

## ***Interactive comment on “Carbon fluxes forced by anticyclonic mesoscale eddies generated by islands at the subtropical NE Atlantic Ocean” by S. Lasternas et al.***

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Actions taken to accommodate the comments of Reviewer#1

Reviewer # 1: Vertical distributions. The authors measured their parameters at either 2 or 5 depths, yet data is seldom differentiated by depth. In particular, in the graphs and charts it is unclear whether the authors are referring to surface concentrations, a mixture of surface and deep concentrations, or vertically integrated biomasses. It would be very useful to see vertical distributions of the measured parameters, or (for parameters that were only measured at surface and DCM) a separate graph for surface and DCM values.

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Author's comment: The reviewer is true. In most of the charts and graphs, it may appear unclear whether parameters were integrated, or averaged... Our graphs show averaged parameters sampled from 5 to 6 depths depending on the station. Chlorophyll a and nutrients concentration and, lysis rates were measured from 6 depths. Only data on micro and nanophytoplanktonic cell abundance and viability, based on samples counting under the microscope, corresponded to two depths (surface and DCM). Figure 5 is showing the vertical distribution of the chlorophyll a concentration, that is a good indicator of the vertical variability.

Author's action: In the M&M section of the revised manuscript we specified the number of depths sampled for the different parameters. P 10246 – L13/16 Text reads: “Seawater samples were collected in 12-liter Niskin bottles mounted on a General Oceanics rosette sampler, at 5 to 6 depths from the surface to 150 m selected as a function to the depth of the deep fluorescence maximum.” P 10246 – L16/17: “Samples for nutrient analysis (phosphate, nitrate + nitrite, ammonium and silicate) were collected at 6 depths down to 150 m” P 10246 – L 23/24: “Samples of 200 ml of water from 6 depths were filtered through Whatmann GF/F filters to estimate total chlorophyll a concentration” P10248 – L 30/31: “Dissolved esterase activity (as FDA hydrolysis) was measured in 3 replicates at 4 to 5 depths sampled above DCM”. We also presented a new Table (Table 3), showing the values of the nano-microphytoplankton populations at the DCM and surface layers (Legend P 10271). In this table, we present the averaged microphytoplankton abundance observed at Anticyclonic (first column), Cyclonic eddies (second column) and Far Field areas (third column):

“Table 3. Abundance of the microphytoplankton groups (average  $\pm$  SE, from the surface and DCM) in the cyclonic and anticyclonic eddies (CE and AEs, respectively) and far field stations studied. Average values for systems connected by different letters are significantly different ( $p < 0.05$ ).” (New Table3)

Reviewer # 1: Cellular lysis rates. The patterns of cellular lysis shown by the authors are compelling and lend credence to their hypotheses about the underlying mecha-

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nisms controlling the AE and CE ecosystems. However, I am concerned about their assumption of a constant PEA:chl ratio and believe that this should be addressed. In particular, in oligotrophic regions with greater water clarity, cells often have reduced pigmentation and thus a higher C:Chl ratio. If esterase concentration scales with biomass rather than pigmentation, this would lead to an increased PEA:Chl ratio, suggesting that utilization of a constant PEA:Chl ratio is underestimating the cellular concentration of esterases. Underestimation of PEA would lead in turn to an overestimation of cellular lysis in the oligotrophic (AE) region. Since this potential artifact could drive the trend that the authors find with respect to lysis, I think they need to address the sensitivity of their results to a variable PEA:Chl ratio. Luckily, I believe the suite of parameters they measured allows them an opportunity to address this question. In particular, their flow cytometry and microscopy measurements should allow them to assess phytoplankton carbon biomass (from biovolume and appropriate conversions – e.g. Menden-Deuer & Lessard, 2000; Garrison et al., 2000) and hence test for cross-system variability in the C:Chl ratio and thus put bounds on the potential variability of the PEA:Chl ratio.

Author's comment: We agree with the reviewer and in the revised version we have applied two different PEA: Chl a ratio varying with Chl a concentration to avoid under/over estimations of lysis rates. The values of lysis rates changed however by less than 10%.

Author's action: We recalculated the phytoplankton lysis rates using a higher PEA:Chl ratio for oligotrophic than for eutrophic waters to avoid over/under estimation of the lysis rates. The new data is described now in the results of the revised version, presented in table 2 and the use of the new calculations are described in the methods section of the revised manuscript: P10249 – L6/16 “The particulate esterase activity (PEA), needed to estimate the lysis rate was calculated from the measured Chl a concentration using a ratio of PEA to Chl a of  $224.5 \pm 83.18$  (Mean  $\pm$  SE) nanoMol Fluoresceine h-1 mg-1 Chl a derived from the particulate esterases of phytoplankton cultures (*Prochlorococcus marina*, *Synechococcus* sp. (strain #1), *Synechococcus* sp. (strain #2), *Chlorella marina*, *Phaeocystis* sp., *Heterocapsa* sp., and *Thalassiosira* sp)

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as described by Agustí and Duarte (2002). To avoid overestimation of the phytoplankton lysis rates in the most oligotrophic waters where pico-phytoplankton dominated phytoplankton biomass, the PEA/Chl a ratio applied was  $377 \pm 101.27$  (Mean  $\pm$  SE) nanoMol Fluoresceine h-1 mg-1 Chl a derived from the PEA of picophytoplanktonic cultures (*Prochlorococcus marina*, *Synechococcus* sp. (strain #1), *Synechococcus* sp. (strain #2), and, *Chlorella marina*).“

Reviewer # 1: The manuscript title begins with “Carbon fluxes”, yet the only carbon fluxes measured and discussed in the manuscript are total primary production and particulate/dissolved primary production, and the only values given are for the ratio of dissolved organic carbon production to total organic carbon production. I believe that the authors should attempt a slightly more comprehensive carbon budget, since they likely have the data for it. In particular, what was the total primary production at each site? How does this compare to total phytoplankton biomass (i.e. what was the turnover rate of the phytoplankton). How does DOC production compare to cellular lysis rates?

Author's comment: Reviewer#2 pointed to the same problem regarding the general focus of the manuscript. We agree and apply several changes to the manuscript and among other, we have changed the title of the manuscript.

Author's action: Title reads: “Forcing of dissolved organic carbon release by phytoplankton by anticyclonic mesoscale eddies in the subtropical NE Atlantic Ocean”. We also included information on the total, dissolved and particulate primary production found at the sampling areas both in the manuscript (P 10253 – L 26/28) and in table 2. The relationship between DOC production and lysis rates is presented in figure 8 and we provide in the results section (P 10254) new information on the significant relationship between PER (the percentage of TPP excreted as PDOC) and lysis rates (PER vs log lysis, ( $R^2 = 0.38$ ,  $p < 0.03$ ), which is also supporting a higher excretion of PDOC associated to high lysis. We also improved the discussion on the biological processes variability as cell mortality, production or PDOC released by phytoplankton, influencing

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the carbon budget we measured.

Table 2 now reads, with averaged values from Anticyclonic, Cyclonic eddies and Far Field areas in first, second and third column, respectively (New Table 2)

Reviewer # 1: Primary production measurements – Some specific details need to be included with these measurements, especially since only 3 hour incubations were used. In particular, at what time of day were the incubations conducted? How were 3 hour incubations (potentially taken at different times of day) used to estimate daily PP rates for comparison across different systems? Time of day could potentially also affect the fraction of viable cells.

Author's comment: The 3 hour incubations were performed at the same time of the day, always including noon so the comparison was established without bias.

Author's action: The time of the day of the incubations was added to the M&M section (P 10248 – L 11/13): "Depending on sea conditions, the incubations were deployed in a mooring buoy system and incubated in situ for 3 hours at the same time of the day (from 12.00 to 4.00 p.m), always including noon." We also (as requested by reviewer#3) included a comment in the revised version indicating the need of short-time incubations (P 10248 – L 13/15): "Short-time incubations were used to minimize the contribution of trophic-related processes to DOC production (Morán and Estrada, 2002). "

Reviewer # 1: Exudation v. lysis. The authors seem to attribute the increased proportion of PP to the dissolved phase in the AE to increased cellular lysis. This seems unlikely to me, since during three hours incubations only a small fraction of cellular carbon will become labeled. Cellular lysis would thus be leading to production primarily of unlabeled DOC. Given the high concentration of <sup>14</sup>C passing to the dissolved pool, it seems more likely to be a result of cellular exudation (which might primarily be of recently fixed – and hence <sup>14</sup>C rich - sugars) in response to nutrient stress (e.g. the "paradox of the phytoplankton" – Bratbak & Thingstad, 1985).

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Author's comment: Studies on cell death mechanisms, including those on microalgae, have progressed much in recent years, and include the description of the morphological and molecular changes that cells undergo when dying by necrosis or by programmed cell death. With the exception of virus-induced lysis or some chemicals (e.g. bacterial cells exploded when exposed to antibiotics) for which we expect a very fast cell lysis, there is a time between the start of the death of the cells and their disintegration (e.g. Dunn et al. 2002, Segovia 2008). The increased cell membrane permeability of compromised cells should lead to the release of recently labelled carbon compounds, as well as fixed sugars and structural carbon as disintegration in the dead cell progress. Veldhuis et al. (2001) reported that cells with a reduced viability (increased membrane permeability) still photosynthesized although photosynthetic activity dropped by as much as 60% relative to that of the viable cells. In the subsequent stages, when photosynthetic pigments were fully degraded, this value dropped further to around 10%. This implies that the recently fixed <sup>14</sup>C could be excreted by dying cells as well as by exudation. The paper of Bratbak & Thingstad (1985) was published before these studies, so it was difficult that these authors considered cell death, as is understood presently, as a probable process. However, we agree that cell exudation of carbon was also occurring. In our study, we were not able to analyse throughout all the mechanisms operating in situ, but our results evidenced the empirical association between the increase of the DOC production and the increasing lysis rates, supported also by a relationship between PER and lysis rates. We also agree that lysis rates were not able to explain all the variability of DOC produced by phytoplankton, and other processes as cell exudation were contributing to.

Author's action: We revised the discussion to increase clarity and include the following paragraph: P 10258 – L16/19 "Even though we were not able to analyze whether other processes as cell exudation may be involved in the DOC production by phytoplankton, we stressed that PDOC production was significantly related to cell lysis rates, indicating an active participation of cell mortality process on the DOC production by phytoplankton. " And in P 10258 – L9/16, we added: "In our study, at the AEs waters, we observed

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higher cell mortality and lysis rates than those observed in the FF and in CE, resulting in higher values of DOC production. Also, the significant relationship between PER and lysis rates observed indicated a significant proportion of the PDOC excreted to be the result of phytoplankton cell death. However, lysis explained less than the 40% of the variability on PER, implying that other processes such as cell carbon exudation, reported to increase when nutrient limitation (e.g. Bratbak and Thingstad, 1985) were also responsible of the PDOC observed.”

Reviewer # 1: Nitrogen limitation – While there is clearly decreased nitrate in the AE, the highly elevated ammonium levels actually lead to relatively similar total DIN concentrations between AE and CE. Since ammonium is typically preferentially taken up by phytoplankton, it thus is not apparent that there is nitrogen limitation in the AE. Do the authors believe that other nutrients are ultimately limiting? They mention silica, but this only holds for diatoms. A more explicit discussion of limitation, potentially broken down into different groups, would be useful. Which groups dominated biomass (not abundance) in each region and what were their nutrient requirements/cellular health?

Author’s comment: We agree with referee#1 considering that other nutrients would be limiting phytoplanktonic populations. In particular, DIP was observed to have lower concentrations at AEs . We agree that we should improve this aspect.

Author’s action: The discussion was modified in order to better discuss limitation by other nutrients and competition between the planktonic groups.

In relation to Si, the revised text reads (P 10256 – L 11/17): “At the cyclonic eddies, the higher diatoms abundance and similar availability of Si in comparison with the far field stations suggest that phytoplankton has already used the nutrient. The low cell mortality (lower % of dead cells) of diatoms in CE systems suggested that they are probably not yet in a post-bloom phase, although phytoplankton lysis rates were similar to those shown in FF, most probably due to the higher cell death at CE of picophytoplankton, which showed higher proportion of dying cells.”

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Regarding P-limitation and competition between groups, the revised text now reads (P 10259 – L 2/13): “Indeed, nutrient supply has been shown to explain variability in bacterial activity in the Atlantic Ocean (Gasol et al., 2009), and the negative relationship between the %DC of *Synechococcus* sp. and the %LC of HB found here may indicate competition for phosphorus in the eddies system (Zubkov et al., 2007; Lasternas et al., 2010) and limitation of picoplankton viability. Flagellates and dinoflagellates may also be limited by DIP in AE, as observed in systems with low DIP availability (Thingstad et al., 1998, Lasternas et al., 2010). Lasternas and co-authors (2010) found in the Mediterranean Sea (a system limited by DIP) that the percentage of living cells of dinoflagellates, heterotrophic bacteria and *Synechococcus* sp. was strongly related to DIP, and competition for this nutrient was important in structuring the phytoplankton community composition. Moreover, the low values of DIP in FF stations indicated that this nutrient must have been limiting phytoplankton biomass in that area.”

Reviewer # 1: Do the authors have any zooplankton measurements? It seems like a comparison of grazing rates or even zooplankton biomasses would help greatly in their interpretation of system dynamics.

Author’s comment: Unfortunately we do not have zooplankton data in order to be included in the present study.

Reviewer # 1: The manuscript seems to be a bit longer than it needs to be. In particular, there is a lot of discussion of the physical generation of eddies that I feel is unnecessary in a manuscript that is really focused on the biology. The authors do not have much physical evidence to address the formation of the eddies, and hence extensive discussion of this topic only dilutes the focus of the manuscript. In particular, I think they could cut back on much of the introduction (for instance lines 10-30 on page 10243) and some of the discussion (for instance line 22 on page 10256 to line 6 on page 10257).

Author’s comment: We agree with referee#1.

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Author's action: In preparing the revised manuscript we made the effort in shortened the text, deleting extensive introduction. We also simplified Table 1 and figures 2 and 3, by suppressing the superfluous information on depth anomalies.

Reviewer # 1: The methods section can be shortened a bit. In particular some of the details of the live/dead staining is repeated/scattered and can be more effectively combined.

Author's action: We followed reviewer recommendation and reduced the methods section, avoiding as well repetitions in the live/dead methods description (P 10247/10248). As commented bellow, we also reduced the physical methodology (P 10245/10246).

Reviewer # 1: End of abstract: "weakness of the carbon pump" – this is a (justifiable) assumption, but is not directly supported by their measurements and hence probably not appropriate in the abstract.

Author's action: Abstract reads now (P 10242 – L 18/20): "The adverse conditions associated to the early-stage anticyclonic systems, mainly triggered by active downwelling, forced photosynthesized carbon to fuel the dissolved pool."

Reviewer # 1: The authors should be explicit when referring to total cells, live cells, and dead cells. I often found myself wondering (when they mention total nanomicrophytoplankton) whether they were referring to total cells or to live cells.

Author's comment: We re-edited the manuscript taking into account the possible confusions referring to the abundance and dead/living cells.

Reviewer # 1: Pg. 10257, line 18. "mortality" was not measured. Mortality differences are inferred from differences in the proportion of non-living cells, but this assumes a similar turnover rate for non-living cells in the different systems. Likely true, but still an assumption.

Author's comment: We agree.

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Author's action: We revised the paragraph to avoid confusion, and modified as follows (P 10256 – L17/21): "Rodríguez et al. (2003) observed that upward velocities favor the growth of large phytoplankton cells. Here in our study, we found a higher microphytoplankton proportion of living cells and lower lysis rates at the upward conditions in the cyclonic stations in agreement with more favorable conditions to increase the proportion of larger phytoplankton cells."

Reviewer # 1: The manuscript has many grammatical errors, too many to enumerate individually. While they do not impair understanding of the science, they are distracting. I would recommend having a native speaker proofread the manuscript.

Author's comment: A native English speaker has proofread the manuscript.

References: Agustí S. and C.M. Duarte. 2002. Addressing uncertainties in the assessment of phytoplankton lysis rates in the sea. *Limnology and Oceanography* 47: 921-924

Bratbak, G. and Thingstad, T.F.: Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. *Mar. Ecol. Prog. Ser.*, 25, 23-30, 1985.

Dunn SR. Bythell JC. Le Tissier MDA et al. 2002. Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *J. Exp. Mar. Bio. Ecol.* 272: 29-53.

Lasternas, S., Agustí, S. and Duarte, C. M.: Phyto- and bacterioplankton abundance and viability and their relationship with phosphorus across the Mediterranean Sea, *Aquat. Microb. Ecol.*, 60, 175–191, doi:10.3354/ame01421, 2010.

Segovia M. 2008. Programmed Cell Death in Dinoflagellates. In: J. M. Perez Martin (ed.) *Programmed Cell Death in Protozoa*. Landes Bioscience and Springer Science+ Business Media, LLC, New York. Pp 126-142.

Thingstad, T.F., Zweifel, U.L., and Rassoulzadegan, F.: P limitation of heterotrophic

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bacteria and phytoplankton in the Northwest Mediterranean. *Limnology and Oceanography*, 43, 88–94, 1998.

Veldhuis, MJW, Kraak, HL, Timmermans, KR. 2001. Cell death in phytoplankton : correlation between changes in membrane permeability, photosynthetic activity, pigmentation and growth. *Eur. J. Phycol.* 36: 167-177.

Actions taken to accommodate the comments of Reviewer#2

Reviewer # 2: The authors have described the physics in details but it would have been nice to have a plot of isopycnal surface to understand the vertical displacement better.

Author's comment: The reviewer is true, the density field will be more appropriate to infer the eddies vertical structure. Unfortunately only one eddy was crossed with CTD stations (CE1) being the rest (CE2, AE1, EA2) crossed with XBT stations. Thereby in order to make comparable the vertical structure of the eddies we have chosen the temperature as XBT data only provides this variable. We have envisaged the possibility to infer the salinity from the XBT data using standard TS algorithms for the region but we have discard this approach because it is not sufficiently accurate at the near surface layers when the mixed layer is deep as it is the case for AE1 and AE2 (eg, Sangrà et al., 2007, Deep-Sea Research)

Reviewer # 2: The age of the eddies sampled is very important for interpreting the data (e.g Bentiez-Nelson et al., 2008). It would be nice to know the time of formation of these eddies based on satellite data with respect to the time of sample collection.

Author's comment: According to the reviewer suggestion we have re-examined our satellite image collection in order to give an estimate for the eddies age. This was only possible for the eddies attached to the islands and not for AE1. For this eddy we have given and estimated based on previous observations on the eddies spin up time and advection.

Author's action: from P10250 – L14/27, we added these estimates to the text, as fol-

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lows: “Cyclones of La Palma (CE2) and Gran Canaria (CE1) were close to the southwest flank of these islands (Fig. 1) and were sampled at their early life stage (spin up stage) having a radius of about 25 km and about 35km respectively. Their signal on the SST was observed approximately 5 days (CE2) and 7 days (CE1) before the sampling, indicating that they are at least one week older. Anticyclone AE1 probably generated by the island of Tenerife, but located far from this island at the time of sampling and hence relatively older, presented the larger observed radius of about 65 km. We may give an estimate of the eddy age considering that previous observations (Sangrà et al 2005) indicate a spin-up time (half of the Strouhal frequency) of one week and once detached a mean advection velocity of ca. 4 km/day. If originated by Tenerife this will result on an age of ca. 4 weeks. Gran Canaria anticyclone, AE2, with a smaller radius of about 50 km, was located close to the island of origin and therefore was younger than AE1, being at its early stage of generation (spin-up stage) and having an estimate age from its SST signal at least of 7 days.”

Reviewer # 2: The depths of integration for some of the parameters are not clearly defined. How was it carried out - up to base of euphotic zone, certain isopycnal surface, mixed layer? This is extremely important and parity must be maintained when comparing cyclonic eddies, anti-cyclonic eddies and far-field regions.

Author's comment: The M&M section may be confusing as none of the parameters were integrated but averaged. Biological processes as Primary production or Phytoplankton lysis rates could not be standardised to fixed depths, since should be sampled at the relevant vertical scale at each station as are influenced by the light conditions.

Author's action: We added a short section in M&M text precisising the depths of sampling and explaining thus how parameters values were averaged by stations. Text reads now: P 10246 – L13/16: “Seawater samples were collected in 12-liter Niskin bottles mounted on a General Oceanics rosette sampler, at 5 to 6 depths from the surface to 150 m selected as a function to the depth of the deep fluorescence maximum.” P 10246 – L16/17: “Samples for nutrient analysis (phosphate, nitrate + nitrite, ammonium and

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silicate) were collected at 6 depths down to 150 m" P 10246 – L 23/24: "Samples of 200 ml of water from 6 depths were filtered through Whatmann GF/F filters to estimate total chlorophyll a concentration" P10248 – L 30/31: "Dissolved esterase activity (as FDA hydrolysis) was measured in 3 replicates at 4 to 5 depths sampled above DCM".

Reviewer # 2: The 3 hour incubation for PP could bias the data and this caveat should be mentioned in the text and why it was done so.

Author's comment: As described by Morán and Estrada (2002), and other authors, a short incubation period should be selected when measuring PDOC because must be a compromise between the time needed to obtain a significant signal in the PP phase, and at the same time, minimize the loss of <sup>14</sup>C-labeled dissolved organic carbon (DOC) due to assimilation by heterotrophic prokaryotes. We then used short-time incubations as recommended to optimise the measurements and minimise the contribution of trophic-related processes to DOC production (Morán and Estrada, 2002: Phytoplanktonic DOC and POC production in the Bransfield and Gerlache Straits as derived from kinetic experiments of <sup>14</sup>C incorporation, Deep-Sea Res. Pt. II, 49, 769–786)

Author's action: (P10248, L13/15) Text reads: "Short-time incubations were used to minimize the contribution of trophic-related processes to DOC production (Morán and Estrada, 2002)." And reference has been added.

Reviewer # 2: The author should add total and particulate PP data in Table 2 or 3. This data is relevant to the discussion.

Author's comment: As presented in P10253, particulate primary production (PPP) rates presented low variation, with no significant differences between zones. Total primary production values do present variations. Higher values were found at the AEs but mostly because of a high DOC production at the zone, indicated by higher PER. This notion would confuse the reader, for that we didn't mention it in the previous version. However as reviewer #2 proposed, we are willing to present these data as table and

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discuss it in the manuscript as needed.

Author's action: We have included TPP, PDOC and PPP values by zones in table 2. And discuss briefly the variation in TPP. Text reads (P 10253 – L26 to P 10254 L3):" While particulate primary production (PPP) rates presented low variation (averaging  $0.062 \pm 0.024$  mg C m<sup>-3</sup> h<sup>-1</sup>), with no significant differences between zones (Table 2, Student's t-test,  $P < 0.05$ ), we observe higher total primary production (particulate plus dissolved) at the AEs (Table 2). This was mostly due to the high production of dissolved organic carbon at this zone. Indeed, PDOC production presented significantly higher rates at the AE and subsequent higher PER, than at the others areas (Table 2). The percentage of extracellular release by phytoplankton averaged  $69.5 \pm 6.5\%$  and ranged from 31 to 98% along the study, with higher averages found at AEs ( $70.3 \pm 7.7\%$ ).

Table 2 reads, with averaged values from Anticyclonic, Cyclonic eddies and Far Field areas in first, second and third column, respectively (New Table2)

Reviewer # 2: The manuscript title should be re-phrased to better represent its focus on biological productivity/ community rather than carbon flux.

Author's action: The title has been re-phrased as "Forcing of dissolved organic carbon release by phytoplankton by anticyclonic mesoscale eddies in the subtropical NE Atlantic Ocean"

Reviewer # 2: Figure 8 and 9 shows the same data and one or the other can be omitted.

Author's comment: We agreed and since reviewer #3 was also suggesting we excluded previous Figure 8 and its legend from the manuscript.

Author's action: Figure 8 and its legend were removed and figures numbering was changed.

Reviewer # 2: It is interesting that for cyclonic eddies the nitrate and phosphate levels are higher where as silicate concentration is similar compared to the far-field station. The diatom population and mortality rate on the other hand are much higher in the

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cyclonic eddies compared to far-field station. Is this indicative of a more mature eddies in decaying phase of a bloom. The author should give some perspective on the how the age of an eddy could impact the biological community.

Author's comment: Reviewer#2 is right. We agree that at the cyclonic eddies, the higher diatoms abundance but similar availability of Si than at FF would suggest that phytoplankton has already used the nutrients and population, may be in a post bloom phase. However, there were low cell diatom mortality at CE as indicated by the lower proportion of dead diatoms cells and the averaged lysis rates were close to FF, most probably due to the larger cell mortality observed for picophytoplankton at CE, indicating that diatoms population growing at CE were not still in late-life cycle stage, on contrary to the FF diatoms population presenting higher mortality.

Author's action: Text has been improved to better appreciate the perspective mentioned by reviewer#2. Text reads (P 10256 – L 11/17): "At the cyclonic eddies, the higher diatoms abundance and similar availability of Si in comparison with the far field stations suggest that phytoplankton has already used the nutrient. The low cell mortality (lower % of dead cells) of diatoms in CE systems suggested that they are probably not yet in a post-bloom phase, although phytoplankton lysis rates were similar to those shown in FF, most probably due to the higher cell death at CE of picophytoplankton, which showed higher proportion of dying cells."

Reviewer # 2: The authors do not have enough data to comment on the carbon flux. As seen by number of earlier papers, the relation between productivity and carbon flux is not linear and often (e.g. Maiti et al 2008) higher productivity did not translate to higher export inside eddies.

Author's comment: This comment is in agreement with those of reviewer#1. We agree that the results presented are not showing the carbon fluxes, only a part of the budget.

Author's action: We have changed the title of the manuscript to the following: "Forcing of dissolved organic carbon release by phytoplankton by anticyclonic mesoscale eddies

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in the subtropical NE Atlantic Ocean". We also have re-edited the text by lightening the discussion focussed on carbon fluxes, and by better focusing on the biological processes studied such as cell mortality, and the production or PDOC released by phytoplankton.

Reviewer # 2: The manuscript is difficult to follow and the authors might consider editing the text under discussion and rewrite it in term of the different parameters (compare and explain the differences between different eddies for each parameter) rather than other way round. The physics part of the eddies can be shorter. Appropriate figures should be referred during discussion.

Author's comment: We agree with the reviewer, however some biological processes need a more complex explanation, for such discussions were not easily accommodate the discussion to a scheme based in eddies comparison alone. However, we agree that the clarity of the manuscript must be improved, and some paragraphs need to better focus in the eddies comparison.

Author's action: Following reviewer comment, we have revised the manuscript to increase the clarity, shorten the physics part and improved the comparison between the different eddies.

Actions taken to accommodate the comments of Reviewer#3

Reviewer # 3: There are frequent grammatical errors throughout the paper. I began to detail them below but found that there are too many to list. Please make sure the manuscript is thoroughly proof read as these errors do detract from the paper. Distributions

Author's comment: We agree.

Author's action: The revised manuscript was proofread by a native English speaker.

Reviewer # 3: The methods section is too long and overly detailed. The authors should attempt to shorten the methods to make them as concise as possible, citing method-

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ological techniques were appropriate.

Author's comment: As already asked by referee#2, we shortened the M&M section.

Author's action: We simplified the physical part of the M&M section by removing the superfluous information on the depth anomaly (in text, table 1 and figures 2&3). In P 10247, we eliminated repetitions in the description of the live/dead methods; finally, we combined the description for nano-micro and picophytoplankton and shortened this methodological part.

Reviewer # 3: Page 10249, the 3 hours incubation period for  $^{14}\text{C}$  seems overly short. Please explain the rationale for this incubation period.

Author's comment: When measuring PDOC it is recommended the use of short incubation periods (e.g. Morán and Estrada, 2002). 3 hours incubation period was a compromise between the time needed to obtain a significant signal in the PP phase, and at the same time, minimize the  $^{14}\text{C}$ -labeled dissolved organic carbon (DOC) assimilation by heterotrophic prokaryotes. Short-time incubations (i.e. 3 h) are recommended to minimise the contribution of trophic-related processes to DOC production (Morán and Estrada, 2002).

Author's action: To increase clarity, we included the following paragraph in the manuscript (P10248, L13/15): "Short-time incubations were used to minimize the contribution of trophic-related processes to DOC production (Morán and Estrada, 2002)."

Reviewer # 3: Page 10256, I am unclear as to the meaning of "rs". Is this the same as "R2"? If so, please keep the notations consistent.

Author's comment: "rs" corresponds to the coefficient of correlation of Spearman and so differ from the R2. To avoid any confusion, we added its meaning in the M&M section.

Author's action: The new version of the manuscript now reads (P 10250 – L2): "Spearman's rank coefficients (rs) were used to determine correlation between variables that

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departed from normality (Siegel and Castellan, 1988)"

Reviewer # 3: The regression in figure 9 is the same as data in figure 8, but is just plotted in a different way. I am also particularly concerned about the regression in figure 9. The single data point in the top right of the plot is most likely driving the regression and consequently is causing the very low p-value associated with the dataset. It is probably unwise to present this regression as it is. The regression could be validated by performing a second regression analysis on the cluster of data points to the lower left of the plot and comparing this with the regression of the whole data set. I suggest that figure 8 is deleted from the manuscript.

Author's comment: We agree that figures 8 and 9 shows similar results, so as suggested by reviewer we have excluded figure 8 from the manuscript. As suggested by Reviewer#1, we have recalculated the lysis rates to avoid over/under estimation, and the new results are now shown in the new Figure 8. The relationship between PDOC and Lysis rates didn't change substantially because the lysis rates values were not so sensitive to the changes in PEA. Reviewer #3 was right about the validation of the relationship between the lysis rates and PDOC. The highest lysis rate and higher PDOC data influenced the relationship, and was not significant when performing the relationship with the lower data points. The low p-value was also a consequence of the low N. We also found a significant relationship between PER (the percentage of TPP excreted as PDOC) and lysis rates (PER vs log lysis,  $R^2 = 0.38$ ,  $p < 0.03$ ), which is also supporting a higher excretion of PDOC associated to high lysis rates. Following reviewer suggestion, we keep Figure 9 showing the relationship (Figure 8 in the revised version of the manuscript) between PDOC production and lysis rates, and eliminate Figure 8 as suggested by the reviewer.

Author's action: In the new version of the manuscript, a) We have eliminated Figure. 8 and re-numbered in consequence the others figures.

b) The significant relationship between PER and Lysis rates was described in the re-

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sults (P 10254 – L 4/12): “The high lysis rates measured at the AE (Table 2) were consistent with the high percentage of dead nano-microphytoplankton cells ( $r_s = 0.76$ ,  $p < 0.05$ ) at these waters where we observed also the highest production of dissolved organic carbon by phytoplankton (PDOC) in these stations (Table 2), as indicated by the significant positive relationship between PDOC concentration and lysis rates ( $R^2 = 0.76$ ,  $p < 0.0005$ ; Fig. 8). Moreover, we also found a moderate but significant relationship between PER (the percentage of TPP excreted as PDOC) and lysis rates (PER vs log lysis,  $R^2 = 0.38$ ,  $p < 0.03$ ), which also supported a higher excretion of PDOC associated to high lysis rates.”

c) We modified the discussion as follows (P 10258 – L9/16): “In our study, at the AEs waters, we observed higher cell mortality and lysis rates than those observed in the FF and in CE, resulting in higher values of DOC production. Also, the significant relationship between PER and lysis rates observed indicated a significant proportion of the PDOC excreted to be the result of phytoplankton cell death. However, lysis explained less than the 40% of the variability on PER, implying that other processes such as cell carbon exudation, reported to increase when nutrient limitation (e.g. Bratbak and Thingstad, 1985) were also responsible of the PDOC observed.”

Reviewer # 3: I recommend starting the discussion section with a short paragraph to remind the reader of what the study aimed to find out, and what hypotheses were to be tested.

Author's comment: We included the paragraph suggested by the reviewer.

Author's action: The discussion section in the revised manuscript start with the following paragraph (P10255 – L3/17): “The counter-paired cyclonic and anticyclonic eddies generated by the Canary Current and Trade Wind perturbation by the Canary Islands have been described to influence biological processes in the NE Atlantic region (Aristegui et al., 1997; Aristegui and Montero, 2005; González-Dávila et al., 2006; Sangrà et al., 2009). Our study aimed to comprehend the influence of anticyclonic/cyclonic

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eddies on biological processes that informed on the cell physiological responses that may influence the path of the carbon photosynthesized by phytoplankton in the system. Our results indicated that the differences in environmental conditions (specially nutrients availability) associated with the upward/downward forcing of these anticyclonic/cyclonic eddies influenced phytoplankton cell physiology and generated differences in cell stress that forced the carbon dynamics by increasing the excretion of primary production as DOC. Our analysis of phytoplankton cell death processes within the eddies system revealed important differences in phytoplankton cell health related to the cyclonic and anticyclonic eddies, with populations experiencing higher cell mortality and cell lysis at the last ones, which stressed both the diatoms and picophytoplankton populations.”

Reviewer # 3: Page 10261, line 6 - It was unclear to me how the authors have come to the conclusion that eddies have influenced carbon flux. There is no carbon flux data contained in the manuscript, which thus make this statement somewhat hard to justify. Furthermore, the title of the manuscript states that the paper is about carbon flux. There are a couple of approaches the authors may use to address this issue. If carbon flux data exists for this project I would strongly encourage the authors to include it, or reference it if it is being written up as another manuscript. Alternatively, the authors could re-focus the manuscript to better describe the datasets contained within the manuscript. This would include rewording the title and any parts of the manuscript that make direct reference to carbon fluxes.

Author's comment: This criticism has emerged also in the comments by reviewers #1 and 2, so we changed the manuscript, by providing a new title, and by lightening the carbon flux discussion focusing now on biological processes such as cell mortality, and the production or PDOC released by phytoplankton.

Author's action: New title reads: “Forcing of dissolved organic carbon release by phytoplankton by anticyclonic mesoscale eddies in the subtropical NE Atlantic Ocean”. The discussion has been modified, eliminating the detailed paragraph on physical struc-

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tures in the section Cyclonic Eddies, and modifying and re-titling the last section as follows (P 10259 – P 10261): “Phytoplankton dissolved organic carbon production within the eddies system and the anticyclonic forcing”. This section has been simplified in order to better focusing on biological process than carbon fluxes.

Reviewer # 3: Specific comments

Page 10242, line 21: suggest rewording “Mesoscale eddies dynamics” to “The dynamics of mesoscale eddies”

Author’s action: Text modified as suggested.

Page 10242, line 25: check abbreviation “Oceanic Vertical Pumping (VOP)”

Author’s action: We deleted this abbreviation from the text.

Page 10243, line 15: “other considers” should be “others consider”

Author’s action: We re-edited the text.

Page 10243, line 18: remove “and”

Author’s action: Text modified as suggested.

Page 10243, line 19: insert “a” to become “pumping as a consequence”

Author’s action: We re-edited the text. Page 10243, line 28: word order “suggests probably” should be “probably suggests”

Author’s action: We re-edited the text.

Page 10244, line 11: “evidences” should be “evidence”

Author’s action: We re-edited the text.

Page 10246, line 15: “bathythermograph” should be plural “bathythermographs”

Author’s action: We re-edited the text.

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Page 10246, line 16: “provide” should be past tense “provided”

Author’s action: We re-edited the text.

Page 10254, line 8: suggest word alteration “but” should be changed to “except”

Author’s action: We re-edited the text.

Page 10255, line 8: word order “observed also” should be “also observed”

Author’s action: We re-edited the text.

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Interactive comment on Biogeosciences Discuss., 9, 10241, 2012.

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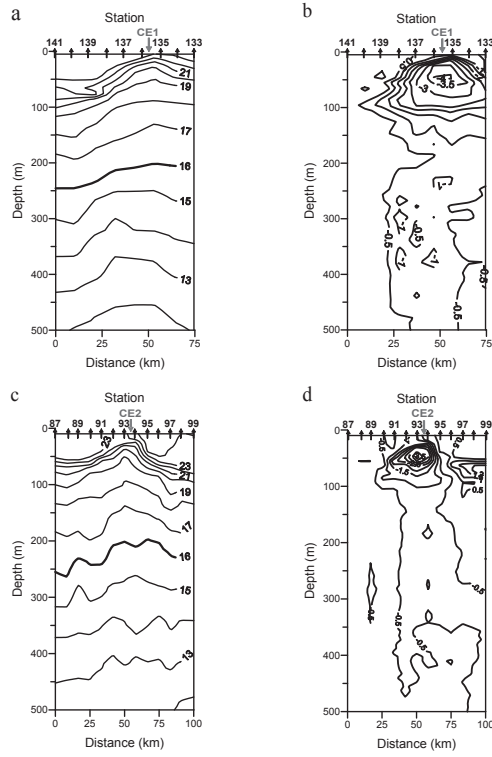


Fig. 1. Figure 2 revised

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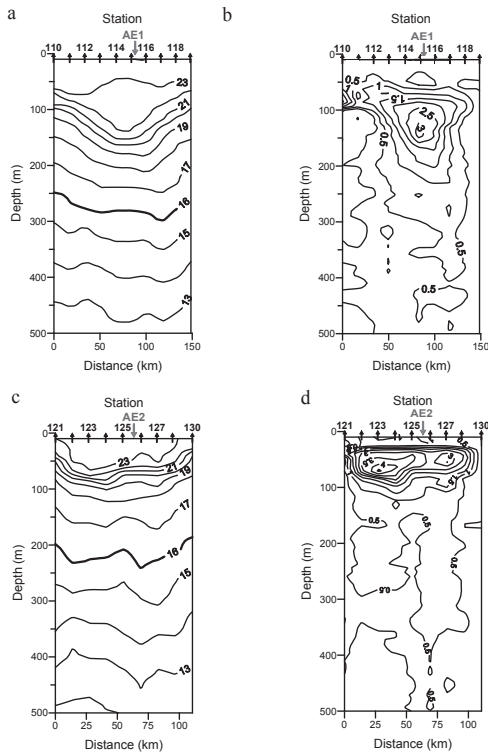


Fig. 2. Figure 3 revised

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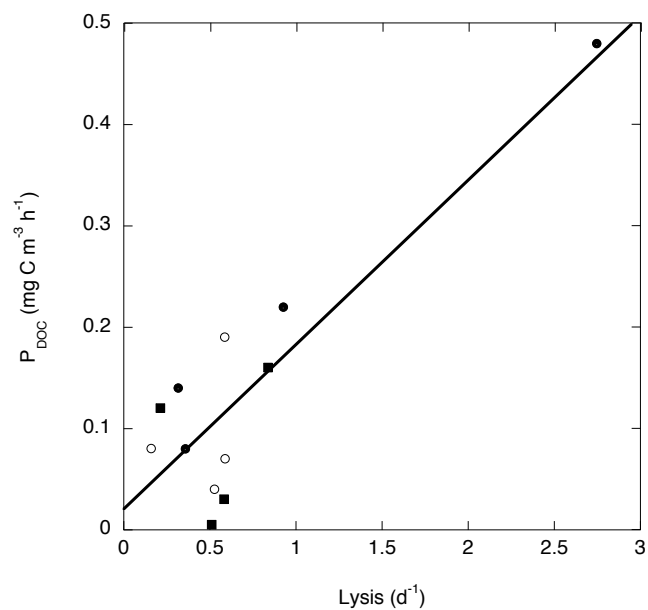


Fig. 3. New Figure 8

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Variable	Anticyclonic eddies	Cyclonic eddies	Far fields
	$0.05 \pm 0.03^b$	$0.29 \pm 0.03^a$	$0.001 \pm 0.00^b$
DIP ( $\mu\text{M}$ )	(0.001 – 0.25)	(0.08 – 0.36)	(0.001 – 0.001)
	$0.44 \pm 0.04^b$	$0.69 \pm 0.12^a$	$0.69 \pm 0.03^a$
Silicate ( $\mu\text{M}$ )	(0.23 – 0.58)	(0.21 – 1.27)	(0.55 – 0.83)
	$0.26 \pm 0.12^b$	$1.21 \pm 0.47^a$	$0.14 \pm 0.05^b$
DIN ( $\mu\text{M}$ )	(0.04 – 0.85)	(0.08 – 3.97)	(0.06 – 0.49)
	$1.26 \pm 0.21^a$	$0.28 \pm 0.04^b$	$0.72 \pm 0.15^b$
Ammonium ( $\mu\text{M}$ )	(0.36 – 2.18)	(0.18 – 0.41)	(0.09 – 1.33)
	$22.4 \pm 0.5^a$	$19.8 \pm 0.8^b$	$21.9 \pm 0.8^a$
Temperature ( $^{\circ}\text{C}$ )	(19.6 – 24.0)	(17.3 – 23.1)	(18.5 – 24.8)
	$0.26 \pm 0.05^b$	$0.52 \pm 0.13^a$	$0.25 \pm 0.06^b$
Chlorophyll a ( $\text{mg Chl } a. \text{ m}^{-3}$ )	(0.07 – 0.49)	(0.13 – 1.38)	(0.11 – 0.72)
Total Primary Production	$0.332 \pm 0.007^a$	$0.142 \pm 0.005^b$	$0.135 \pm 0.003^b$
( $\text{mg C m}^{-3} \text{ h}^{-1}$ )	(0.15 – 0.49)	(0.04 – 0.26)	(0.08 – 0.23)
Particulate Primary Production	$0.100 \pm 0.007^a$	$0.062 \pm 0.028^a$	$0.042 \pm 0.004^a$
( $\text{mg C m}^{-3} \text{ h}^{-1}$ )	(0.01 – 0.31)	(0.01 – 0.14)	(0.03 – 0.05)
Lysis rates	$0.86 \pm 0.23^a$	$0.47 \pm 0.08^{ab}$	$0.42 \pm 0.06^b$
( $\text{d}^{-1}$ )	(0.31 – 2.75)	(0.15 – 0.84)	(0.11 – 0.66)

Fig. 4. New Table 2

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Average $\pm$ SE (cells. L <sup>-1</sup> )	Anticyclonic eddies	Cyclonic eddies	Far fields
Surface			
DCM			
Nano-microphytoplankton	$3.3 \pm 0.1 \times 10^3$ <sup>b</sup>	$6.4 \pm 1.2 \times 10^3$ <sup>a</sup>	$1.5 \pm 0.3 \times 10^3$ <sup>b</sup>
Surface	$3.3 \pm 0.2 \times 10^3$	$6.5 \pm 2.8 \times 10^3$	$1.3 \pm 0.3 \times 10^3$
DCM	$3.2 \pm 0.1 \times 10^3$	$6.3 \pm 1.1 \times 10^3$	$1.6 \pm 0.7 \times 10^3$
Nanoflagellates	$1.6 \pm 0.5 \times 10^3$ <sup>ab</sup>	$2.3 \pm 0.6 \times 10^3$ <sup>a</sup>	$6.6 \pm 0.2 \times 10^2$ <sup>b</sup>
Surface	$2.0 \pm 0.8 \times 10^3$	$1.5 \pm 0.7 \times 10^3$	$5.9 \pm 2.3 \times 10^2$
DCM	$1.2 \pm 0.7 \times 10^3$	$3.1 \pm 0.7 \times 10^3$	$7.3 \pm 2.6 \times 10^2$
Diatoms	$6.4 \pm 1.8 \times 10^2$ <sup>ab</sup>	$1.3 \pm 0.5 \times 10^3$ <sup>a</sup>	$1.8 \pm 0.4 \times 10^2$ <sup>b</sup>
Surface	$4.7 \pm 2.6 \times 10^2$	$1.8 \pm 0.8 \times 10^3$	$1.5 \pm 0.02 \times 10^2$
DCM	$8.1 \pm 2.3 \times 10^2$	$7.5 \pm 2.6 \times 10^2$	$2.2 \pm 0.9 \times 10^2$
Dinoflagellates	$2.0 \pm 0.3 \times 10^2$ <sup>b</sup>	$7.6 \pm 1.1 \times 10^2$ <sup>a</sup>	$2.2 \pm 0.6 \times 10^2$ <sup>b</sup>
Surface	$1.9 \pm 0.6 \times 10^2$	$6.6 \pm 2.4 \times 10^2$	$2.1 \pm 0.5 \times 10^2$
DCM	$2.1 \pm 0.4 \times 10^2$	$8.5 \pm 0.5 \times 10^2$	$2.4 \pm 1.4 \times 10^2$

**Fig. 5.** New Table 3

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