Reviewer 1 :

Petit et al. "Assessing phytoplankton community composition using in-situ multispectral excitation fluorescence and potential for application to BGC-Argo profiling floats" investigated the potential of the multispectral excitation fluorometer (MXF) to assess the phytoplankton community structure. The MXF approach is mandatory for the BGC-Argo observations, and thus, this work is important. However, there were several disadvantages in this study: one was the quality of the MXF, and the other was the approach (clustering analysis). First, they used the three excitation channels MXF; however, there are two major MXFs which have five excitation channel (FluoroProbe) and nine channels (MultiExciter). Both of them were started to apply the ocean observations (Garrido et al, 2019 https://doi.org/10.1007/s10661-019-7349-8; Wang et al. 2016 https://doi.org/10.1364/OE.24.023635; Kodama et al. 2022 https://doi.org/10.1371/journal.pone.0257258; Xu et al. 2022 https://doi.org/10.3389/fmars.2021.756180; Zhang et al. 2025 https://doi.org/10.1016/j.algal.2025.104155; Taniuchi et al. 2026 https://doi.org/10.6090/jarq.24S30). Thus, I cannot understand why the authors evaluate the ability of the ECO 3M1M. The small number of channels could identify the community structure so roughly, and thus I strongly believe that FluoroProbe and MultiExciter had an advantage for the aims of the authors' study. Second, the authors did the clustering analysis. I agree that this analysis is useful if the clusters are representative of the ocean environments. For example, when clusters A, B, and C are grouped into diatom blooms, dinoflagellate blooms, and Prochlorococcus-rich water, this analysis is very useful; however, the authors' results are not. Thus, this manuscript's contribution to the scientific community is very limited in its current form, and if the authors promote ECO 3M1M for BGC-Argo observations based on this manuscript, I cannot find any advantage to ocean science.

Hence, my recommendation for this manuscript has two options: 1) if the authors want to keep their conclusion (it looks like ECO 3M1M is useful for BGC-Argo observations), the authors should show the advantage of this fluorometer compared to the others, except for the identification of phytoplankton community composition. 2) If the authors will fairly describe the caveats of ECO 3M1M compared to the other MXF (FluoroProbe and MultiExciter), this manuscript could be worthy for publication. The manuscript should be fully revised for them.

We thank the reviewer for this insightful and critical comment. We have substantially revised the manuscript to address both points raised.

On the choice of the ECO 3X1M sensor:

We fully acknowledge that higher-resolution instruments such as the FluoroProbe (5 channels) and MultiExciter (9 channels) offer enhanced taxonomic discrimination, as demonstrated by recent studies in coastal and open-ocean environments (Garrido et al., 2019; Kodama and Taniuchi, 2022; Xu et al., 2022). This is now explicitly stated in the revised Introduction and conclusion.

L.84: "M*ulti-channel instruments such as the FluoroProbe (bbe Moldaenke, 5 excitation wavelengths) have also been widely used for in-situ taxonomic discrimination in freshwater and coastal environments (Beutler et al., 2002; Catherine et al., 2012). More recently, multi-wavelength excitation fluorometers' applications have extended to open ocean discrimination of phytoplankton assemblages, demonstrating their capacity to resolve major taxonomic groups including diatoms, dinoflagellates, as well as phycoerythrin-containing (Synechococcus, Trichodesmium, etc.) or lacking (Prochlorococcus) cyanobacteria. cyanobacteria, and prochlorophytes (Xu et al., 2022; Kodama and Taniuchi, 2022). Concurrently, laboratory-based studies have demonstrated that portable, low-cost multi-excitation devices combined with machine learning classifiers can achieve high-accuracy taxonomic discrimination across a wide range of phyla (Magalhães et al., 2025; Zhang et al., 2025), further highlighting the potential of this approach for scalable, cost-effective phytoplankton monitoring.*"

However, the central motivation of this study is not to maximise taxonomic resolution from all available fluorescence sensors, but to evaluate what phytoplankton community information can realistically be retrieved from the miniaturised sensors currently deployed or deployable on BGC-Argo profiling floats.

The FluoroProbe and MultiExciter, while scientifically powerful, currently do not meet the requirements for integration onto profiling floats in terms of size, weight, energy consumption, and data flow. The SeaBird ECO, by contrast, is already integrated on BGC-Argo floats in a three-channel configuration including two-fluorescence-channels configuration, and the full three-fluorescence-channel version represents a realistic near-term upgrade for global autonomous observations. We therefore respectfully argue that the comparison with FluoroProbe and MultiExciter, while scientifically interesting, is not the appropriate benchmark for three-channel-oriented study, and that the ECO 3X1M represents a deliberate and well-motivated instrumental choice. We attempted to make this clearer in the revised version of the manuscript.

In the introduction:

L95: "*Higher-resolution multispectral fluorometers, such as the FluoroProbe and MultiExciter (9 channels), offer enhanced taxonomic discrimination (e.g., Garrido et al., 2019, Kodama and Taniuchi, 2022; Xu et al., 2022). However, their significant weight, large physical footprint, and high-power consumption limit their use primarily to shipboard or short-term logging applications. In contrast, the SeaBird ECO sensor used in the present study is miniaturized and optimized for the stringent power and payload constraints of the global BGC-Argo fleet (Bittig et al., 2019; Organelli et al., 2017). Our study therefore evaluates whether a reduced 3-channel spectral subset can still provide sufficient biological resolution for global-scale autonomous observations."*

And in the method section:

L163: *"This three-channel design has been chosen for its immediate capacity to be integrated on a BGC-Argo float, or equivalent autonomy asset, due to its miniaturisation and low power consumption, the ECO series sensor already being part of the BGC-Argo payload."*

In response to the reviewer's broader concern, we have followed the second recommended path: we have carefully toned down the claims made throughout the manuscript, particularly in the abstract and conclusion, to more fairly reflect the capabilities and limitations of the three-channel approach relative to higher-resolution instruments.

The term "phytoplankton community composition" has been changed to "phytoplankton community structure indices".

On the clustering analysis and ecological interpretability of clusters:

We agree with the reviewer that the ecological interpretability of the clusters is central to the value of the classification approach. We have revised Section 3.2 to provide a clearer ecological description of each cluster and to explicitly link them to well-documented phytoplankton succession patterns at the BOUSSOLE site and more broadly in the NW Mediterranean (Marty et al., 2002; Mayot et al., 2017). Specifically: Cluster 1 corresponds to winter and deep autumn communities with a significant Chl b contribution from green flagellates and prochlorophytes; Cluster 2 corresponds to the spring bloom dominated by diatoms as indicated by high fucoxanthin; Cluster 3 is associated with summer deep chlorophyll maximum communities with mixed nano- and microphytoplankton; and Cluster 4 represents surface summer picophytoplankton communities dominated by zeaxanthin-rich picocyanobacteria. These clusters thus represent ecologically meaningful and recurrent community types directly relevant to seasonal biogeochemical dynamics in temperate open-ocean regions. We have also added explicit text noting that MXF alone captures pigment-based taxonomic differences rather than size per se, and that size-related discrimination emerges when bbp and cp are incorporated into the model, as these variables carry information on particle size distribution (Section 3.4).

Title: The clustering analysis could not show the phytoplankton community composition.

The title has been revised accordingly to better reflect the evaluative and exploratory nature of the study rather than implying a demonstrated capability for community composition retrieval.

The new title is: "*Evaluating a multispectral miniaturised fluorometer with three excitation channels for predicting phytoplankton community structure indices from BGC-Argo float observations.*"

L31: Are there any evidences that 532 nm is useful for chlorophyll b? Also, for phycoerythrin, the signal from this wavelength is obscure (Palenik, 2001

https://doi.org/10.1128/AEM.67.2.991-994.2001)

We thank the reviewer for this precise and insightful comment. The reviewer is correct that 532 nm does not directly target the absorption spectrum of chlorophyll b, whose excitation maximum lies closer to 470 nm (French et al., 1977; Kume et al., 2018). We acknowledge that the original text was misleading on this point. In the revised version of the abstract we only mention good performance of the 532 excitation channel for discriminating phycoerythrin rich taxa.

L38: "*The different excitation channels contributed unequally: 440 and 470 nm provided robust pigment sensitivity across communities, while 532 nm was particularly informative for detecting phycoerythrin-rich taxa.*"

Regarding the reviewer's point that the phycoerythrin signal at 532 nm is obscure, we note that Palenik (2001) specifically discusses the variability in phycourobilin to phycoerythrobilin (PUB:PEB) ratio across *Synechococcus* strains but could not find any mention of the point mentioned by the referee. We have added a caveat in the revised text acknowledging that the F532 signal may not be a reliable indicator of phycoerythrin content across all Synechococcus strains, particularly those with low or absent phycoerythrobilin content. We have revised the relevant sentence in the manuscript to accurately reflect these nuances and to avoid overstating the mechanistic basis of the F532 contribution.

L362: "*It should be noted, however, that the phycoerythrobilin-to-phycourobilin ratio in open-ocean Synechococcus strains can vary in response to ambient light quality through chromatic acclimation (Palenik, 2001; Humily et al. 2013Grébert et al., 2018), a capacity which may modulate the F532 response of individual strains and introduce variability in the signal beyond what is captured by our laboratory measurements under fixed white light conditions*."

L70: The authors must refer to the more recent papers.

We thank the reviewer for this suggestion. We have expanded the MXF introduction paragraph to include more recent references demonstrating the growing application of multi-wavelength excitation fluorometry for phytoplankton community discrimination. We now cite Beutler et al. (2002) and Catherine et al. (2012) in the context of FluoroProbe applications in freshwater and coastal environments; Xu et al. (2022, Frontiers in Marine Science), who applied a multi-excitation fluorometer to resolve phytoplankton groups at high vertical resolution in the East China Sea and Tsushima Strait; Kodama and Taniuchi (2022, PLOS ONE), who estimated

phytoplankton assemblages across coastal and offshore Japanese waters using a nine-channel multi-wavelength excitation fluorometer; Zhang et al. (2025, Algal Research), who combined multi-excitation fluorescence with an XGBoost machine learning model to quantify eight phytoplankton groups with high predictive accuracy, including tracking diel vertical migration in dinoflagellates; and Magalhães et al. (2025, Sensors and Actuators), who developed a portable, low-cost multi-excitation device achieving 97% classification accuracy at the phylum level across 16 microalgae species using machine learning. The last two studies are of particular relevance as they share the conceptual approach of the present work, combining compact multi-excitation fluorescence measurements with machine learning for phytoplankton community classification, and collectively demonstrate that this field has advanced considerably in recent years, further motivating the present study.

Table1: What is the order of main pigments?

We have adjusted the table so that pigment order reflects their relative contribution importance in each taxon.

L184: What is "pure seawater"?

"Pure seawater" was a typo and has been corrected to "Pure water", which is a commonly used term in bio-optics studies, describing water without any particulate component, reflecting theoretical optical properties of water for a given temperature and salinity.

L191: Please check the citation style.

The citation style has been adjusted to journal standards.

L201: The description of HPLC was too short. Please provide more details. Also, I'm not sure that the authors used the same HPLC machine as that of 15 years ago.

To address the Reviewer's comment, we provide additional details on the HPLC analyses, including the type of HPLC chain used for the analysis

L. 232 of revised manuscript: "*Briefly, seawater from discrete field samples (~2L) or cultures (0.1 to 0.5L, depending on culture biomass concentration) was filtered onto glass fibre filters (GF/F Whatman 25 mm), stored in liquid nitrogen during cruises and then transferred at −80°C in the laboratory until further analysis at the SAPIGH HPLC analytical facility of the Institut de la Mer de Villefranche (IMEV). Phytoplankton pigments were extracted by sonication in 3 mL 100% methanol (100%) at -20°C for 2 hours, clarified by vacuum filtration (GF/F Whatman 25 mm), and finally separated and quantifiedanalyzed within 24 hours by HPLC using an Agilent Technologies 1200 Series system. More details about the analytical protocol can be found in Ras et al. (2008). The*

total chlorophyll a concentration, [Chla], is defined as the sum of chlorophyll a, divinyl-chlorophyll a , and chlorophyllid a concentrations. ".

L215: The authors did not assess the reliability of the results in the present study. Also, the actual information on the size classes could not be provided from the pigments. This sentence is plastic.

We thank the reviewer for this comment. We agree that the original sentence was overly general and did not adequately justify the reliability of the approach as applied in the present study. We have revised the sentence in two ways. First, we have clarified that the diagnostic pigment method is used here as a reference framework for comparison with MXF-derived estimates, rather than as an independent ground truth. Second, we have added a sentence noting that the BOUSSOLE site has been the subject of extensive prior characterization of phytoplankton seasonal dynamics (Marty et al., 2002; Organelli et al., 2013; Latasa et al., 2022), providing a well-documented ecological context against which the outputs of this method can be interpreted. We also acknowledge, following Chase et al. (2020), that the inference of size classes from diagnostic pigments relies on assumed taxon-size relationships that may not always hold, and have made this caveat explicit in the revised text.

L247: "*Although it has limits because some phytoplankton taxa may occasionally span over several size classes and some DP may be found in several taxa (e.g. Vidussi et al., 2001; Chase et al., 2020), this approach provides a widely used information about the phytoplankton community structure at large spatial and temporal scales (e.g., Vidussi et al., 2001; Bricaud et al., 2004; Uitz et al., 2006; Brewin et al., 2014). Moreover, the BOUSSOLE site and the NW Mediterranean Sea have been the subject of extensive prior characterization of phytoplankton seasonal dynamics (e.g., Marty et al., 2002; Organelli et al., 2013; Latasa et al., 2022), providing a well-documented ecological context against which the outputs of this method can be interpreted.*"

L280: I could not understand "the model has a learning rate of 0.05,l 400 estimators and a maximum depth of 8." This description is too brief.

While describing and explaining all hyperparameters of a boosted trees model is beyond the scope of the study, we agree with the lack of explanation behind those technical choices. The sentence has been revised to explain what guided the choice of the hyperparameter tuning.

L320: "*The hyperparameters of the model, i.e. the parameters influencing the learning process, were defined using a cross-validation grid search. In brief, the model has a learning rate of 0.05 to ensure stable convergence and l 400 estimators to capture non-linear relationships without overfitting, constrained by a maximum depth of 8 to maintain model interpretability and generalizability. "*

L321: Did Six et al. (2007) and Grébert et al. (2018) describe that 532 nm is most excited at 532 nm? I could not find such descriptions in them.

This citation referred to the description of the respective excitation maxima of phycourobilin (PUB) and phycoerythrobilin (PEB). The "most excited at 532 nm" meant that 532 nm being closer from the PEB excitation peak than PUB excitation peak, PEB is likely more excited than PUB by this excitation wavelength of the 3x1m, even though both chromophores are excited. The sentence has been revised and the citation has been placed at the appropriate location to avoid any misunderstanding.

L359"*The higher F*532 / F*470 values observed for the Synechococcus taxon may be explained by their higher fluorescence at 532 nm, induced by the presence of phycoerythrin. Indeed, this phycobiliprotein is systematically found in open ocean Synechococcus and binds two chromophores, phycourobilin (λmax ~495 nm) and phycoerythrobilin (λmax ~545 nm), the latter thus being the most excited at 532 nm (Six et al., 2007; Grébert et al. 2018).*"

L326: If so, phytoplankton that has chlorophyll b could not divide in this method.

We thank the reviewer for this comment. We believe there may be a misunderstanding. Among all strains measured in this study, Prochlorococcus is the only taxon containing DV-Chlb. Therefore, the distinctive F440/F470 signature of Prochlorococcus is not a source of confusion with other taxa, but rather what makes it uniquely identifiable in our dataset. We have nonetheless strengthened the mechanistic justification by explicitly citing French et al. (1977) and Kume et al. (2018), which establish that Chl a and Chl b have excitation maxima around 440 and 470 nm respectively, and softened the wording to "may be explained" to avoid overstating the mechanistic interpretation.

L366: "*Consequently, the phycourobilin-rich strain RCC2379 expectedly exhibited a lower average F*532 / F*470 ratio than the two other Synechococcus strains both being chromatic acclimaters which, in white light, exhibit a low phycourobilin to-phycoerythrobilin ratio (Palenik, 2001; Six et al. 2004; Humily et al. 2013). The fairly high F*532 / F*470 ratio observed in Prochlorococcus strains are harder to explain, given their very low phycoerythrin content (Steglich et al. 2003, 2005). In contrast, the higher F*440 / F*470 ratio of the HL-adapted PCC 9511 compared to the LL-adapted RCC156 may be explained by the much higher DV-Chla (λmax ~450 nm) to DV-Chlb (λmax ~475 nm) ratio of the former strain (Moore et al. 1995).*"

L328: The authors only mentioned chlorophyll, but they should consider the carotenoids.

The reviewer is correct that carotenoids were insufficiently considered in the original text. We have revised the sentence at L328 to specifically identify the carotenoids most relevant to our three excitation wavelengths: fucoxanthin in diatoms and

pelagophytes, and peridinin in dinoflagellates, both absorbing in the 440–532 nm range and capable of transferring excitation energy to chlorophyll a via fluorescence resonance energy transfer (Bidigare et al., 1989; Das et al., 2002; Bricaud et al., 2004; Menehegin et al., 2018). This energy transfer mechanism means that the F440, F470, and F532 signals in these taxa reflect not only chlorophyll absorption but also the indirect contribution of carotenoid light harvesting, which partly explains the taxon-specific fluorescence ratios observed in Figure 1. We acknowledge that the efficiency of this energy transfer varies across taxa and physiological states, which explains why fluorescence differences among diatoms, dinoflagellates, and pelagophytes remain more subtle and harder to interpret quantitatively than those driven by phycobiliproteins or the Chla/Chlb ratio.

L372 : "*The differences in fluorescence responses among diatoms, dinoflagellates, and pelagophyceae are also likely related to their distinct content in accessory chlorophylls and carotenoids. In particular fucoxanthin in diatoms and pelagophytes, and peridinin in dinoflagellates, absorb light in the 440-532 nm range and transfer energy to Chla via fluorescence resonance energy transfer, potentially contributing to the fluorescence signal measured at these excitation wavelengths (e.g., Bidigare et al., 1989; Ras et al., 2002; Bricaud et al., 2004; Menehegin et al., 2018). However, the efficiency of this energy transfer varies across taxa and physiological states, making the contributions of these carotenoids more challenging to interpret quantitatively than those of chlorophylls.*"

Figure 1: This shows that MXF did not show the size. Also, I cannot find the results of Chaetoceros. Why?

We thank the reviewer for these two comments. Regarding *Chaetoceros*: its absence from Figure 1 was due to an unintended filtering error in the data processing code, which has now been corrected. *Chaetoceros* has been added back to Figure 1 in the revised manuscript. Regarding size information: the reviewer is correct that MXF alone captures differences in pigment composition between taxonomic groups rather than cell size directly. However, we show later in the manuscript that when additional bio-optical indices, specifically the particulate backscattering coefficient (bbp) and beam attenuation coefficient (cp), are incorporated into the model for in-situ community discrimination, size-related information becomes accessible, as these variables carry information on particle size distribution. This point is discussed in Section 3.4 of the manuscript.

Figure 2: The cluster 1 and 4 look very similar. Johnsen et al. (1997, https://doi.org/10.4319/lo.1997.42.5_part_2.1166) showed that peridinin exhibited high fluorescence with excitation wavelengths between 480 nm and 510 nm. However, ECO 3M1M does not cover this range. So, I considered that ECO 3M1M is unable to detect the presence of peridinin (dinoflagellates).

The clusters 1 and 4 differentiate from each other by a significantly larger contribution of Zeaxanthin for cluster 4 and a larger contribution of Chlb for cluster 1. Cluster 4 represents summer surface *Synechococcus* communities, while cluster 1 represents mixed winter communities, with nanophytoplankton and *Prochlorococcus.* Peridinin always contributed to a minor part of each cluster, hence it is not of significant concern for this specific dataset but could be a caveat of that clustering construction in different environments.

We've clarified the distinction between clusters 1 and 4 in section 3.2 "*Although Clusters 1 and 4 both represent picophytoplankton-dominated communities, they are chemically and ecologically distinct. Cluster 1 is characterized by a significant contribution of total Chlorophyll-b, typically associated with green microalgae and prochlorophytes in deeper or mixed waters (Bustillos-Guzmán et al., 1995; Moore et al., 1995). In contrast, Cluster 4 is dominated by zeaxanthin-rich picocyanobacteria, here Synechococcus, in surface waters (Barlow et al., 1997). This distinction is critical for the MXF sensor; the high phycoerythrin content in Cluster 4 is expected to produce a significantly stronger F532 response compared to the TChlb-dominated signature of Cluster 1 (Grébert et al., 2018; Six et al., 2007; Veldhuis et al., 2004)*. »

L395: Figure 2 did not show that their MXF distinguishes diatoms and dinoflagellates. This is not based on objective data.

We thank the reviewer for pointing out this potential misunderstanding that could lead to an overstatement. We have rephrased the sentence to tone it down and explicitly mentioned the results shown in the first figure.

Figure 5: Please revise the quality for the manuscript level.

The quality of the figures of the manuscript has been revised to the journal's standard.

L487: I could not understand why the authors describe the model tuning after presenting the results from field observations.

We respectfully clarify that the section on model tuning was intentionally placed after the initial results obtained with four phytoplankton clusters because it builds upon the previously presented classification results. Specifically, this section explores how reducing the number of clusters (i.e., decreasing the classification complexity) influences the predictive performance of the model under different sensor configurations.

To clarify this logic for the reader, we have revised the beginning of Section 3.4 to explicitly state that this analysis aims to evaluate the sensitivity of the classification model to the level of ecological complexity represented by the number of clusters.

L536: "*In the previous section, the classification model was evaluated for the prediction of four phytoplankton clusters representing the main communities observed over the annual cycle. Here, we further explore the sensitivity of the model to the predictive complexity by progressively reducing the number of clusters from four to three and two. This allows us to assess how model performance varies under simpler ecological scenari.*"

L551: Did Morel and Saito report that 532 nm excitation is expected to significantly improve classification performance?

We thank the reviewer for pointing this out. The studies of Morel (1997) and Saito et al. (2005) did not explicitly state that the addition of a 532 nm excitation channel would improve phytoplankton classification performance. Their work highlighted the potential of green excitation to better target accessory pigments such as phycoerythrin and other pigment groups that are less efficiently excited at shorter wavelengths. Our statement, therefore overstated their conclusion. We have revised the sentence to clarify that the expected improvement in classification performance is an inference based on the pigment excitation properties discussed in their work rather than a direct claim from these authors.

L594: "*From a broader perspective, this sensitivity also explains why the usefulness of F532 will vary geographically: in regions where Synechococcus or green flagellates are recurrent and occasionally abundant (e.g., coastal upwelling systems), the inclusion of a 532nm excitation channel may improve the discrimination of these specific groups by enhancing the excitation of accessory pigments that are less efficiently excited at shorter wavelengths, as discussed in previous studies (Morel, 1997; Saito et al., 2005). In contrast, in persistently oligotrophic waters such as the subtropical gyres, the added value of F532 is likely reduced.* "

Reviewer 2:

I strongly support the development of new optical approaches for resolving phytoplankton community structure, particularly the combined use of hyperspectral radiometry and multispectral excitation fluorescence (MXF). The present manuscript represents an important step toward evaluating MXF-based methods for this purpose.

However, I was expecting a more detailed and structured discussion regarding the feasibility and necessity of deploying instruments such as the ECO 3X1M on BGC-Argo platforms, or more generally, a clearer framework outlining what level of phytoplankton discrimination can realistically be achieved with different sensor configurations.

At present, the manuscript demonstrates that MXF adds information under certain configurations, but it does not sufficiently articulate ***(i) under what ecological or***

observational conditions MXF becomes essential rather than complementary, and (ii) what minimum or optimal sensor characteristics are required to achieve specific biological resolution targets. *Without such a framework, it remains difficult to assess the broader implications for future BGC-Argo instrumentation strategies.*

It is encouraged that the authors expand the discussion beyond the specific instrument tested and provide a more general conceptual and operational framework linking sensor capability to achievable phytoplankton classification performance. This would substantially strengthen the manuscript's impact on the community.

We thank the reviewer for this valuable and constructive comment. Providing a general framework linking sensor capability to achievable phytoplankton classification performance was indeed the original intention behind the design of the last part of the study, which systematically evaluated classification performance across six sensor configurations and three levels of community complexity (Table 2 and Fig. 6). However, we acknowledge that this framework was not made sufficiently explicit in the original manuscript, and we thank the reviewer for helping us identify and articulate this key contribution more clearly.

In brief, our results support the following framework. When phytoplankton communities differ primarily in size structure, bio-optical proxies (bbp and cp) provide sufficient discrimination and MXF adds limited value, the standard BGC-Argo configuration already achieves recall scores above 70% in this regime. MXF becomes essential when communities share similar size structures but differ in pigment composition, such as when distinguishing nano- and microphytoplankton assemblages with contrasting carotenoid signatures, or separating distinct picophytoplankton communities. In terms of minimum sensor requirements, two fluorescence wavelengths (440 and 470 nm) combined with bbp and cp represent an optimal and technically feasible configuration, achieving above 75% recall for two and three-cluster scenarios, a configuration already partially deployed on the BGC-Argo fleet. A third channel at 532 nm provides incremental benefit specifically in regions where phycoerythrin-rich or chlorophyll b-containing taxa are ecologically important. We believe this framework, now made explicit in the revised manuscript, directly addresses the reviewer's request and substantially strengthens the broader impact of the study.

We have added a new paragraph at the end of Section 3.4 that addresses both points explicitly.

L601: "*More broadly, our results allow us to draw a general framework linking sensor configuration to achievable phytoplankton classification performance in the context of BGC-Argo observations. The level of community discrimination that can realistically be achieved depends on two interacting factors: the ecological contrast between the communities to be distinguished (e.g., differences in pigmentation, size, or photoacclimation status), and the spectral and optical information available from the*

sensor suite. When communities differ primarily in size structure, such as pico- versus microphytoplankton dominance, bio-optical proxies alone (i.e., bbp and cp) provide sufficient discriminatory power, while the MXF contributes only limited additional value. In this regime, the standard BGC-Argo configuration (configuration F) already achieves recall scores above 70%. MXF becomes essential when communities share similar size structures but differ in pigment composition, for instance, when distinguishing between nano- and microphytoplankton assemblages characterized by contrasting carotenoid signatures, or between distinct picophytoplankton communities dominated by Prochlorococcus vs. Synechococcus. In these cases, adding even a single fluorescence channel (440 or 470 nm) to the standard configuration provides a statistically significant improvement in classification performance. The 532 nm channel provides additional value specifically in environments where phycoerythrin-rich taxa such as Synechococcus or Chlb-containing organisms are recurrent and abundant, but its contribution diminishes when such groups are absent or merged into broader assemblages. In terms of minimum sensor requirements, our results suggest that combining two fluorescence channels (440 and 470 nm) with bbp and cp represents an optimal and achievable configuration for BGC-Argo platforms. This configuration achieves over 75% recall in scenatios distinguishing (1) two phytoplankton clusters (i.e. picophytoplankton vs mixed nano- plus microphytoplankton assemblages) or (2) three clusters (i.e. a mixed nano- plus microphytoplqnkton, a Synechococcus like and a Prochlorococcus like assemblages). This configuration corresponds to dual-channel fluorometers already implemented on part of the BGC-Argo fleet, suggesting that meaningful phytoplankton community information could be extracted from existing deployments without requiring new instrumentation. Adding a third fluorescence channel at 532 nm provides incremental benefit specifically for pigment-based discrimination at finer taxonomic resolution, and would be the recommended configuration for regions or seasons where Synechococcus or green flagellates are ecologically important."

L60: There are more works using bbp for inferring phytoplankton community structure, for example: https://doi.org/10.1029/2021JC018195. Please update the brief review.

We thank the reviewer for pointing out this additional relevant study. We have updated the paragraph reviewing approaches that infer phytoplankton community structure from BGC-Argo bio-optical observations to include the work of Robert J. W. Brewin et al. (2022), which proposes an analytical framework to partition vertical chlorophyll profiles measured by BGC-Argo floats into contributions from distinct phytoplankton communities using chlorophyll and particulate backscattering observations. We also mention the subsequent extension of this framework to three communities in oxygen-minimum-zone conditions (Cox et al., 2023). This addition further illustrates the range of approaches that use optical proxies such as particulate backscattering to infer phytoplankton community structure.

L72: "*More recently, Brewin et al. (2022) proposed an analytical framework to partition vertical chlorophyll profiles measured by BGC-Argo floats into contributions from two phytoplankton communities associated with the mixed layer and the deep chlorophyll maximum, combining chlorophyll and particulate backscattering observations. This model was later extended to three communities in an oxygen minimum zone context (Cox et al., 2023).* "

L83: According to https://vocab.nerc.ac.uk/collection/R27/current/, the ECO_FLBBFL and ECO_FLBBFL_AP2 models use excitation wavelengths at 470 nm and 435 nm. The manuscript, however, refers to 440 nm. Please clarify this discrepancy and explain whether the 5 nm difference has any practical or spectral implications for the analyses presented.

The reviewer is right that this excitation channel can be described as having a maximum light emission at 435 nm. The nominal excitation wavelength of the blue channel for these fluorometers is sometimes reported as 435 nm in instrument metadata, such as the NERC vocabulary entry for the ECO_FLBBFL sensors. In the manuscript, we referred to 440 nm, which corresponds to the value reported in the instrument characterisation sheet used in this study and is commonly used in the literature to describe the same excitation band. We have clarified this point in the manuscript to indicate that the excitation wavelength is around 440 nm (sometimes reported as 435 nm) and that both values refer to the same blue excitation band.

L105: "*Of note, the nominal wavelength of the blue excitation channel is sometimes referred to as 435 nm in instrument metadata (see https://vocab.nerc.ac.uk/collection/R27/current/) but both values correspond to the same blue excitation band used in these fluorometers.* "

L86: Vidussi et al. 2001 is about the Eastern Mediterranean

We thank the reviewer for this correction. Vidussi et al. (2001) is indeed focused on the Eastern Mediterranean and is therefore not the most appropriate reference in this context. We have replaced it with Mayot et al. (2017), which explicitly describes phytoplankton ecological succession in the Northwestern Mediterranean Sea.

L105: It would be better to know whether the phytoplankton communities considered in this section are representative of the NW Mediterranean. What criteria were used to justify the selection?

We thank the reviewer for this comment. We agree that the selection criteria were not sufficiently explicit. The strains were chosen to represent the major taxonomic groups known to dominate the seasonal phytoplankton succession at the BOUSSOLE site, as documented in previous studies of the NW Mediterranean (Marty et al., 2002; Mayot et al., 2017). Specifically, diatoms dominate the spring bloom, dinoflagellates and pelagophytes contribute to winter and mixed communities, and

picocyanobacteria (Synechococcus and Prochlorococcus) are prevalent during summer stratification. We have revised the text at line 105 to make these selection criteria explicit.

L133: "*These strains were selected as being representative of the taxonomic diversity of the main eukaryotic and prokaryotic phytoplankton organisms encountered in open-ocean waters, and particularly at the BOUSSOLE site in the NW Mediterranean Sea, based on previous pigment-based studies in the region (Marty et al., 2002 ; Mayot et al., 2017). The selected strains include three diatom species, one pelagophyte, one dinoflagellate and five photosynthetic prokaryotes (three Synechococcus and two Prochlorococcus strains; Table 1).* "

We also note that the representativeness of the selected strains is further validated a posteriori by the correspondence analysis in Figure 3, which shows that the cultured strains span the same pigment composition space as the field samples collected over the annual cycle.

Table 1: Caption "HPLC" better follow with the full name.

We thank the reviewer for this suggestion, which we have added to the text accordingly.

L131: How was the ECO 3X1M calibrated before the measurements? In addition, laboratory temperature, etc., can influence optical sensors. Were these factors monitored and accounted for during the experiments?

We agree that these specific details on the sensor calibration were not sufficiently described in the manuscript. The ECO 3X1M was operated using factory calibration coefficients. Regarding temperature effects, we note that only the optical window of the sensor was immersed in the culture medium, and never for more than one minute continuously. Furthermore, the stability of the fluorescence signal over each one-minute acquisition period (recorded at 1 Hz) confirmed that no thermal drift occurred during the measurements. We have added a sentence to section 2.1.2 to clarify these points.

L177: "*The ECO 3X1M was used with factory calibration coefficients. Temperature effects on the sensor were considered negligible as only the optical window was immersed in the culture medium, and never for more than one minute continuously. The stability of the signal over each one-minute acquisition period confirmed the absence of any thermal drift.* "