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Interactive comment on "Evolution of dissolved and particulate chromophoric materials during the VAHINE mesocosm experiment in the New Caledonian coral lagoon (South West Pacific)" *by* M. Tedetti et al.

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Anonymous Referee #2

Overview

In this manuscript, Tedetti et al. investigated the temporal changes in the optical properties of CDOM and particulate matter during a mesocosm study located outside a lagoon in New Caledonia. P-fertilization of the mesocosm was carried out during the study in order to stimulate diazotrophs and N2 fixation, and the evolution of the optical

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properties was studied in this context.

The study led to the following conclusions: 1) A strong connection was observed between the abundance of synechococcus and the absorption of particulate matter and CDOM during the mesocosm, suggesting synechococcus was a strong contributor to the particulate absorption and was strongly involved in the production of CDOM. 2) The data also support the idea that N2 fixation by diazotrophs enhanced the synechococcus bloom and indirectly contributed to the production of the chromophoric material in the mesocosm, suggesting the existence of an indirect link between N2 fixation and the production of chromophoric material. 3) There was a surprising decoupling between FDOM and CDOM during the mesocosm study, which the authors attributed to the two components being regulated by different processes. Overall, the study provided convincing evidence of a strong link between the dynamics of synechococcus bloom and that of the chromophoric material, and provides good reasoning supporting the idea (although it does not provide hard evidence of it) that there is a link between N2 fixation and the production of chromophoric via the stimulation of the synechoccocus bloom.

Overall the manuscript is well written and referenced. The figures and tables are generally of high quality and clear. The methods are adequate and clearly explained, and the conclusions drawn are generally well supported by the data presented. The results and conclusions advance our understanding of the processes regulating CDOM and chromophoric particulate matter in the ocean. The topic and scientific contribution are appropriate for “Biogeosciences” and for this special issue. I recommend the manuscript for publication after the following comments are addressed (minor revisions).

Major comments

1. The Sg+p data: I do not see the value in presenting the ag+p spectra or the corresponding spectral slope coefficients (Sg+p). The results are shown but the implications are never adequately discussed in the manuscript. The point of presenting these data

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remains unclear, and the data are more distracting than anything. Furthermore, I don't find it suitable to calculate a S-value from ag+p spectra that are not really exponential. I would recommend removing the ag+p and Sg+p data, unless they are used in a meaningful way in the discussion and they enhance the conclusions of the manuscript.

Answer: We agree with the Reviewer #2. In the revised ms, we thus removed all the data concerning ag+p(λ) and Sg+p (in the text, Fig. 5, Fig. 6, Table 1 and Table 2).

2. Decoupling between CDOM and FDOM: The authors attribute the lack of correspondence observed between CDOM and FDOM to the fact the dynamics of these two components are probably driven by different processes. While this conclusion is not erroneous, another possible explanation is that the components that are fluorescing are not major components of the CDOM (meaning they absorb but not strongly enough to affect the CDOM variability in a significant way). I think this could be included in the discussion of this result.

Answer: We agree with the Reviewer #2.

- We added the sentence "Also, these fluorophores could be not major components of the CDOM. Consequently, they would absorb but not strongly enough to significantly affect the CDOM variability." (discussion section, page 29, lines 711-713 in the revised ms).

3. Link between N2 fixation and chromophoric material: The following paper might provide some useful insights about the role of N in the formation of CDOM/FDOM: Biers et al. (2007) The role of nitrogen in chromophoric and fluorescent dissolved organic matter formation. Marine Chemistry. doi:10.1016/j.marchem.2006.06.003

Answer: We agree with the Reviewer #2. Biers et al. (2007) highlighted the role of dissolved organic nitrogen (DON), specifically amino sugars and aromatic amino acids, in the microbial production of CDOM and FDOM. This result is very interesting because in our paper, we suggest that the labile DOM released by Synechococcus spp.

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cyanobacteria is utilized and converted into CDOM by heterotrophic bacteria. Well, Bronk et al. (1999) reported the production of DON by Synechococcus spp. Consequently, the works by Biers et al. (2007) and Bronk et al. (1999) support our assumption of the CDOM production by heterotrophic bacteria consecutive to their utilization of DOM (that would be in part in the form of DON) issued from Synechococcus spp. cyanobacteria.

- We added the sentence "Interestingly, Biers et al. (2007) highlighted the role of DON, specifically amino sugars and aromatic amino acids, in the microbial production of CDOM and FDOM while Bronk et al. (1999) reported the production of DON by Synechococcus spp. Consequently, the works by Biers et al. (2007) and Bronk et al. (1999) support the assumption of the CDOM production by heterotrophic bacteria consecutive to their utilization of labile DOM (that would be in part in the form of DON) released by Synechococcus spp. cyanobacteria." (discussion section, page 26, lines 636-641 in the revised ms).

- We added Biers et al. (2007) in the reference list.

4. Figure 5: I think Figure 5 could be improved. The presentation of all the spectra in the left panels makes it difficult to discern any spectra. For each variable, I would recommend the authors show only 3-4 spectra from distinct times during the mesocosm study (e.g., Initial ; P1 ; P2). In order to show the full range, the average of all spectra could be shown with the range shown as a gray area (instead of showing the standard deviations). Again, I don't think adding the ag+p spectra adds to the paper, and I would suggest removing these data unless the authors can use the data in a meaningful way.

Answer: We modified Figure 5 accordingly. As mentioned in Answer to comment n°1, we removed the ag+p spectra. For CDOM and particulate data, we removed the graphs with all spectra and we modified the graphs showing the full range according to the Reviewer's comment: We used black lines to represent the average of all spectra, and grey areas to represent the measured minimal and maximal values. Please note that

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along with these full range graphs, we do not think necessary to add graphs showing 3-4 spectra from distinct times during the mesocosm study, neither small graphs representing spectra over the range 370-430 nm.

5. New figure: I leave this to the discretion of the authors, but I think adding a figure showing the plots of the relationship between ag vs synechococcus and ap vs synechococcus would help emphasize to the readers (who often don't read the entire manuscript and just look at figures) that there is strong connection between ag/ap and synechoccoccus. Showing this in a figure would help getting the point across (this is probably one of the most important finding in the paper).

Answer: We agree with the Reviewer #2.

- We added in this new figure in the revised ms, as Figure 9: Linear relationships between absorption coefficient of CDOM at 370 nm [ag(370) in m-1] or absorption coefficient of particulate matter at 442 nm [ap(442) in m-1] and Synechoccocus spp. abundance ($\times$ 103 cell mL-1) for samples collected in the mesocosm M1 from day 5 to day 20, i.e. from the day after the dissolved inorganic phosphorus fertilization to almost the end of the experiment (P1 + P2) (n = 36).

- We refer to this Fig. 9 in the results part, section 3.7 and in the discussion part, section 4.2.

6. Abstract: I think it would be worthwhile to expand and clarify the last sentence of the abstract. This is an important point of the paper, but the last sentence will be a little unclear to someone who hasn't (and might never have time) to read the entire paper. I suggest replacing last sentence with something like that: "Finally, the results of this work support the idea there is indirect coupling between the dynamics of N2 fixation and that of chromophoric material via the stimulation of synechococcus bloom."

Answer: As suggested by the Reviewer #2:

- We replaced the sentence "Finally, this works indicates a coupling between the dy-

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namics of the N2 fixation and that of chromophoric material in the South West Pacific through Synechococcus bloom." by the sentence "Finally, the results of this work support the idea there is indirect coupling between the dynamics of N2 fixation and that of chromophoric material via the stimulation of Synechococcus bloom." (abstract, pages 2-3, lines 49-51 in the revised ms).

Minor comments

Abstract, Line 45: I would suggest using a more specific term than "activities"

Answer: We replaced "activities" by "biomass" (abstract, page 2, line 45 in the revised ms).

Abstract, Line 48: Replace "proving that these were" by "suggesting they were". Also, see Major comment 2) shown above.

Answer: Done (abstract, page 2, lines 48 in the revised ms).

Line 171: Please explain what EVA is here.

Answer: EVA is ethylene vinyl acetate. Thus, we replaced "one vinyl acetate (EVA, 19 %)" by "one ethylene vinyl acetate (EVA, 19 %)" (page 7, lines 169-170 in the revised ms).

Line 203: I suggest adding "(see section 2.2)" after "onboard"

Answer: Done (page 9, line 202 in the revised ms).

Line 278: Replace "With regard to our" by "Considering the"

Answer: Done (page 12, line 277 in the revised ms).

Line 306: Please add citation for fluorometry method

Answer: We added the reference "(Lantoine and Neveux, 1997)" (page 13, line 305 in the revised ms) and in the reference list: Lantoine, F., and Neveux, J.: Spatial and seasonal variations in abundance and spectral characteristics of phycoerythrins in the

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tropical northeastern Atlantic Ocean, Deep-Sea Res. I, 44, 223–246, 1997.

Line 314-316: Can you provide a citation and expand briefly about the clustering approach used.

Answer: We added the part “according to their optical properties (light scattered and ïňĆuorescence emission by the cells) (Marie et al., 1999)” (page 13, lines 317-318 in the revised ms).

Line 324: Weird sentence structure. Please change to “BP was calculated. . ..leucine, and is shown here in ng C L-1 h-1.”

Answer: Accordingly, we replaced the sentence “BP, calculated from leucine incorporation rates using the conversion factor of 1.5 kg C mol-1 leucine, is given in ng C L-1 h-1.” by the sentence “BP was calculated from leucine incorporation rates using the conversion factor of 1.5 kg C mol-1 leucine, and is shown here in ng C L-1 h-1.” (page 14, lines 326-328 in the revised ms).

Line 336-339: Please provide name of instruments used for DIN and TN analysis.

Answer: We replaced the part “DIN concentration was determined on a segmented flow auto-analyser according to Aminot and Kérouel (2007). TN concentration was determined according to the wet oxidation procedure described in Pujo-Pay and Raimbault (1994). Samples for PON concentrations were collected by filtering 1 L of water on GF/F filters and analyzed according to the wet oxidation protocol (Pujo-Pay and Raimbault, 1994) with a precision of 0.06 μM.” by the part “TN concentration was determined according to the wet oxidation procedure described in Pujo-Pay and Raimbault (1994). Samples for PON concentrations were collected by filtering 1 L of water on GF/F filters and analyzed according to the wet oxidation protocol (Pujo-Pay and Raimbault, 1994) with a precision of 0.06 μM. DIN concentration was determined according to Aminot and Kérouel (2007). Measurements were conducted using a segmented flow auto-analyser (AutoAnalyzer AA3 HR, SEAL Analytical).” (page 14, lines 339-345 in

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the revised ms).

Line 380-382: I know no replicates were measured, but is there any information about the typically uncertainty in this measurement that could be added here.

Answer: The measurement precision of PE from replicates $\sim$ 16 %. We rather added this information in the material and methods (page 13, lines 305-306 in the revised ms).

Line 382: I suggest using “outside the mesocosm” here and throughout the manuscript instead of “OUT”

Answer: The term “OUT” is used in the other papers from this special issue in order to define the surrounding waters/waters outside the mesocosm, and we think it is more appropriate to keep it for maintaining certain homogeneity among papers.

Line 397: Change to “No significant difference”

Answer: Done (page 17, line 401 in the revised ms).

Line 402: Please provide value correspond to “much higher” (10 times)

Answer: We replaced the part “Hence, the abundance of UCYN-C was much higher in M1 during P2” by the part “Hence, the abundance of UCYN-C was much higher in M1 during P2 than in M1 during P1 (14 times higher) and than in OUT during P1 and P2 (22-53 times higher)” (page 17, lines 406-408 in the revised ms).

Line 425-427: Again, consider removing the ag+p data as they are simply a combination of ap and ag features and they are not really insightful

Answer: All data concerning ag+p were removed from the revised ms.

Line 436-439: This kind of presentation makes it hard to match values to compare them. Please consider writing this sentence so each variable is directly shown with to its corresponding range.

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Answer: We replaced the sentence “In M1, ag(370), ag(442), ap(442), ap(676), ag+p(370) and ag+p(442) decreased from day 4 to day 9, when they were as low as $\sim$ 0.041, 0.011, 0.009, 0.003, 0.047 and 0.020 m-1, respectively, and then increased from day 9 to the end of the experiment to reach $\sim$ 0.067, 0.020, 0.025, 0.012, 0.075 and 0.046 m-1 at day 23, respectively [Fig. 6a,b,e,f; data not shown for ag+p(λ)].” by the sentence “In M1, absorption coefficients decreased from day 4 to day 9 and then increased from day 9 to the end of the experiment (day 23), leading to variations in the ranges 0.041-0.067 m-1 for ag(370), 0.011-0.020 m-1 for ag(442), 0.009-0.025 m-1 for ap(442) and 0.003-0.012 m-1 for ap(676) (Fig. 6a,b,d,e).” (page 18, lines 438-441 in the revised ms).

Line 483-484: The high correlation between either ag or ap and ag+p should be somewhat expected considering ag+p is a combination of ag and ap.

Answer: We agree. As mentioned above, the part dealing with ag+p has been removed in the revised ms.

Line 546-548: The data in Figure 8 support this statement, but I did not see a statement in the results that explained that the combined fluorescence values of the Tryptophan like and Tyrosine-like component were substantially higher than that of the humic-like component. I think this needs to be mentioned in the results to substantiate the claim made here.

Answer: In the result section, we added the sentence “Overall, the FDOM pool was dominated by protein-like material: the combined fluorescence of tryptophan and tyrosine fluorophores ranged from 9.1 to 22.3 QSU, while the fluorescence of humic fluorophore ranged from 1.9 to 6.2 QSU.” (pages 19-20, lines 472-474 in the revised ms).

Line 553: It would be good to cite one of Benner’s paper considering its contribution to the topic. (e.g., David and Benner (2007) Limnol. Oceanogr.)

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Answer: We added this reference in the text (pages 22-23, lines 546-547 in the revised ms) and in the reference list: Davis, J., and Benner, R: Quantitative estimates of labile and semi-labile dissolved organic carbon in the western Arctic Ocean: A molecular approach, Limnol. Oceanogr., 52, 2434–2444, 2007.

Line 565: I don't think you can claim it is no longer photodegradable because it no longer absorbs in natural solar radiation range. There could be secondary photochemical reactions that could still photodegrade it (e.g, via reaction with radicals produced from other photochemical reactions).

Answer: We agree with the Reviewer #2. Reviewer #1 also mentioned this point.

- In the revised ms, we thus replaced the part "...this humic-like component is recognized as a photodegradation product of marine organic matter that is no more photodegradable due to its absorption solely in the UVC wavelengths (Yamashita et al., 2008; Ishii and Boyer, 2012). Besides its resistance to photodegradation, the UVC humic-like fluorophore appears to be resistant to biodegradation (Balcarczyk et al., 2009; Fellman et al., 2010)." by the part "this humic-like component is recognized as a photodegradation product of marine organic matter (Yamashita et al., 2008; Ishii and Boyer, 2012) and appears to be resistant to biodegradation (Balcarczyk et al., 2009; Fellman et al., 2010; Lønborg et al., 2015)." (page 23, lines 556-559 in the revised ms).

- We also removed the part: "...which would represent a kind of "ultimate" refractory humic compound in marine waters (no more photodegradable, no more biodegradable),..." (page 29, lines 703 in the revised ms).

Line 605-607: Rephrase with something like "Several observations suggest the observed change in particulate matter absorption (ap) during the experiment was mainly driven by Synechococcus".

Answer: As suggested, we replaced the sentence "During the experiment, we may assume that the absorption of particulate matter was mainly driven by Synechococcus

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spp." by the sentence "Several observations suggest the observed change in particulate matter absorption [ap(λ)] during the experiment was mainly driven by Synechococcus spp." (page 25, lines 596-598 in the revised ms).

Line 634-635: Rephrase as follows: "...Table 2), thereby suggesting CDOM was produced by heterotrophic bacteria..."

Answer: As suggested, we replaced the part "...Table 2). Therefore, we can make the assumption that CDOM was produced by heterotrophic bacteria..." by the part "...Table 2; Fig. 9), thereby suggesting CDOM was produced by heterotrophic bacteria..." (page 26, lines 626-627 in the revised ms).

Line 660-663: How was the decrease that attributed to N limitation? Is this from another work in this special issue?

Answer: Yes this statement is from other works in the special issue (Bonnet et al., 2015; Berthelot et al., 2015). As mentioned in the revised ms (page 27, lines 806-810 in the revised ms), during the first days of the experiment, NO3- concentration was low in the mesocosm (< 0.04 μM), and because there was no external supply of NO3-, phytoplankton was N-limited. In addition, DDAs would not have been a significant source of N for its surrounding environment because Richelia would have given the major part of the N that they had fixed to their host diatoms (Berthelot et al., 2015).

Line 669: replace "putting forward a rapid... by "suggesting there is a rapid..."

Answer: Done (page 27, line 666 in the revised ms).

Line 687-690: Replace "submitted to" by "affected by". Also, please remove "which led to modifications in the CDOM molecular weight". There is no evidence of that in the data. A link between S and MW has been mainly shown for land-derived CDOM and for S275-295.

Answer: Done (page 28, line 686 in the revised ms).

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Line 693: Replace “CDOM in total” by “CDOM to the total”. Also, did the contribution of pure water included in the total absorption here, or is this the contribution of ag to ag+p? Please clarify.

Answer: We replaced “CDOM in total” by “CDOM (ag) to the total absorption (ag+p)” (page 28, line 689 in the revised ms).

Line 745-746: Wording is a little strong and definitive for this part of the study. Please rephrase with something like” Finally, this study strongly supports the idea of an indirect link between the dynamics: : :Pacific.” You might want to mention that more work is needed to directly demonstrate the role of N2 fixation in the production of chromophoric material.

Answer: Done (page 30, lines 741-742 in the revised ms).

Interactive comment on Biogeosciences Discuss., 12, 17453, 2015.