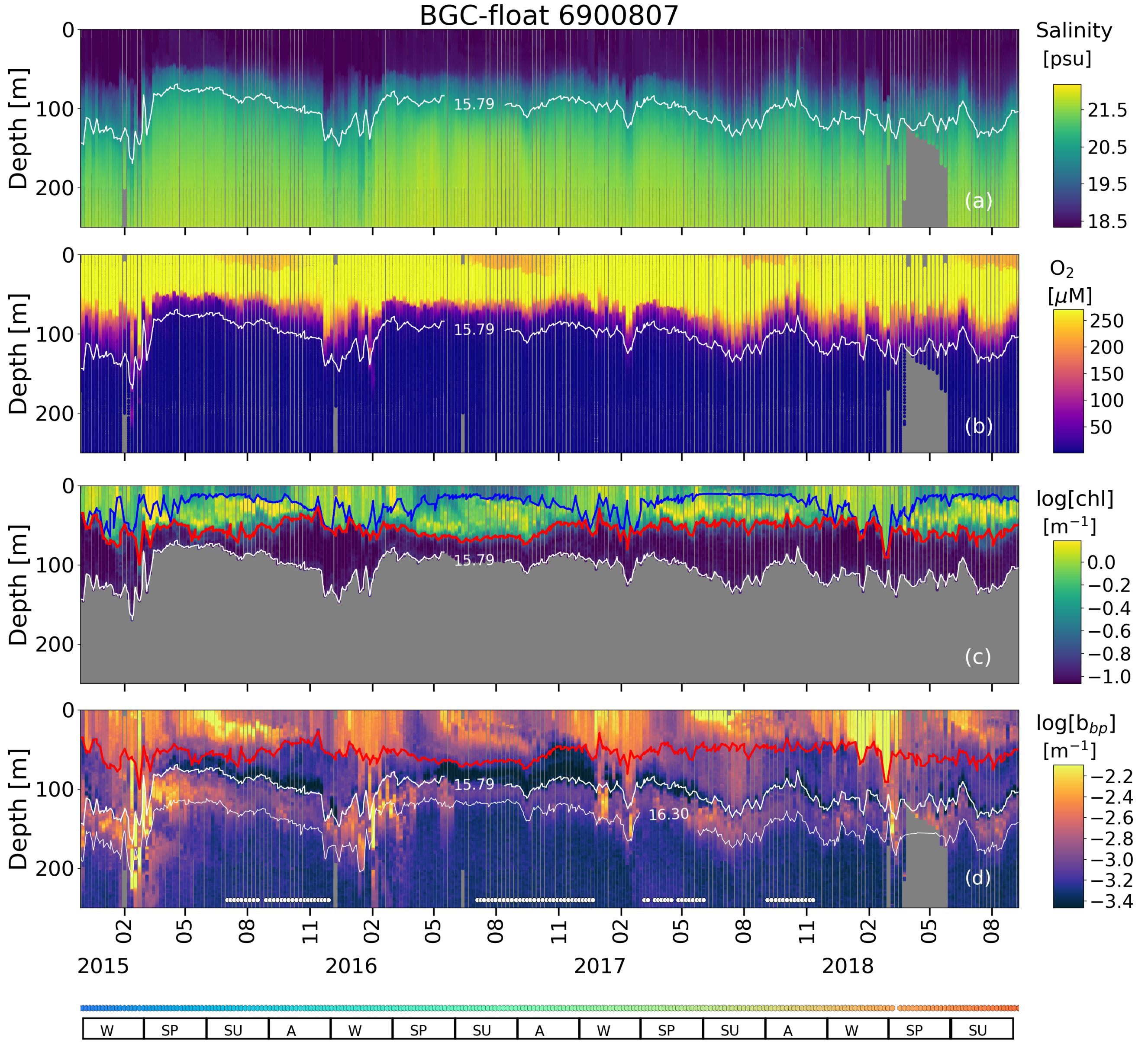

Figure A3: Same as Figure A2 but for float 6900807

Data availability. Data from Biogeochemical-Argo floats used in this study are freely available at ftp.ifremer.fr/ifremer/argo. These data were collected and made freely available by the International Argo Program and the national programs that contribute to it (http://www.argo.ucsd.edu; the Argo Program is part of the Global Ocean Observing System). Data on N_2:Ar ratios are freely available at https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1029/2018GB006032.

Author contributions. R.R. conceptualized the study, wrote the original draft, and generated all figures. H.C. contributed to tuning the study's conceptualization and figures design. A.P. processed all BGC-Argo float data. R.R. and H.C. reviewed and edited the final manuscript. We finally thank Dr. Clara A. Fuchsman and the anonymous reviewer for their accurate and constructive feedback, which allowed us to significantly improve the original version of the manuscript.

Acknowledgments. This study was conducted in the framework of the *Noceanic* project. This project is funded by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Individual Fellowship awarded to

Rafael Rasse (grant agreement 839062). This study is a contribution to the remOcean project (European Research Council, grant agreement 246777, Hervé Claustre).

Competing interests. The authors declare that they have no conflicts of interest.

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<u>Point by point response to the reviewers</u>

***<u>First iteration-Major revision</u>*-[*First reviewer*]**

Dear Dr. Clara A. Fuchsman

Thank you very much for providing your valuable time to review our manuscript. We also thank you for your constructive feedback because it allowed us to improve the original version of the manuscript. Please find below answers and related actions for all your comments and recommendations.

King regards,

Rafael Rasse
Hervé Claustre
Antoine Poteau

General Comment

In "The suspended small-particles layer in the suboxic Black Sea: a proxy for delineating the effective N_2-yielding section" by Rasse et al, the authors analyze particle back scattering, oxygen, HS- and nitrate float data from the Black Sea. The authors can thus delineate the suboxic zone of the Black Sea with the float, and see productivity and export events. The authors assume that the particle back scattering data in the suboxic zone indicates the presence of anammox and heterotrophic denitrifying bacteria. This assumption may be problematic in the Black Sea where it is known that there are high manganese oxide concentrations in the suboxic zone, and it is known that there is an organic matter maximum at the top of the sulfidic zone composed of S oxidizers. However, the data in this paper is useful. The writing just needs to be shifted.

Answer. We agree and understand the main reviewer's concern about the interpretation of the b_{bp}-*layer*.

Actions taken. We changed the writing at the required sections, and added the information needed to explain the role that other particles (inorganic & biogenic) have on the formation of the b_{bp}-*layer*. We are confident you will find this revised version satisfactory (see details below).

Main Comment #1

Large issues.

The introduction needs a section at the beginning describing the Black Sea. This is particularly important because the Black Sea differs from oxygen deficient zones in several important ways. The Black Sea has a sulfidic zone. There are fluxes of reduced species out of the sulfidic zone: reduced S, ammonium, reduced manganese, and methane among other reduced species. Additionally, the zone above the sulfide is suboxic rather than anoxic. Oxygen deficient zones, on the other hand, are mid-water zones that have oxygenated water below them. They are truly anoxic (Revsbech et al., 2009). They don't have fluxes of reduced species entering them. In fact, ammonium is usually below detection (Widner, Fuchsman, et al., 2018; Widner, Mordy, et al., 2018). In the Black Sea, the flux of ammonium from the sulfidic zone determines the importance of anammox and its place in the water column. A comparison of depth profiles of anammox bacteria, ammonium flux and N2 gas can be seen in Fuchsman et al 2012a. Linkage between aerobic ammonium oxidation of the upward flux of ammonium and anammox can be found in (Lam et al., 2007) Additionally, the Black Sea is known to have an organic matter maximum in the redoxycline and quite a bit of information is known about this maximum. (see below) The authors have data from the Black Sea and they need to be more focused on understanding that unique system.

Answer. We agree, the Black Sea is an ecosystem that clearly differs from oxygen deficient zones as we indicated very briefly between lines 215-216 (original manuscript).

Action taken. We removed the phrase oxygen deficient zones (ODZs) from the introduction and sections required. Instead, we used the term: "poorly-oxygenated water masses" (e.g. O_2 < 3 uM), which refers to those water masses at which N_2 can be produced independently of the biogeochemical mechanisms driving it. Thus, including basins (e.g. Black Sea and Cariaco basin) and oxygen deficient zones (e.g. ETNP, ETSP, and AS).

We also included a "background section" to describe the key biogeochemical processes and associated inorganic-biogenic particles contributing to the formation of the b_{bp}-*layer* (see more details below).

The changes mentioned above are highlighted in yellow in the following lines of the new manuscript:

- term: “poorly-oxygenated water masses”: 1, 8, 23, 25, 30, 35, 40, 42, 49, 57, 60, 63, 64, 69…165, etc.
- background section: 71-99.

Main Comment #2

What can this float data tell us about the Black Sea? Are the particle maxima larger on the edges than in the middle of the Sea? Is there a correlation between euphotic zone particles and size of the suboxic zone particle max? Are particle flux events correlated to a season? Not that these particular questions need to be answered. At the moment, this paper tells us things that we already know (there is a particle maximum between 3uM oxygen and 10 uM sulfide), but I think it could easily tell us more.

Answer. We agree, there are many interesting aspects that can be easily explored with our data set. However, such aspects are out of the scope of our study. In addition, we consider that our findings tell more than a single maximum of particles between 3 uM O_2 and 10 uM sulphide. This is mainly because they highlight that the b_{bp}*-layer* can be exploited as a combined proxy to efficiently *delineate and track* the effective N_2 yielding section. The latter was in part demonstrated by the *novel and robust* b_{bp} vs NO_3^- correlations computed with *high spatial and temporal* resolutions. The data of optical particles maxima are only used as complementary information to support our findings.

Finally, from our point of view, we consider that the key questions would be: how this optically derived layer of suspended small-particles can be exploited – by first time- to improve current estimates of N_2 yielding via the growing BGC-Argo float network? For instance, what does this b_{bp}*-layer* can tell us about how physical forcing drive spatial-temporal changes in the location and thickness of the *effective* N_2 yielding sections of ODZs? What can occur to the b_{bp}*-layer* and related N_2 yielding rates, if O_2 is injected from the bottom instead of Mn^{2+}, NH_4^+ or H_2S? How can we exploit the latter two aspects to improve oceanic estimates of N_2 production? Overall, this work is only a step forward to build the foundations that we need to achieve the main goal of our ongoing research. We hope the reviewer can also understand our perspective.

Action taken. We did not take actions about this.

Main Comment #3

I think it is important to note that manganese oxides are quite abundant in the Black Sea and that manganese oxides are also particles in the 0.2-20 um size range. Particle backscattering detects particles of all kinds. In the Black Sea, both ammonium and Mn^{2+} have an upward flux from the sulfide zone. Anammox bacteria use the ammonium flux to produce N2 gas (Fuchsman et al 2012a) and the Mn2+ is oxidized to manganese oxides under very low oxygen levels in the same zone (Clement et al., 2009). Thus excess N2 gas and manganese oxides are correlated. That correlation is not due to causation however. The authors need to consider how the manganese oxides affect their results. See (Clement et al., 2009; Dellwig et al., 2010; Yakushev et al., 2009) for more information about manganese oxides in the Black Sea.

Answer. We agree with the reviewer. The role of manganese oxides and its link with the suspended small particles layer should be described better.

Action taken. The role of manganese oxides (mainly as MnO_2) was included in both the new “background section” and discussion.

The changes indicated above are highlighted in yellow in the following lines of the new version:

- 71-99, 175-202, 209-235, 264-270.

Main Comment #4

[a] The ability to detect particles in the water is not a measurement that only exists on floats, but is also present on CTD packages. Thus, let us look at a station in the Black Sea where we have all the relevant dataâ˘A˘ T the Western Gyre station in 2005. **[b & c]** Here the maximum in organic C associated with microbes is found at sigma theta 16.3 (Figure 1 Fuchsman et al 2011). The maximum in anammox bacteria at the same cruise/station is at sigma theta 16.0-16.1 and the maximum in biologically produced N2 gas is at sigma theta 15.9-16.3 (Fuchsman et al 2012a Figure 1d). The maximum in MnO2 is at sigma theta 15.85 (Fuchsman et al 2011 Figure 6c). There is a small minimum in transmission from 15.8-15.85. The transmission signal corresponds to the manganese oxide peak not the peak in anammox bacteria or organic matter. However, that particular station didn’t have a large organic matter signal in the redoxycline. From looking at the authors' data, I would guess that they often see the organic matter maximum in the redoxycline. The organic matter maximum in the Black Sea redoxycline is from S oxidizing bacteria, which may or may not be autotrophic denitrifiers (Glaubitz et al., 2010; Kirkpatrick et al., 2018). These organic matter maxima can be dominated by S utilizing autotrophic denitrifiers of the genus Sulfurimonas (Kirkpatrick et al., 2018 Figure 7). And thus they could be involved in N2 production, but it has not been proven. Some useful papers about the organic matter maximum in the redoxy- cline of the Black Sea (Coban-Yildiz et al., 2006; Ediger et al., 2019; Glaubitz et al., 2010; Yilmaz et al., 2006).

[d] Though anammox and denitrification are very important biogeochemically, they aren't actually the most abundant bacteria found in the Black Sea or oxygen deficient zones. In the ETNP oxygen deficient zone, anammox bacteria reached 10% of the community and complete denitrifiers reach _5% in the water and 14% of the community on particles (Fuchsman et al., 2017). The most abundant bacteria in oxygen deficient zones, by far, are nitrate reducing SAR11, reaching 60% of the community (Fuchsman et al., 2017; Tsementzi et al., 2016). In the Black Sea, once again SAR11 are the most abundant bacteria (Fuchsman et al., 2011 Figure 2). The SAR11 cannot make N2 gas. They just reduce nitrate to nitrite. I am just trying to note that for heterotrophic denitrifiers and anammox, the authors are using a bulk measurement to look for changes in bacteria that are rarely more than 10% of the community.

Answers.

[a] CTD. We agree, CTD packages provide very valuable information about the physical, optical, and biogeochemical properties of the ocean. For instance, they generate variables that cannot be measured by the current float sensors as well as of those variables used to calibrate them. However, they can only provide a snapshot for a given time of the sampling day, while discrete samples are often collected with poor vertical resolution. We thus consider that alternative methods need to be developed to complement CTD packages, and ultimately better understand biogeochemical cycles in poorly oxygenated regions as proposed here (please see also our answer in the **main comment #1**, section 4.4. e.g. and refs: Chai et al., 2020; Claustre et al., 2020; Martin et al., 2020).

Action taken. We did not take actions about this.

[b] Comparison between the vertical profiles of the particles measured by CTD and floats. We reviewed all figures and articles cited by the reviewer. Again, this is a very interesting description of the vertical profiles of N_2, and particles using data collected via CTD packages. Even though we used the profile of March 2005 to highlight the *qualitative* relationship between N_2 excess and optical particles *only* in the *effective N_2* production section; we consider these data are not the most suitable to compare the vertical profiles of particles derived from CTD and floats (including those from Coban-Yildiz et al., 2006; Ediger et al., 2019; Glaubitz et al., 2010; Yilmaz et al., 2006). The main reason is that the vertical profiles of particles cited by the reviewer are representative of the region impacted by the Bosporus plume. In this region, lateral advection via the Bosporus plume drives a maximum of particles ~ 16.3 kg m^{-3} (or higher) because it fuels chemoautotrophic activities by injecting NO_3^- (e.g. ~ 700 m depth, Stanev et al. 2017). Thus, these CTD-profiles of particles must be similar to those excluded in our analysis (e.g. see our Figure 2 between May-June). Note that we focus on the in-situ 1D processes driving the local formation of the *b_{bp}-layer* (this was indicated between lines 83-84 of the original manuscript). Thus, our data set is more representative of the profile described in Figures 1c-1d of Lam et al. 2017 indicated below. Also note that our b_{bp} maxima are located between the isopycnals 15.79 kg m^{-3} and 16.3 kg m^{-3} (Figure 2, and Figure 3g of the manuscript).

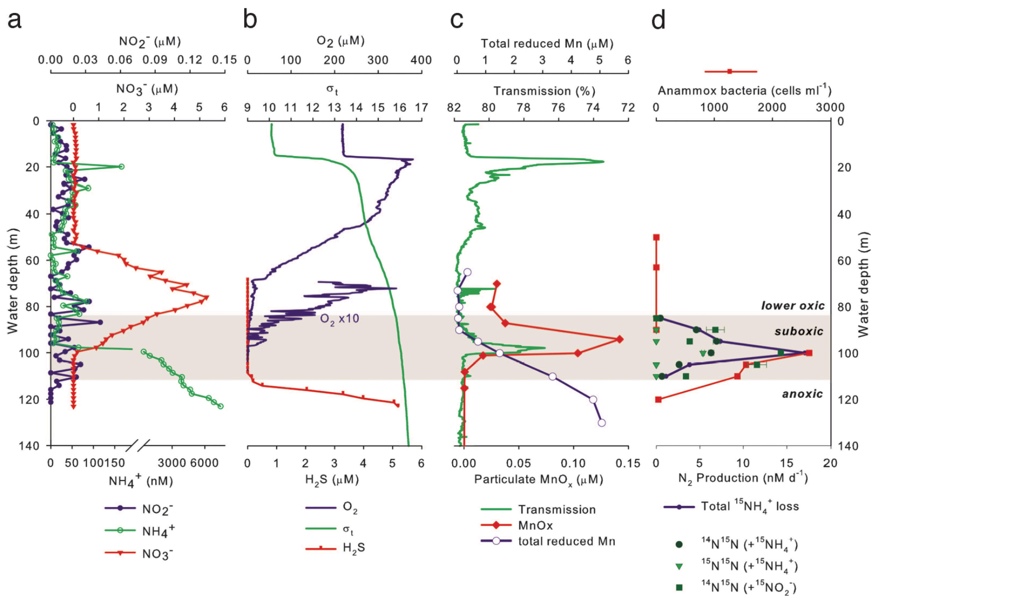
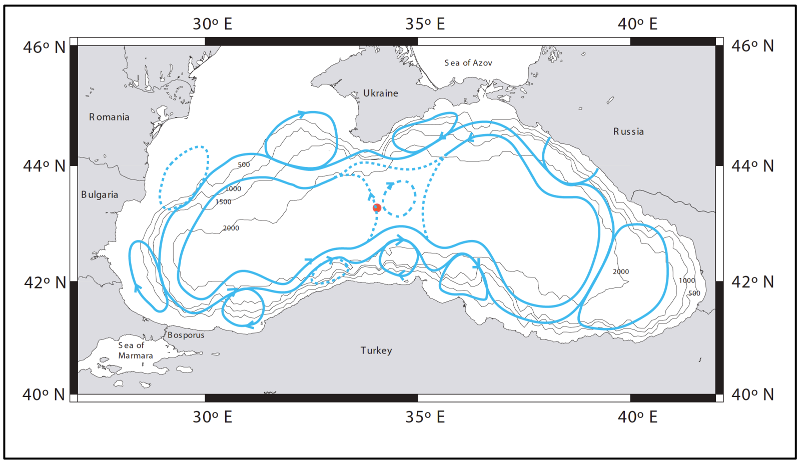


Fig. 1. Vertical distribution of inorganic nitrogen (*a*), O_2 and sulfide (*b*), light transmission, particulate MnO_x, and total reduced Mn (*c*), and anammox bacterial abundance and $^{15}N_2$ production rates (*d*).



Action taken. We did not take actions about this.

[c, d] **Vertical profile of biogenic and organic particles**. We thank the reviewer for this didactic and constructive comment.

Again, we agree. There are different biogenic and inorganic particles that contribute to the b_{bp}-*layer*, as we briefly recognize between lines 196-197 of the original manuscript . We only indicate that annamox-denitrifying bacteria are *at least partial contributors of the* b_{bp}-*layer* because they should have the major contribution to N_2 yielding and removal rates of NO_3^-. Hence, we did not suggest that such bacteria are the main microbial component (or organic particles) that contribute to the b_{bp}-*layer*. It is clear that b_{bp} signal is a black box that cannot be attributed to a single type of organic-inorganic particles.

As mentioned above, we are only trying to highlight two aspects: (1) the b_{bp}-*layer* is systematically *delineating* and *tracking* the effective N_2 yielding section, independently of the physical (e.g. NH_4^+, Mn^{2+}, H_2S pumped from the sulfidic zone) and biogeochemical mechanisms driving both N_2 yielding and the small-particles content, and (2) similar results should also be expected for the b_{bp}-*layer* of the ODZ because this layer must be mainly due to the microbial communities involved in N_2 yielding (see also the answer of the **main comment # 1, section 4.4, and refs:** Martin and Knauer, 1984; Johnson et al., 1996; Lewis and Luther, 2000).

Action taken. We added information about the other inorganic and biogenic particles that contribute to the formation of the b_{bp}-*layer*, and modified the writing at the required sections.

Such changes are highlighted in yellow in the following lines of the new manuscript:

- 71-99, 175-202, 209-235, 264-270.

Main Comment #5
Thus, in the Black Sea, I think the assumption that the particle layer represents anammox and heterotrophic denitrifiers is not ideal. First, there are high concentrations of particulate metals in the Black Sea, particularly mananese oxides. Second, the organic matter maximum in the redoxycline is from S oxidizers. Some of these S-oxidizers may be autotrophic denitrifiers. Some aren't. Thus I think the way the particle maximum is talked about in the paper needs to be shifted. Additionally all this information should be in the introduction and discussion.

Answer. We agree
Action taken. We changed how the maximum of optical particles is described (see all details above and in the new version).

Specific [ES].

ES comment # 1
Was the oxygen data from the floats calibrated? See the work of Seth M. Bushinsky to understand the importance of calibration. This information is glossed over in the methods. I think that in previous float work in the Black Sea, scientists used the sulphide zone as a zero to at least track the drift of the oxygen optode over time. Also, it would be good to have a detection limit for all the different float sensors. Bushinsky et al 2016 Limnology and Oceanography Methods

Answer. Yes, the optodes 43330 are multi-point calibrated. The oxygen data is adjusted and corrected following the Argo quality control manual for dissolved oxygen concentration as cited in the manuscript (Thierry et al. 2018).

Action taken. We added both the range of O_2 concentrations that can be measured by the Optode sensors and their accuracy. This information is reported by the manufacturer**.** In addition, we cited the work related to the O_2 Optode Drift correction applied to the sensors (Bittig, and Körtzinger. 2015).

ES comment # 2
Line 8: This sentence is not accurate as written.

Answer. Agree
Action taken. We modified this sentence.

ES comment # 3
Line 22-23: I am confused what this sentence is trying to say. I note that N2 gas concentrations can be between 400 and 500 microM in the water due to abiotic gas exchange of N2 from the atmosphere. So the authors really mean to say N2 production not concentration. The use of the word respectively in line 23 implies that denitrification is 20% of N2 production and anammox is 40%. Rather, I think the authors are talking about how 20-40% of N2 production occurs in the water column as opposed to in the sediments. The best citation for this is (DeVries et al., 2013).

Answer. We did not understand this observation because N_2 concentration is not mentioned in this sentence. We only indicate that N_2 is mainly produced via anammox-denitrification.
Action taken. We modified this sentence and removed the word "respectively".

ES comment # 4
Line 25: perhaps "where the bacteria that mediate the process mainly reside"
Answer. OK.
Action taken. We did not take actions about this because this is only a semantic issue**.**

ES comment # 5
Line 26-27: I am confused as to the meaning of this sentence? Are the authors trying to say that 90% of the N2 production occurred in the upper ODZ? Perhaps it would be better to say that 90% of N2 production occurred in the upper 50 meters of the ODZ. Additionally, one should either say N2 production or N loss. N loss refers to the

loss of nutrients. The N2 is produced not lost. I also note that anammox rates are not always highest at the top of the ODZ. See (De Brabandere et al., 2014)

Answer. OK.

Action taken. The term ODZs was removed from the introduction. We now use the term "shallower poorly-oxygenated water masses" and replaced the word "loss" by "yielding" throughout the manuscript.

ES comment # 6

Paragraph 1: I am having issues with oxygen deficient zones being called suboxic. The deficient part of oxygen deficient zone implies that the system is anoxic. No oxygen. The word was coined to differentiate these anoxic systems from suboxic systems which are called oxygen minimum zones.

Answer. We agree, the application of the term suboxic is not accurate and very confusing because the O_2 levels used to define it can potentially overlap with hypoxic (O_2 < 60 uM, e.g. Stramma et al. 2008) and anoxic conditions (O_2 < 1-2 uM). Example, for the Black Sea, the suboxic zone is set between O_2 levels ranging between 1.8 uM and 39 uM (Fuschman et al., 2011; Stanev et al., 2018 and references therein).

Action taken. We now use the term "poorly oxygenated water masses" to describe the section where N_2 is effectively produced. We also introduced the term, "chemical zones" because this is more suitable (or accurate) to explain the biogeochemical reactions that drive N_2 yielding and the small-particle content in the poorly oxygenated water masses (Canfield and Thamdrup, 2009).

The changes indicated above are highlighted in yellow in the following lines of the new manuscript:
- Lines: 71-99, 209-235, and 209-235.

ES comment # 7

Line 93: The best citation is (Dalsgaard et al., 2014). The authors do cite this paper later. To be consistent it should be noted here as well.

Answer. OK

Action taken. We cited Dalsgaard et al., 2014 as well.

ES comment # 8

Line 121-122: This sentence needs clarification for two reasons. The authors are comparing depth and density. The Black Sea is much more consistent in density space than depth. It would be good to give the density range as well as the depth in line 121. Additionally, the authors compare a depth where sulfide is 11 uM to a depth where it is 10 nM. It is not surprising that the 11 uM depth is deeper than the 10 nM depth. That's an order of magnitude different in concentration. What is the HS- detection limit of the float?

Answer. OK

Action taken. We reported the detection limit of HS-, as well as the ranges of depth and density. We also modified the sentence between lines 121-122 of the old manuscript.

ES comment # 9

Lines 133-148: The particle layer is between 3 uM oxygen and 11 uM sulfide. Both manganese oxides, and S oxidizers are also found in this range as well as methane oxidizers (Kirkpatrick et al 2018 Figure 6D)– not just anammox and denitrifiers. It is true however, that lots of microbial activity is occurring in this zone. These processes also could all affected by intrusions of oxygen. Lines 142-144: This is interesting.

Answer. OK

Action taken. This sentence was modified. We included the presence of other bacteria and MnO_2, and described their links with O_2 and NO_3^- levels according to the case.

Such changes are highlighted in yellow in the following lines of the new manuscript:
- Lines: 71-99, 175-202, 209-235, 264-270.

ES comment # 10

Line 150-151: This sentence is confusing. I am glad that the authors acknowledge manganese oxides existence. However, manganese oxides are formed by manganese oxidizing bacteria not by denitrifiers. Perhaps autotrophic denitrifiers and manganese oxides, as concepts, should be separated into two sentences

Answer. OK

Action taken. This sentence was removed.

ES comment # 11

Line 171: Are the authors that confident in their oxygen concentrations? This would only be true if the sensors are calibrated. Can the optode see the difference between 0.2 uM and 0 uM??

Answer. Yes. According to the manufacturer (https://www.aanderaa.com/media/pdfs/d378_aanderaa_oxygen_sensor_4330_4330f.pdf), the Optode sensors can measure O_2 concentrations between 0 to 1000 uM with an accuracy of 1.5%. Thus, in theory, we should be able to see the difference between 0.2 uM and 0 uM. However, the minimum value of O2 measured by the sensor and used in our calculation was 0.22 uM. Thus, we cannot confirm (or regret) that such sensors can see such difference.

Action taken. We added the range of O_2 concentrations and accuracy of the Optode sensor reported for the manufacturer. Finally, we indicated that 0.22 uM was the minimum value of O2 measured by the sensor, and suggested that the O_2 level can be also lower (see line 224 in the new one)

ES comment # 12

Line 190: Can you actually differentiate correlations with temperature from correlations with density in these deep layers? There is no biological reason that a change < 0.1 in temperature should matter. However, I think many things, such as sulfide, correlate with temperature in this basin.

Answer. Correlations between b_{bp} vs T were computed between two reference isopycnals delineating the two sub-zones of the b_{bp}*-layer*. The same principle was applied for b_{bp} vs NO_3^-. Thus, these correlations are consistently found in the water layers of the two sub-zones (or "chemical zones") defined for the b_{bp}*-layer*. which ultimately validates -in part- our main hypothesis. However, our data cannot explain why such correlation is found.

Action taken. The following sentence was added:

Finally, more information is needed to investigate the physical and/or biogeochemical processes driving the correlation between the increase rates of T, and declines rates of NO_3^- in the first sub-zone. The former is, however, out of the scope of our study. (lines 243-245 in the new version)

ES comment # 13

Line 235: (Cavan et al., 2018) Line 237: (Margolskee et al., 2019)

Answer. OK

Action taken. We cited Margolskee et al., 2019 and Cavan et al., 2017 because the latter is more suitable for this context (see also Rasse and Dall'Olmo, 2019 for the ETNA-OMZ case; http://dx.doi.org/10.1029/2019GB006305).

ES comment # 14

3.4 New perspectives for studying N2 losses in suboxic ODZs : This section would be more compelling if the floats measured N2 gas. There is such a deviceâ˘A ˇTReed et al 2018 Deep Sea Research Part I 139: 68-78.

Answer. OK

Action taken. We cited Reed et al., 2018 and indicated that N_2 can be measured by BGC-floats.

References not cited in the new version of the manuscript

Chai, F., Johnson, K. S., Claustre, H., Xing, X., Wang, Y., Boss, E., ... & Sutton, A. (2020). Monitoring ocean biogeochemistry with autonomous platforms. *Nature Reviews Earth & Environment*, 1-12. https://doi.org/10.1038/s43017-020-0053-y

Claustre, H., Johnson, K. S., & Takeshita, Y. (2020). Observing the global ocean with biogeochemical-Argo. Annual Review of Marine Science, 12, 23-48. https://doi.org/10.1146/annurev-marine-010419-010956

Stramma, L., Johnson, G. C., Sprintall, J., & Mohrholz, V. (2008). Expanding oxygen-minimum zones in the tropical oceans. *science*, *320*(5876), 655-658. DOI: 10.1126/science.1153847

Martin, A., Boyd, P., Buesseler, K., Cetinic, I., Claustre, H., Giering, S., ... & Robinson, C. (2020). The oceans' twilight zone must be studied now, before it is too late. doi: 10.1038/d41586-020-00915-7

***Second iteration-Minor revision*-[*First reviewer*]**

General Comment

I thank the authors for expanding the introduction and discussion to include manganese oxides as a source of particles. I think this addition is important to the paper. However, the authors still need to add in references to previous work on the organic matter maximum at the redox interface In the Black Sea. Just one or two sentences acknowledging this previous work seems important since this organic matter maximum is the focus of the present paper.

Important papers about the organic matter maximum in the Black Sea redoxycline: (Coban-Yildiz et al., 2006; Yilmaz et al., 2006; Glaubitz et al., 2010; Ediger et al., 2019) Perhaps add these new sentences around line 29 of the introduction.

First, we would like to thank Dr. Clara A. Fuschman for her constructive, positive, and accurate feedback. The latter allowed us to improve the original version of this manuscript. We are confident that you will find this revised version satisfactory.

Answer. We agree, Epsilonproteobacteria have the most significant contribution to the formation of organic particles *mainly* in the sulfidic zone**.**

Action taken. We added the information related to the role of such bacteria in the formation of organic particles and N_2 yielding. This information is in the section 2.0 between lines 83-88 of the last version (text highlighted in green). Related references were also included.

Specific [ES].

ES comment #1

Line 31: N2 yielding bacteria—the noun is necessary
Line 77: N2 yielding bacteria

Answer. OK
Action taken. The noun N_2 yielding bacteria was added (text highlighted in green at lines 31 and 78, respectively)

ES comment #2

Poorly oxygenated isn't a scientific term. I think the term you are looking for is suboxic**.**

Answer. Oxygen-poor waters is a scientific term that was already used by Stramma et al. 2008 (abstract), 2010 (Introduction, 2nd paragraph). We thus consider that the latter term is equivalent to the one used here (poorly-oxygenated).

Action taken. To be consistent with what is reported in the literature, we changed the terms poorly-oxygenated by oxygen-poor throughout the manuscript. These changes are highlighted in green at the respective lines of the revised manuscript.

ES comment #3

Line 174: The epsilonproteobacteria Sulfurimonas is one of the most important sulfur oxidizers in the Black Sea. See (Glaubitz et al., 2010) also (Kirkpatrick et al., 2018) figure 7. This epsilon proteobacteria is likely an autotrophic denitrifier (Fuchsman et al., 2012). Might be a better sulfur oxidizer to single out than SUP05—or name them both.

Answer. OK
Action taken. We included the information requested between lines 178-179 of the revised manuscript (highlighted in green).

ES comment #4
Line 28: the fact that some SAR11 can reduce nitrate is from (Tsementzi et al., 2016). I know you cite this paper later, but it would be good to cite it here too.

Answer. OK
Action taken. The reference was included at the line specified.

ES comment #5
Line 312: Actually, your results do not suggest that the particle layer is due to the list of bacteria. Previous work suggests this. For example (Glaubitz et al., 2010) or (Kirkpatrick et al., 2018). You assume that this is the case. Please rephrase.

Answer. OK
Action taken. This sentence was rephrased.

References

Stramma, L., Johnson, G. C., Sprintall, J., & Mohrholz, V. (2008). Expanding oxygen-minimum zones in the tropical oceans. *science*, *320*(5876), 655-658. DOI: 10.1126/science.1153847

Stramma, L., Schmidtko, S., Levin, L. A., & Johnson, G. C. (2010). Ocean oxygen minima expansions and their biological impacts. *Deep Sea Research Part I: Oceanographic Research Papers*, *57*(4), 587-595. https://doi.org/10.1016/j.dsr.2010.01.005

***First iteration-Minor revision*-[*Second reviewer*]**
Dear reviewer,

Thank you very much for spending part of your valuable time reviewing our manuscript. We also thank you for your constructive feedback because it allowed us to improve the original version of the manuscript. Below, you will find our answers and actions taken for each of your comments.

King regards,

Rafael Rasse
Hervé Claustre
Antoine Poteau

Specific [ES].

ES comment #1

What is the typical depths ??

Are these depths vary among different ODZs?

Answer. OK

Action taken. This sentence was modified. We indicated the depths at which this layer can be found. This information is based on data from the Black Sea (this study), and the ODZs of the Arabian Sea and ETSP (Whitmire et al. 2009; Wojtasiewicz et al. 2018).

ES comment #2

Are these factors listed in order of their importance?

Answer. According to the literature, we consider this is the most likely order.

Action taken. We did not take actions about this.

ES comment #3

Will not the chemical composition, salinity and temperature of water column would also matter for resultant optical visibility / abundance of anammox and denitrifying bacteria ??

Answer. Organic matter composition should be key driving the microbial activity (e.g. anammox and denitrifying bacteria, e.g. Van Mooy et al. 2002) but this not be critical for our case (see line 165 in the old manuscript and the cited work). We mentioned an array of chemical variables (levels O2, NO3, and HS, OM) at the line 34 of the old version. We don't have information about salinity but T can affect their activity in sediments (e.g. Rysgaard et al. 2004; Canion et al., 2014).

Action taken. We did not take actions about this.

ES comment #4

Here authors are attempting to investigate measured Bbp layer (absorption ?) with chemical parameters such as O2, NO3 H2S and N2 produced.....all chemical parameters is there any way to provide Bbp thickness and its absorption correlation with actual density of microbial mass...(just wondering samples collected on filters??)

Answer. We did not have such data .
Action taken. We did not take actions about this.

ES comment #5

How much thick it is?

Answer. It can be highly variable with time, and between ODZs and anoxic basins**.** Please see section 4.1, where we indicate the thickness of the *b_{bp}-layer*' for the case of the Black Sea.

Action taken. We did not take actions about this..

ES comment #6

Suppose this factor is negligible in some locations ??

Answer. Please**,** see how the ventilation of subsurface O_2 defines the characteristics of the *b_{bp}-layer* and how we used such information to explain what are the main particles contributing to its formation (e.g. section 4.2).

Action taken. We did not take actions about this.

ES comment #7

why ? what is another factor for second sub-zone?

Answer. This is related to the biogeochemical processes that control the content of suspended small particles and N_2 excess in the chemical zones of the poorly-oxygenated water masses. This is better described in the new version of the manuscript.

Action taken. We included a new "background section" to describe the key biogeochemical processes and associated inorganic-biogenic particles contributing to the formation of the b_{bp}-*layer*. The interlinks among biogeochemical processes, and the vertical profiles of small-particles and N_2 excess are described in the discussion as well.

These changes are highlighted in yellow in the following lines of the new version:
- 71-99, 175-202, 209-235, 264-270..

<u>ES comment #8:</u> Sentences highlighted in yellow without suggestions

- of chl and bbp and due to particle

Answer. Both spikes are due to particles-aggregates. We thus consider this sentence is OK

Action taken. We did not take actions about this.

- hypothesized

- Optical proxies of tiny particles can be applied as an alternative approach to assess the vertical distribution of N2-yieldingmicrobial communities in upper suboxic ODZs

- particle content inferred from bbp and N2 produced by microbial communities are at least qualitatively correlated microbial communities in upper suboxic ODZs

- bbp and O2 can be exploited as a combined proxy for defining the N2-producing section of the suboxic Black Sea

- fluorescence and total backscattering were converted into Chlorophyll concentration (*chl*) and particle backscattering (b_{bp}) following standard protocols

- HS- was not used to delimit the bottom of this zone because the maximum concentration of H2S that denitrifying and anammox bacteria tolerate is not well established.

- NO_3- and O_2 are two of the key factors that modulate the presence of denitrifying and anammox bacteria

- bbp-layer is partially composed of N2-yielding microbial communities such as anammox and denitrifying bacteria.

- bbp-layer is at least partially composed of anaerobic microbial communities involved in the production of N2

Answer. OK

Action taken. The sentences above were modified.

- o free-living bacteria (0.2-2 µm), and those associated with small-suspended particles (> 2-20 µm).

Answer. This particle size is explained in the introduction

Action taken. We did not take actions about this.

<u>ES comment #9:</u> Other sentences highlighted in yellow without suggestions

- How key drivers of anammox-denitrifying bacteria dynamics impact on the vertical distribution of bbp and the thickness of the bbp-layer.

- Optical proxies of tiny particles can be applied as an alternative approach to assess the vertical distribution of N2-yielding

- Slightly sulfidic conditions of the deepest isopycnal at which anammox bacteria can be still recorded

- It is still debated whether the oceanic nitrogen cycle is in balance or not

Answers. Because it is not specified what are the issues with the sentences above; we assumed that these are only semantic issues**.**
Action taken. We did not take actions about this.

<u>References.</u>

Canion, A., Kostka, J. E., Gihring, T. M., Huettel, M., Van Beusekom, J. E. E., Gao, H., ... & Kuypers, M. M. (2014). Temperature response of denitrification and anammox reveals the adaptation of microbial communities to in situ temperatures in permeable marine sediments that span 50? in latitude. *Biogeosciences*, *11*(2), 309.

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Van Mooy, B. A., Keil, R. G., & Devol, A. H. (2002). Impact of suboxia on sinking particulate organic carbon: Enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta*, *66*(3), 457-465.