

# ***Interactive comment on “Particulate trace metal dynamics in response to increased CO<sub>2</sub> and iron availability in a coastal mesocosm experiment” by M. Rosario Lorenzo et al.***

**M. Rosario Lorenzo et al.**

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RESPONSE TO REVIEWERS Anonymous Referee #1 1)... Generally, the topic of the change of particle trace metal during the marine environment changing such as Ocean acidification (OA) and different Fe availability is very interesting for ocean biogeochemists. I believe the data set is valuable in this field. I feel that, however, authors need to regard more about how they can present their data set to induce conclusion above, which they claimed in conclusion section in this paper. The present contents of this manuscript are not well organized for presenting their data set to conclude the claimed conclusions. General comments: In construction of this manuscript, "results"

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section is not constructed only by result, and "discussion" section is not well explained by this study's result (data) ("Discussions" are only like a review of previous knowledge). I recommend that authors should re-construct and re-organized whole part of the manuscript. "Results" section should be used some "Figures" for presenting their data. It makes more easily to understand for readers. "Discussion" section should be related more to data from this study, including which data induce which conclusion more clearly. In view of both reviewers comments we have substantially changed the Ms attending to their requirements. The abstract has been modified, as well as the results and discussion sections. We would like to remark that specifically the Discussion section has been fully re-structured and re-discussed. We believe it is more focused now in order to get good conclusions. Specific answers to the comments raised follow below. 2) The effect of CO<sub>2</sub> did not follow a clear trend in this study, as authors mentioned in the text. The effect of controlled Fe availability by DFB addition/non-addition to phytoplankton bloom is also not clear. How authors induced these their claimed results is not clearly understandable for readers. This has been modified accordingly in 2.1 Experimental set-up section, Lns 119-124: "To induce changes in Fe availability, and analyse its effects on the plankton community, 70 nM (final concentration) of the siderophore desferrioxamine B (DFB) (+DFB and -DFB treatments) (Figure 1b) was added to half of the mesocosms on Day 7, when the community was already acclimated to high CO<sub>2</sub>. The initial dFe concentration before DFB addition was about 4.5 nM. Even though DFB is a strong Fe-binding organic ligand often used to induce iron limitation in phytoplankton (Wells 1999), DFB additions may also increase the dissolved Fe pool in environments with high concentrations of colloidal and/or particulate Fe, such as fjords (Kuma et al. 1996, Öztürk et al. 2002). By day 17, dissolved iron concentrations were significantly higher (by ~3-fold) in the high CO<sub>2</sub> and DFB treatments than in the control (Segovia et al. 2017). These results support an increase in the solubility of Fe in seawater by either lowering its pH (Millero 1998; Millero et al. 2009) and/or the addition of DFB (Chen et al. 2004)." We have added Figure 1, comprising 3 panels referring to CO<sub>2</sub> concentration, dFe and dCu concentrations to better

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understand the chemical scenario that lead the experiment to the obtained results. 3) For discussion OA influence, I think authors should focus on to show “How particle trace metal concentrations and its ratio changed by CO<sub>2</sub> concentrations” by more well presented their own data set. We have changed the tables by bar plots (now figures 3 and 4) according to reviewer’s suggestions and we think it is much clearer now. We have re-written section 3.3-The effects of increased CO<sub>2</sub> and the DFB addition on particulate metal concentrations. Lns 233-242. “Increased CO<sub>2</sub> and the DFB addition did not significantly affect the concentrations of particulate Al, Ti, Cu, and Pb (Tables 1 and S2). Similarly, the addition of DFB did not directly influence particulate concentrations of Fe, but high CO<sub>2</sub> had a significant negative impact on particulate Fe (Tables 1 and S2, Figure 3). Particulate Cd concentrations were also inversely affected by CO<sub>2</sub>, but only in the presence of DFB (CO<sub>2</sub>; and CO<sub>2</sub> x DFB effect, Tables 1 and S2, Figure 3). All other elements (P, Co, Zn, Mn and Mo) exhibited significant effects by CO<sub>2</sub> and by DFB, but there was also a significant interaction between these two factors (Table 1, S2). This indicates that, for example, particulate Mn, Zn, Mo, Co, and P concentrations were significantly decreased by high CO<sub>2</sub>, but only in the +DFB treatments (Figure 3, Table 1, S2,). Similarly, the addition of DFB significantly increased pZn and pMn, but only at ambient CO<sub>2</sub> levels (Figure 3, Table 1, S2)” Data are now supported by new Fig 3, Table 1 and Suppl.Table S2. 4) For Fe availability, they need to discuss that “did DFB addition influence positive/negative to Fe availability?”. It depends on natural dissolved Fe concentrations. See answer to point 2 above, and point 5 below. 5) Additionally, authors should show more clearly about relationship between Fe availability and *E. huxleyi* bloom response, with figure etc. It is very difficult for readers to understand the relation only from the “Tables” number. We have substituted the Table by a new figure 2 with permission from Segovia et al. Mar. Ecol. Prog. Ser. 2017 for a better understanding. This figure shows the temporal development of chlorophyll a ( $\mu\text{g L}^{-1}$ ) and phytoplankton biomass ( $\mu\text{g C L}^{-1}$ ) in the mesocosms exposed to different CO<sub>2</sub> and dissolved iron (dFe) treatments. (a) Chlorophyll a, (b) *Emiliania huxleyi* (5–10  $\mu\text{m}$ ), (c) *Synechococcus* (0.6–2  $\mu\text{m}$ ), (d) picoeukaryotes (0.1–2  $\mu\text{m}$ ), (e) small nanoeukary-

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otes (prasinophytes, small haptophytes, 2–7  $\mu\text{m}$ ), (f) large nanoeukaryotes (small single-celled diatoms and flagellated forms, 6–20  $\mu\text{m}$ ), (g) diatoms (chain-forming *Skeletonema* sp. 20–500  $\mu\text{m}$ ), (h) dinoflagellates (20–200  $\mu\text{m}$ ). This is also discussed in a new 4.1 Section: “The effects of CO<sub>2</sub> and dFe in the plankton community In this experiment we investigated changes in particulate trace metal concentrations, in response to increased CO<sub>2</sub> and/or an addition of the siderophore DFB in a coastal mesocosm experiment. For a better understanding of the processes affecting these stressors, we briefly summarise the mesocosm experiment results originating from Segovia et al. (2017). High CO<sub>2</sub>, as well as the DFB addition increased dFe concentration. The higher dFe concentrations were sustained in the DFB treatments. A bloom of the coccolithophore *Emiliana huxleyi* was observed in the ambient CO<sub>2</sub> treatments, and was especially massive in the one with the addition of DFB (LC+DFB). On the contrary, the biomass of *E. huxleyi* was negatively affected by increased CO<sub>2</sub>. However, increased dFe partially mitigated the negative effect of elevated CO<sub>2</sub>, indicating that the coccolithophore was able to acclimate better to ocean acidification when Fe availability was high. High dFe also had a positive effect on the cyanobacterium *Synechococcus* sp, while the rest of the plankton food web did not response to the treatments (Segovia et al. 2017)” 6) DFB addition inducing more dissolved fraction of TM is artificial response. This is different story from Fe bio-availability. Important for bio-availability is how much free Fe exist under each condition. As one of author well know that DFB-Fe uptake by phytoplankton need very complex mechanisms. Authors should discuss more detail about this part. This was fully discussed in Segovia et al. 2017 and it is not the aim of this paper which is focused in pMe. However, we suggest to consult Segovia et .2017 for further details. See Lns 423-427. “Under control conditions at present CO<sub>2</sub> concentration (LC, 380  $\mu\text{atm}$ ) and no DFB amendment, the globally important coccolithophore *Emiliana huxleyi* was experiencing Fe limitation (Segovia et al. 2017). The shift between particulate and dissolved Fe promoted a massive bloom of *E. huxleyi* in the treatments with ambient CO<sub>2</sub> due to increased Fe bioavailability (for further details on Fe-bioavailability in *E. huxleyi* please see Segovia et a. 2017)”.

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difficult to understand which data indicate this fact. Line 197-198: Authors described “All other elements (P, Co. . . . . .these two factors was clear (Table 5)”. Please explain how clear as like Cd description in previous sentence. Only showing Table 5 is not enough explanation to reader. We have rewritten 3.3-The effects of increased CO<sub>2</sub> and the DFB addition on particulate metal concentrations, as follows: “Increased CO<sub>2</sub> and the DFB addition did not significantly affect the concentrations of particulate Al, Ti, Cu, and Pb (Tables 1 and S2). Similarly, the addition of DFB did not directly influence particulate concentrations of Fe, but high CO<sub>2</sub> had a significant negative impact on particulate Fe (Tables 1 and S2, Figure 3). Particulate Cd concentrations were also inversely affected by CO<sub>2</sub>, but only in the presence of DFB (CO<sub>2</sub>; and CO<sub>2</sub> x DFB effect, Tables 1 and S2, Figure 3). All other elements (P, Co, Zn, Mn and Mo) exhibited significant effects by CO<sub>2</sub> and by DFB, but there was also a significant interaction between these two factors (Table 1, S2). This indicates that, for example, particulate Mn, Zn, Mo, Co, and P concentrations were significantly decreased by high CO<sub>2</sub>, but only in the +DFB treatments (Figure 3, Table 1, S2,). Similarly, the addition of DFB significantly increased pZn and pMn, but only at ambient CO<sub>2</sub> levels (Figure 3, Table 1, S2)” We believe it is much clearer now. Note that Table 5 has been substituted by figure 3 to make it easier, and that Table 1 and S2 also support these data. 10)For “3. Results” section, all subtitle is not well organized. Some contents can be compiled to one (For example, 3.1 and 3.2 can merge for “biological chemical response in mesocosms”. And particle trace metal variation in different treatment in 3.2, 3.3, and 3.4 can merge to one section. Some of the sub-headings have been reorganised. We have accepted the suggestion of “3.1 biological chemical response in mesocosms”. However, we have not merged the following sections as we think it will be rather confusing to the reader. 11)Title and contents of subsection in 3.6 and 3.7 are part of “discussion”. These results parts have been re-structured and part of the text has been moved to the Discussion section. 12) Discussion Line 247-248: “Our results demonstrate that in the studied fjord, particulate Ti and Fe concentrations were dominated by lithogenic material.”. Authors need explanation how they judged this. The explanation is ap-

peared in section 3.6 result (actually this is discussion). Please indicate clearly “this data is shown in Figure 1”. Line248-250: “In contrast, particulate Cu, Co, Mn, Zn, Mo and Cd concentrations were correlated with P concentrations, as well as phytoplankton biomass, suggesting strong biogenic influence on their distribution (Table 6)”. Authors need explanation how they judged this. The explanation is appeared in section 3.7 result (actually this is discussion). Please indicate clearly “this data is shown in Figure 1”. Only showing Table 6 is not kind for reader. This part is overlapped to 4.2 section. It should be in to 4.2 section with detailed explanations with Tables and figures. This is one of the parts that has changed most. This section is supported by Fig 5 and Table 4. Thus, we have fully restructured and re-discussed 4.2 Particulate Fe and Ti are associated with lithogenic sources, while particulate Co, Cu, Zn, Cd, Mo and Mn are associated with biogenic sources, as follows, Lns 265-326: “The particulate trace metal concentrations (nM, mean of all treatments and dates) during the experiment were highest for Al, Fe and Zn, and lowest for Co and Cd, following this trend:  $Al \approx Fe \approx Zn > Ti > Cu \approx Mn > Mo \approx Pb > Co > Cd$ . Lithogenic particles are enriched in Al and low in P (average crustal Al and P content is 2.9 mmol Al and 0.034 mmol P g<sup>-1</sup> dry weight, Taylor 1964), while biogenic particles are enriched in P and low in Al (average plankton Al and P content is 0.001 mmol Al and 0.26 mmol P g<sup>-1</sup> dry weight, Bruland et al. 1991). Therefore, the distinct high abundance of Al and P in lithogenic and biogenic particles, respectively, can be used to evaluate the relative contribution of lithogenic and biogenic material in our particulate samples. In order to do this, first, it is important to establish that the vast majority of the measured particulate P is associated the biogenic fraction. In this study, the abiotic P was estimated using particulate Al concentrations (nM) and the P:Al ratio in crustal material, and was calculated to be negligible (< 1% of the total measured particulate P). In addition, a significant correlation ( $p < 0.003$ ) was found between particulate P concentrations and phytoplankton biomass (Table 4). Therefore, we assume a constant trace metal composition in biogenic particles (assuming they are rich in phytoplankton) and lithogenic particles (assuming they are rich in crustal material). We then calculated the expected

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metal concentrations in the particulate samples assuming that all the P measured in the particles is associated with a biogenic fraction, and that all Al in the particles is associated with the lithogenic fraction. Thus, for a given trace metal, its expected particulate trace metal concentration in seawater (mol L<sup>-1</sup>) can be calculated as the sum of the contribution from biogenic and lithogenic particles, so that:  $[Me] = a [P] + b [Al]$  where  $[Me]$  is the total concentration of the metal (mol L<sup>-1</sup>) expected in the particulate sample;  $[P]$  is the P concentration measured in the particles (mol L<sup>-1</sup>);  $[Al]$  is the Al concentration measured in the particles (nM L<sup>-1</sup>);  $a$  is the average, well-known metal content in biogenic particles, normalized to P (i.e. mol Me: mol P in marine plankton) and  $b$  is the average, well known metal content in lithogenic particles, normalized to Al (mol Me: mol Al in the Earth crust). For example, on day 21 in the HC-DFB treatment, the concentrations of particulate Al and P were 8.22 and 134.8 nM, respectively (Table S2). Assuming a constant 0.0051 mol Fe: mol P in biogenic particles and 0.331 mol Fe: mol Al in lithogenic particles (Table 2), we calculated an expected particulate Fe concentration of 3.41 nM, where 80% was associated with lithogenic material and 20% with biogenic material. Similar calculations were made for the bioactive metals Mn, Co, Cu, Zn, Cd, and Mo (Table 5). Our calculations indicate that on average, particulate Fe was dominated by the lithogenic component (accounting for an average of 78% of the total expected particulate Fe), while for particulate Co, Cu, Zn, Cd, and Mo the biogenic fraction dominated (accounting for 94, 95, 99, 94 and 98%, respectively, of the total expected concentration; Table 5). Particulate concentrations of Mn were also dominated by the biogenic fraction (65% of the total), but the lithogenic fraction was also significant (35%). Moreover, the expected particulate Mn and Fe concentrations closely matched the particulate Mn and Fe concentration we measured (accounting for an average of  $\sim$  71% of the measured Mn, and 115% of the measured Fe). For other metals (i.e. Cu, Mo and Zn), the expected particulate concentrations (nM) were lower than measured (23% of the measured pCu, and 8% of measured pZn; Table 5). This suggests that the particles were enriched in Cu, Mo, and Zn relative to what is expected based on natural marine plankton metal quotas (Bruland et al. 1991) and

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crustal ratios (Taylor 1964). To further establish the lithogenic or biogenic source of the pMe in the particles, the particulate metal concentrations were normalized to the concentrations of particulate P and Al (Figure 4, and Table 2). These ratios were then compared with well-known molar ratios of metal to Al in the crust (Taylor 1964) and of metal to P ratios in marine plankton samples (Ho 2006) and cultures (Ho et al. 2003) (Table 2). The average Fe: Al (506 mmol Fe: mol Al) and Ti:Al ratios (119 mmol Ti: mol Al, Table 2) were relatively similar to crustal molar ratios (331 mmol Fe: mol Al and 39 mmol Ti: mol Al; Taylor 1964). Additional evidence for the significant lithogenic component in particulate Ti and Fe was gathered from Figure 5, where we plotted the molar ratios of the metals relative to P in the collected particles against the Al:P ratios measured in those same particles. The slope of these data  $[(\text{Fe:P})/(\text{Al:P}) = \text{mol Me: mol Al}]$  is the ratio of Me:Al in the particles and can be compared to well-known Me:Al crustal ratio. Visually, if the data nicely fit the Me:Al line for crustal material, these metals are mainly associated with the lithogenic component, as evident for Fe and Ti (Figure 5). These combined results suggest that in our experiment, particulate Fe and Ti concentrations were enriched by lithogenic material. In support of this finding, we also found no significant correlation between particulate Fe and Ti concentrations (nM) and either the total plankton (phytoplankton and microzooplankton) or *E. huxleyi* biomass ( $\mu\text{g C L}^{-1}$ ; Table 4). In contrast, when the P-normalized metal ratios in the particles collected from the mesocosms were plotted against the Al:P ratios in these particles, there were no correlations for the following metals Co, Cu, Zn, Cd, Mn and Mo (Figure 5), indicating that these particulate metals were not enriched in lithogenic material. Our measured metal: P ratios were comparable to plankton ratios in natural samples and in cultures (Table 5). The concentrations ( $\text{mol L}^{-1}$ ) of these metals (i.e. Cu, Co, Zn, Cd, Mn, Mo), as well as P, also showed significant correlations with the biomass ( $\mu\text{gC L}^{-1}$ ) of *E. huxleyi* and that of total plankton cells ( $p < 0.05$ , Table 4), supporting a significant influence of the phytoplankton in the distribution of these particulate elements. 13)Line 251-252: "Changes in CO<sub>2</sub> and/or Fe levels affected total particulate and biogenic metal concentrations for some metals.". This part of results

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is not well presented in manuscript overall. Authors should regard to present some figures which can compare particle and biogenic metals concentrations among each treatment. We have included Figures 3 and 4, and eliminated the corresponding tables that were unclear. 14)Line 255-263, 4.1 Efficacy of the oxalate-EDTA wash removing lithogenic trace metals from particles: First half part of this section is should be move to “results”. Especially from line 260-263, “In general, the concentrations of Fe and Co in the particles were decreased the least by the oxalate wash (by ~25%), while Mo and Pb concentrations were decreased the most (by ~70%). The concentrations of particulate Cu, Zn, Cd and Mn were reduced by 50% by the oxalate wash. As shown previously (Sanudo-Wilhelmy et al. 2004), the oxalate reagent also removed extracellular P (by ~20%).”. This has been moved to material and methods and now reads as follows, Lns 176-195: “2.3.3 The effect of oxalate-EDTA wash on particulate trace metal concentrations To better estimate the biogenic fraction of the particulate metals, the filters were washed with an oxalate-EDTA solution, which removes extracellular metals and oxyhydroxides (Tovar-Sanchez et al., 2003; Tang and Morel, 2006). In our study, the oxalate wash significantly decreased the concentration of all particulate metals, with the exception of Al and Ti (Tables S2 and S3), as observed by Rauschenberg and Twining (2015). The quantity of metal remaining after the oxalate wash (i.e. biogenic fraction) varied among elements (Tables S2 and S3). In general, the concentrations of Fe and Co in the particles were decreased the least by the oxalate wash by ~ 25%, while Mo and Pb concentrations were decreased the most by ~70%. The concentrations of particulate Cu, Zn, Cd and Mn were reduced by 50% by the oxalate wash. As shown previously (Sanudo-Wilhelmy et al. 2004), the oxalate reagent also removed extracellular P (by ~20%, Table S2 & S3). Compared to Rauschenberg and Twining (2015), the estimates of the biogenic fraction, after the oxalate wash, were in agreement for Co, Cu and P, and lower for Fe, Mn, Zn and Cd concentrations. However, the efficacy of the oxalate wash to dissolve Fe, and other metals, from lithogenic particles is not well constrained (Frew et al. 2006, Rauschenberg and Twining, 2015, King et al., 2012). Therefore, the results obtained after the oxalate-EDTA wash should be

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interpreted with caution because we do not know whether the removed metal fraction is a) only lithogenic; b) mainly lithogenic but some biogenic fraction is also removed, or c) whether metals absorbed onto particles are equally labile to the wash on biogenic and lithogenic particles. Given that many of the trends we observed were identical for the oxalate-EDTA washed and non-washed particles [i.e. higher Me concentrations in the LC+DFB treatments (Table S2 & S3) and positive correlations between phytoplankton biomass and Me concentrations (Lorenzo-Garrido 2016)], below we present and discuss only the non-oxalate wash results” So that, oxalate-wash is not further discussed. Yet, it is important to maintain this paragraph in the Ms. 15) Line 282:” Me:P ratios we measured in the particles are similar to those of natural phytoplankton assemblages (Ho, 2006) and of *Emiliana huxleyi* cultures (Ho et al., 2003).”. If authors want to compare their filed data to previous reported data by Ho, 2006, and Ho et al., 2003, authors should show the previous study’s number with their data on to Tables or Figures with citation. Otherwise, authors just state “similar” to natural plankton but did not show any evidence. This has all been re-organised and re-discussed as required by both reviewers. Please see point 12 above. 16) Line 311-312, 319-320: “Interestingly, we also found a putative ZIP-transporter gene. ZIP-transporters are. . . . . . . . . ., such as tRNA synthetase, reverse transcriptase, metallo-carboxypeptidase, ABC-Zn-transporter and CDF-Zn-transporter.”. If authors want to say “we found”, they should show their data and discuss with using their data. If this “gene part” is part of other study, they should cite the other study appropriately. This discussion section is very strange for this aspect. It is written like author’s original data for this study. We have removed the paragraph in agreement with referee comments. 17) Line 329- : Discussion on Cu:P should construct by using their data, what their data’s characteristics, what their data indicate, what is authors claim from the data, which previous knowledge supports their claims. This section 4.2 is like just a review of other papers. We have re-written this part and better discussed our own data as follows, Lns 352-362: Similarly, the Cu:P ratios in the collected particles were relatively elevated ( $1.4 \pm 0.8$  mmol Cu: mol P) compared to those of other phytoplankton, including *E. huxleyi* (Ta-

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ble 2). The dissolved ( $7.7 \pm 0.41$  nM Cu, Figure 1) and particulate Cu concentrations ( $0.35 \pm 0.25$  nM, Table S2) in our experiment were high, and similar to those previously measured in this fjord (Muller et al., 2005). Rain events (or wet deposition of anthropogenic aerosols) in this fjord result in high dissolved Cu and the active production of strong organic ligands by *Synechococcus* to lower the free Cu concentrations (Muller et al., 2005). Therefore, high Cu might be a general condition in this fjord, and indigenous plankton might have developed physiological mechanisms to deal with high Cu, such as the production of organic ligands to prevent uptake (Vraspir and Butler, 2009), or of heavy-metal-binding peptides (phytochelatins) to lower Cu toxicity inside the cell (Ahner and Morel, 1995; Ahner et al., 1995; Knauer et al., 1998). Since we measured high particulate Cu, and Cu:P in our experiment, *E. huxleyi* might have been relying mainly on phytochelatins to buffer high intracellular Cu (Ahner et al., 2002). 18) Line 344: “The Cd:P were significantly lower than those found in phytoplankton and *E. huxleyi*.”. Reader can not understand clearly which data they compared. Is this sentence mean that “The Cd:P were significantly lower than those found in individual phytoplankton and *E. huxleyi* which was reported by previous studies (Ho, 2006, Ho et al., 2003)”?. If so, they should show the comparable data from previous study. We have clarified this in section 4.3 Particulate metals with a strong biogenic component: their P-normalized ratios, Lns 364-372 :” The Cd:P ratios (average  $0.024 \pm 0.01$  mmol Cd:mol P, Figure 4 and 6) were significantly lower than those in phytoplankton and *E. huxleyi* ( $0.36$  mmol Cd:mol P, Figure 4 and 6). This was surprising, because Cd quotas are normally higher in coccolithophores than in diatoms and chlorophytes (Sunda and Huntsman, 2000; Ho et al., 2003). High Cd quotas in coccolithophores have been suggested to result from accidental uptake through Ca transporters and channels (Ho et al., 2009). The low Cd quotas here may be explained by the antagonistic interaction between Mn and Cd or Zn and Cd under high Mn and Zn, respectively (Sunda and Huntsman, 1998, 2000; Cullen and Sherrell, 2005). Since high Zn:P ratios were common in this study ( $34.02 \pm 18.05$  mmol Zn:mol P, Figure 4 and 6), we hypothesize that high Zn levels antagonistically interacted with Cd, resulting in low Cd:P ratios in the par-

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ticles” 19)Line 377: “The decrease in particulate Fe might have been due to enhanced solubility of Fe- oxides at low pH.”. The author should show scientific basis. They have to show relation between pFe and PH in each treatment. It is now shown in Figure 1 and Figure S2 and discussed in 4.4 The effects of increased CO2 and the DFB addition on particulate metal concentrations and P-normalized ratios, Lns 373-395: “Iron enrichment is common in coastal waters, due to sediment resuspension, rivers input, aeolian deposition and mixing or upwelling of deep water. Indeed, Fe was the essential metal with the highest particulate concentrations in our study (Figure 3, Table 3). Furthermore, in this study particulate Fe was characterized by a strong lithogenic component, and was not correlated with phytoplankton biomass. Iron was also unique, in that it was the only trace element whose particulate concentration was significantly and uniquely affected by CO2, regardless of the presence or absence of DFB (no interaction between CO2 and DFB, Table 1). Furthermore, particulate Fe concentrations (nM) decreased steadily between days 12 and 21, with the exception of the control treatment (LC-DFB; Figure 3, Table 2S). This suggests that the increase in CO2 and/or the DFB addition reduce the concentration of pFe, despite the phytoplankton bloom. Such a decrease in pFe (range 2.3-fold in LC-DFB, vs. 13.7-fold in HC+DFB; Table S2) might be mediated by the dissolution of particulate Fe by low pH or by the presence of strong organic chelators as observed in this very experiment (Segovia et al. 2017 and references therein). where dFe notably increased in treatments with high CO2 and/or the addition of DFB (Figure 1). Furthermore, the dissolution of particulate Fe in the treatments with high CO2 and/or the addition of DFB was evident in the Fe partitioning coefficients—the molar ratio between particulate and dissolved concentrations. On day 21, the Fe partitioning coefficients varied by 22-fold between the highest for the control (LC-DFB: 1.039) and lowest for the HC+DFB treatments (HC+DFB: 0.047; Figure S2). Thus, either the DFB addition or high CO2 promoted the dissolution of pFe. However, at the end of the experiment, high dFe concentrations were only observed in the treatments with the DFB additions, suggesting that the presence of strong organic Fe chelators, such as DFB, mediated the maintenance of high dissolved Fe concen-

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trations, as previously observed (Segovia et al. 2017). Thus, in our future oceans, high CO<sub>2</sub> (low pH) will increase dissolved Fe concentrations in regions rich in particulate Fe, and in strong organic Fe chelators.” 20)Line 378: “the concentration of the elements P, Co, Zn, Mn and Mo were influenced by CO<sub>2</sub> and Fe levels”. Which data indicate those results? Authors should present with their dataset. Line 380-381: “where the addition of DFB resulted in higher dissolved Fe, and optimal pH enhanced *E. huxleyi* growth.”. Authors should present this relation, between dissolved Fe, pH and *E. huxleyi* growth, with figures which are constructed by their dataset. This is now solved by the inclusion of Fig 1, Fig 2 , by changing Tables to Figs 3 and 4 and also answered in points 3,6,9 and 19. 21) conclusion remarks Please consider for my “general comment”. It is necessary to describe more specifically what was understood in each argument (claim) a)-d).Authors should present what are difference/similarity of their data among four mesocosms treatment more clearly, and what they can find from the difference/similarity? How they induced the conclusion of this study form the difference/similarity? This aspect is not clear overall in this manuscript. We believe that this requirement is now met attending to the deep-structural changes we have done in the ms. 22) Others Authors used “pFe”, “particulate Fe”, “particulate iron”, “dFe”, and “dissolved Fe” in the text. They should use same words through the manuscript. Changed accordingly

We thank the reviewers for their comments and their time, and hope that our responses are satisfactory.

Yours sincerely,

Maria Segovia & Maite Maldonado

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2018-448/bg-2018-448-AC1-supplement.pdf>

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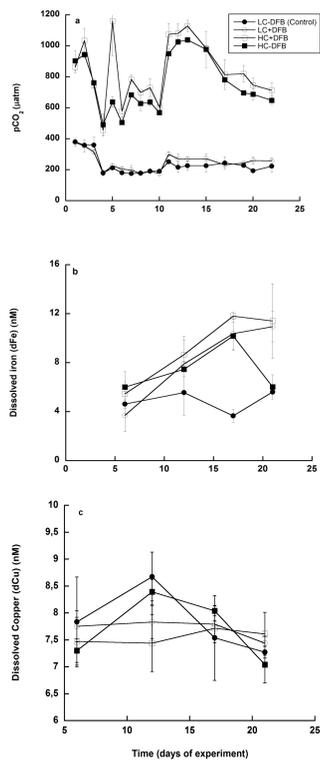
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**Fig. 1.** Temporal development of (a) CO<sub>2</sub> partial pressure (pCO<sub>2</sub>) and (b) pH within the mesocosms. Ambient pCO<sub>2</sub> and ambient dFe (LC-DfB, grey); ambient pCO<sub>2</sub> and increased dFe (LC+DfB, red filled circle); increased pCO<sub>2</sub> and increased dFe (HC+DfB, red open circle), increased pCO<sub>2</sub> and ambient dFe (HC-DfB, black open circle). Symbols indicate means of measurements in 3 independent mesocosms (n = 3) except for LC-DfB where n = 2. Error bars indicate SD. Figure reproduced with permission from Segovia et al. *Mar. Ecol. Prog. Ser.* 2017

**Fig. 1.** Figures

527 **Table 1.** Statistical analyses (Split-plot ANOVA) of the effects of high CO<sub>2</sub>, the addition of DFB, and their interaction, as well as the effect of  
 528 time, on the concentrations of particulate metals (mmol L<sup>-1</sup>, data in Table S2, and Figure 3) in particles collected from the different mesocosms  
 529 treatments.

Factor	Al	Ti	P	Fe	Cu	Co	Zn	Cd	Mn	Mo	Pb
CO <sub>2</sub>	ns	ns	**	*	ns	**	***	***	**	***	ns
DFB	ns	ns	*	ns	ns	*	**	ns	*	*	ns
CO <sub>2</sub> x DFB	ns	*	**	ns	ns	*	**	*	**	**	ns
Time	ns	ns	ns	***	*	***	***	***	***	***	**

530 *ns: not significant; \* p <0.05; \*\* p <0.01; \*\*\* p<0.001*

Fig. 2. Tables

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