Reply to Referee #2

General comments

This is a good manuscript, well written and very informative about TEP distribution in surface waters of the Atlantic Ocean, taking into account many other studies. The main limitations, in my opinion, rely on the number of data collected and the depth, only at 4 m, which probably underestimates other processes related to TEP presence especially close to the depth of the chlorophyll maximum.

> *We thank the reviewer for his/her positive general comments. We fully agree that it would be more informative to show vertical TEP profiles within the euphotic layer, but we consider that the study, which was carried out during a transit (i.e. no opportunities for CTD stations), adds valuable information for many processes happening at the ocean surface, as pointed out in the introduction.*

As pointed out by the other referee, conversions factors may be approximative and a proper critical consideration on any limitation should be included in the manuscript.

> *We will add information about the uncertainty of phytoplankton and heterotrophic prokaryotes biomass estimate (see Referee#1).*

Another general recommendation: TEP importance in processes such as air-sea gas exchange, aerosol formation, marine snow and carbon export and cycling should be better addressed in the whole study, as focal points of TEP influence on carbon dynamics, please see my comments in the introduction and discussion. I recommend that the following points are addressed before publication.

Abstract:

Lines 37-38: The authors should be aware that air-sea gas exchange and aerosol emissions are complex processes, which are not properly explained in the manuscript. I would thus remove this sentence that in the abstract appears a bit vague and would concentrate on the role of TEP in channeling the carbon produced by primary productivity (see Mari et al. 2017).

> *We appreciate the reviewer's comment. We will remove the sentence about aerosol emission and change it. We will mention the role of TEP channeling the biological pump.*
>
> *Line 36: "Transparent exopolymer particles (TEP) are a class of gel particles, produced mainly by microorganisms, which play important roles in biogeochemical processes such as carbon cycling and export. TEP (a) are colonized by carbon-consuming microbes; (b) mediate aggregation and sinking of organic matter and organisms, thereby contributing to the biological carbon pump; and (c) accumulate in the surface microlayer (SML) and affect air-sea gas exchange."*

Lines 50-51: it could also be an inhibited TEP-aggregation by UV, not just breaking. I would rephrase the sentence.

We will change this sentence in the revised version of the MS as follows: "suggesting that sunlight, particularly UV radiation, is more a sink than a source for TEP. "

We will also mention it in the introduction (see comments below), and the discussion:

Line 451: "Ultraviolet (UV) radiation causes TEP loss by photolysis (Ortega-Retuerta et al., 2009a) and inhibits TEP formation from precursors (Orellana and Verdugo, 2003."

Line 454: "Our results suggest that the roles of UV radiation in breaking up TEP and/or limitating their formation from precursors overcome UV stress–induced TEP production."

Introduction:

We thank the reviewer for his/her thorough effort to improve the introduction section of our manuscript.

Lines 63-75: The introduction is a bit vague, I would introduce the concept of a marine gel, the composition and cross-links in the molecule that make TEP water insoluble but still subject to fragmentation and further aggregation processes, and the size distribution in the ocean, mentioning the size range we are talking about. 0.4 µm falls into the truly dissolved phase, and a discussion on the continuum of sizes linking DOM and POM should be added.

We will add the concept of DOM-POM continuum and evoke the gel polymer theory introducing a sentence like this in the revised version of the MS: "TEP are gel-like substances mainly formed by the spontaneous assembly from dissolved precursors, namely some acidic polysaccharides, which are stabilized as TEP either by covalent links or ionic strength. Therefore, the formation and fragmentation of TEP from/to dissolved precursor material spans the dissolved to particulate continuum of organic matter in the sea". However, one could consider 0.4 µm as a fraction included in the particulate phase if the 0.2 µm cutoff (one most widely used) is taken into account.

Line 80: "Regarding the sources, TEP are released by organisms, mainly microorganisms, during production and decomposition processes, either directly as detritus (Hong et al., 1997; Berman-Frank et al., 2007), or indirectly as dissolved precursors that can self-assemble to form TEP (operationally defined as particles > 0.4 µm) (Passow and Alldredge, 1994; Chin et al., 1998; Thuy et al., 2015) TEP are stabilized by covalent links or ionic strength (Cisternas-Novoa et al., 2015) and therefore, the formation and fragmentation of TEP from/to dissolved precursor material spans the dissolved to particulate continuum of organic matter in the sea."

Several species can directly release TEP or macrogels, but such macromolecules can also form from dissolved abiotic material in the absence of phytoplankton (Chin, W.-C., Orellana, M.V., Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391, 568–572.)

We already mentioned this process in line 82 but we will rephrase it in order to clarify the spontaneous assembly of DOM into TEP (See comment above).

Moreover, the importance of TEP and marine snow should be mentioned. The role of TEP in the sea-surface microlayer should be either expanded or left out. The description presented here about air-sea gas exchange and aerosol is a bit vague and not precise. I would suggest spending more words on it, especially because 4 m depths is close to the surface so surface ocean processes and air-sea interaction should be properly mentioned. The role of TEP in the sea-surface microlayer depends on many factors: wind speed, primary productivity, and they are not the only class of gel particles present (e.g., highly productive region, see Engel and Galgani 2016, Biogeosciences, Wurl, O., Miller, L., Röttgers, R., and Vagle, S.: The distribution and fate of surface-active substances in the seasurface microlayer and water column, Mar. Chem., 115, 1–9, 2009. Wurl, O., Miller, L., and Vagle, S.: Production and fate of transparent exopolymer particles in the ocean, J. Geophys. Res., 116, C00H13, doi:10.1029/2011JC007342, 2011).

We will add a few sentences to better introduce why TEP accumulates in the sea surface, implications and factors affecting this accumulation.

Lines 63-75: "Transparent exopolymer particles (TEP) are defined as a class of non–living organic particles in aqueous media, mainly formed by acidic polysaccharides, that are stainable with Alcian Blue (Alldredge et al., 1993). Due to their stickiness, TEP favour the formation of large aggregates of organic matter and organisms (typically named marine snow), enhancing particle ballast and sinking in the ocean (Logan et al., 1995; Kumar et al., 1998; Passow et al., 2001; Burd and Jackson, 2009). The presence of TEP also affects the microbial food–web, as they can be used as a food source for zooplankton (Decho and Moriarty, 1990; Dilling et al., 1998; Ling and Alldredge, 2003) and heterotrophic prokaryotes (HP) (Passow, 2002b) through microbial colonization of aggregates (Alldredge et al., 1986; Grossart et al., 2006; Azam and Malfatti, 2007). On their way to aggregation, and due to their low density, TEP and TEP–rich microaggregates formed near the surface may ascend and accumulate in the sea surface microlayer (SML) (Engel and Galgani, 2016), a process that is largely enhanced by bubble-associated scavenging (Azetsu-Scott and Passow, 2004; Wurl et al., 2009; Wurl et al., 2011). This accumulation in the SML, also contributed by local direct production (Wurl et al., 2011) can supress the air-sea exchange of CO_2 and other trace gases by acting as a physicochemical barrier or modifying sea surface hydrodynamics at low wind speeds (Calleja et al., 2008; Cunliffe et al., 2013; Wurl et al., 2016). Sea surface TEP can also be released to the atmosphere by bubble bursting (Zhou et al., 1998; Aller et al., 2005; Kuznetsova et al., 2005), contributing to organic aerosol and possibly acting as cloud condensation nuclei and ice nucleating particles (Orellana et al., 2011; Leck et al., 2013; Wilson et al., 2015). All in all, TEP play important roles

in microbial diversity, carbon cycling, and carbon exports to both the deep ocean and the atmosphere."

Line 69: specify what do you mean by "affect air-sea gas exchange".

Some studies, revised in Cunliffe et al. (2013), show the influence of surface active components of the SML (including biogenic polysaccharides) on air-sea gas exchange, either acting as a physicochemical barrier or modifying sea surface hydrodynamics, which in turn results in a suppression of air-water gas exchange. For example, Calleja et al. (2008) found that the organic matter content of the surface water supressed CO_2 gas exchange between the air and the ocean at low and intermediate wind speeds (> 5 m s^{-1}). Wurl et al. (2016) found enrichments of TEP, POC, PON, total prokaryotic cell numbers and picophytoplankton abundances in sea microlayers at multiple stations of different regions, compared to the underlying bulk water, being higher in slick surfaces than non-slick ones, and estimated that slicks could reduce CO_2 fluxes by up to 15 %, which highlight the importance of slicks in regulating air-sea interactions. Jenkinson et al. (2018) reviewed recently known and suspected mechanical aspects of how biologically produced organic matter modulates air-sea fluxes of CO_2.

We will briefly add some of this information in the introduction section. See comment above.

Lines 70-71: caution is needed here. Orellana et al. discuss about micro and nanogels, determined with a different method with respect to the one reported here. When gels are present in the sea-surface microlayer, it will depend on their size distribution whether they will be part of the organic aerosol fraction or not. Aerosol particles smaller than 1 µm will be part of the accumulation mode of sea-spray aerosols, but when further aggregating and reaching sizes above 2.5µm they won't actually stay in the atmosphere longer than a few hours – as their size distribution is described as coarse mode aerosols. TEP as macromolecules are between accumulation and coarse mode but not ice-nucleating particles or cloud condensation nuclei. Another consideration is that if high wind speed are present (above 5 m/s), there might be increased aggregation rates of TEP with solid particles which will favour the formation of negatively buoyant aggregates that will sink out of the surface microlayer and surface waters in general.

We agree with the reviewer that the microgels measured by Orellana et al. (2011), defined as those stabilized with calcium bridges, may not fully correspond to TEP, defined by their stainability with Alcian Blue (thus on their polysaccharide composition). However, some studies have demonstrated that some TEP (about 30 %) are also stabilized by divalent cations (Passow, 2002; Cisternas-Novoa et al., 2015). In addition, even though TEP were measured in the particulate phase, we believe that TEP precursors could be measurable whenever TEP are present if they are in a dynamic equilibrium with their precursors (Verdugo, 2012). Thus exopolymers in the dissolved and colloidal phases, i.e. those potentially acting as CCN, would covary with TEP concentration (hypothesis yet to test). Furthermore, the exopolymer particles could depolymerise in the atmosphere due to ultraviolet light (Orellana and

Verdugo, 2003) or acidification (Chin et al., 1998) and form nano-sized particles (Karl et al., 2013). It is also worth mentioning that Kuznetsova et al. (2005) found the presence of TEP (i.e. Alcian Blue-stained polymers) in natural and simulated marine aerosols, and Russell et al. (2010) showed the high carbohydrate composition of submicron aerosols in remote regions of the North Atlantic and Arctic oceans that contained organic hydroxyl groups from primary emissions of the ocean.

Since this is not the subject of the manuscript, we will not include this discussion but will tone down a bit the statement referring to aerosol and clouds:

Line 70: "contributing to organic aerosol and possibly acting as cloud condensation nuclei and ice nucleating particles (Orellana et al., 2011; Leck et al., 2013; Wilson et al., 2015)."

Line 80: not just photolysis but also UV inhibited aggregation of precursor polymers limits TEP formation.

We will add the following information in the revised version of the manuscript:

Line 101: "high solar radiation can stimulate TEP production by Prochlorococcus during cell decay (Iuculano et al., 2017), but also can limit TEP formation inhibiting the aggregation of the precursor polymers (Orellana and Verdugo, 2003)."

Line 102: What does this sentence mean? Please explain how HP affect TEP production and assembly of precursors.

Several experiments have found that the presence of bacteria stimulate or are necessary for TEP production by diatoms. Specifically, Guerrini et al. (1998) observed that the presence of bacteria during phosphate limitation conditions in batch cultures stimulated the production of polysaccharides by the diatom Cylindrotheca fusiformis. Gärdes et al. (2011) demonstrated that specific bacterial strains attached to the diatom Thalassiosira weissflogii was necessary for TEP production and suggested that direct interaction between bacteria and diatoms could be required for TEP formation.
Moreover, through different mechanisms, HP seem to facilitate the self–assembly of dissolved precursors into TEP. In a seawater culture experiment, Sugimoto et al. (2007) observed that TEP formation appeared to be related with increases in bacterial abundance. Bacterial TEP production was not enough to explain the overall TEP formation and they suggested the self-assembly of TEP precursors coupled with bacterial growth. Ding et al. (2008) demonstrated that the amphiphilic exopolymers released by the bacterium Sagitula stellata induced DOM self-assembly and formation of marine microgels.
We will add some of this previous information to better explain the processes involving prokaryote-TEP relationships.

Line 102: "HP have been found to stimulate TEP production by diatoms, suggesting that HP-diatom interaction is required for TEP formation (Guerrini et al., 1998; Gärdes et al., 2011). HP may also facilitate TEP production from

DOM self-assembly (Sugimoto et al., 2007), e.g., through the release of amphiphilic exopolymers that induce microgel formation (Ding et al., 2008)."

Line 106: I suggest introducing the concept of biological carbon pump and the importance of TEP in ocean carbon cycle, as this is a central idea of the study. How much estimated primary production carbon is channeled into the TEP pool? (See Mari et al., 2017). This could also help making confrontations with phytoplankton-derived carbon, still estimates but could be interesting.

We will introduce the concept of biological carbon pump and the importance of TEP in the ocean carbon cycle. Beginning of the Introduction:
"Due to their stickiness, TEP favour the formation of large aggregates of organic matter and organisms (typically named i.e. marine snow), enhancing particle ballast and sinking and thereby contributing to the biological carbon pump."

As for how much PP is channelled into TEP, we will add the following:
"The aforementioned importance of TEP in carbon fluxes in the pelagic ocean can be further stressed by considering the following rough numbers: if the percentage of extracellular carbon release during planktonic primary production is generally constrained within 10-20 % (Nagata, 2000) but can reach >50% (López-Sandoval et al., 2011), and half of the extracellular release is in the form of reactive polysaccharides (Biddanda and Benner, 1997), then the production rate of TEP precursors may represent 5-10 %, but reach >25%, of planktonic primary production, without considering production by heterotrophs."

Lines 107-108: As mentioned already, TEP span over a wide range - DOC or POC is just an operational definition. From colloids (dissolved) to macrogels (particulate) (see Verdugo 2012 Annual Rev. of marine sciences).

We thank the reviewer for her/his comment. We will make the following changes to clarify it:

Line 107: "It is also important to determine the contribution of TEP as a constituent of the organic carbon pool to better understand its role in the organic matter cycling."

In the objectives section (end of introduction section), we will change the first sentence (line 110) to "we described the horizontal distribution of TEP (> 0.4 µm) in surface waters across a North–South transect in the Atlantic Ocean …"

Methods:

If you have DOC data, I think it would be worth showing them and looking for the missing fraction that drives POC underestimation with respect to TEP, as TEP are connecting both pools of organic matter. Can you provide a standard deviation or error estimation for POC filters?

Unfortunately, we don't have DOC data and there was only one replicate per POC measurement. However, we can add that the reproducibility of the elemental analyser used to measure POC (based on the coefficient of variation of the calibration slopes) is about 1 % for carbon. Regarding the coefficient of variation of the replicates, that takes into account the reproducibility of the whole process (sampling, filtering and analysis), we have obtained, in previous studies, a value of around 5 %.

We will add the following:

Line 153: "No POC replicates were run, but replication in a previous study yielded a coefficient of variation of around 5%."

Do you have wind speed information? This would be useful in estimating whether TEP could accumulate in the surface layer.

We have wind speed information but we can't estimate TEP relative accumulation in the surface layer as we only have data at one depth. The regression of TEP vs wind speed gave R^2=0.2 in OAO and 0.3 in the SWAS, both with a negative slope. Contrasting results have been found in previous studies: Engel and Galgani (2016) found depletion of TEP in the SML above 5 m s^{-1}, while earlier observations found enrichment in the microlayer also at higher wind speed (Wurl et al., 2009; Wurl et al., 2011).

Discussion:

Lines 317-319: Can you provide any reason why you think your values are higher than those observed in the Mediterranean Sea and Pacific Ocean? Is it related to nutrient concentration/time of year, different analysis method (e.g. spectroscopy vs microscopy for gel particles identification), depth?

We believe that one of the reasons is the depth. Mean TEP values in some of them (Ortega-Retuerta et al., 2010; Kodama et al., 2014; Ortega-Retuerta et al., 2017) correspond to the upper mixed layer depth or from 0 to 200 m. As TEP tend to accumulate in the surface and our values correspond only to the surface, this could explain the higher values obtained in our dataset. In fact, if we had provided integrated measurements within the photic layer, we would probably have obtained a lower mean TEP concentration.
Another reason seems to be the different Chl a concentrations, as the main TEP producer is phytoplankton. Chl a concentration in the OAO (0.4 ± 0.2 mg m^{-3} (0.2-0.6 mg m^{-3})) was generally higher than in the other studies referred in the Table. For example, in Iuculano et al. (2017) Chl a ranged 0.05-0.31 mg m^{-3}, and in Kodama et al. (2014) Chl a averaged 0.05 ± 0.01 mg m^{-3}. In some cases it is a pity that we don't have the average values, as the range could be a little bit misleading. However, in Ortega-Retuerta et al. (2010), TEP:Chl a ratio was higher than ours, suggesting that Chl a values were also low and gave rise to lower TEP. We can't forget either that differences in TEP chemical composition could cause differences in staining capacity. Regarding analytical methods, all

the studies gathered in the table used the spectroscopic method, so this can't be the reason for the contrasting TEP concentrations.

We will briefly include these arguments in the discussion:

Line 320: "Mean TEP values in some of them (Ortega-Retuerta et al., 2010; Kodama et al., 2014; Cisternas-Novoa et al., 2015; Ortega-Retuerta et al., 2017) correspond to the above mixed layer depth or from 0 to 100 or 200 m. As TEP tend to accumulate in the surface and our values correspond only to the surface (4 m), this could explain the higher values obtained in our dataset. Another reason seems to be the different Chl a concentrations, as the main TEP producer is phytoplankton. Chl a concentration in the OAO (0.4 ± 0.2 mg m^{-3} (0.2-0.6 mg m^{-3})) was generally higher than in the other studies referred in the Table 2. For example, in Iuculano et al. (2017) Chl a ranged 0.05-0.31 mg m^{-3}, and in Kodama et al. (2014) it averaged 0.05 ± 0.01 mg m^{-3}. We can't discard either that differences in TEP chemical composition could cause differences in staining capacity."

Lines 355-356: The authors should mention here any limitation of the conversion factors.

We will mention it as follows:

Line 364: "Furthermore, conversion factors carry quite an uncertainty as pointed out in the Methods section".

Line 331: is the organic matter that influences HPA concentration and their TEP production or ..? How does the organic matter pool influences TEP formation? If you mean, by abiotic assembly of a pool of dissolved precursors, this concept should be mentioned early in the introduction.

We realize this sentence was ambiguous and we will change it. In the revised MS we now clarify the concept of abiotic formation. What we meant is that heterotrophic prokaryotes can be discharged directly with freshwater outflow, but also autochtonous microbes can be stimulated due to allochtonous DOM inputs. On the other hand, DOM inputs from freshwaters could also contain TEP and their precursors.

We will do the following change:

Line 328: "The nutrient–rich water in the region is responsible for the proliferation of phytoplankton and HP, which could partly explain the high TEP concentrations in this region. It is also known that large freshwater discharges occur in the shelf (Piola, 2005). These discharges could bring allochtonous HP directly to the shelf or bring DOM loads, which would stimulate autochtonous microbes. Besides, DOM inputs associated to freshwater discharges could also contain TEP and their precursors."

Lines 370-374: Again, the fate of TEP depends on further aggregation processes. Generally less dense than water could accumulate in the surface microlayer but wind speeds, high heterotrophic activity, coagulation with other organic and mineral particles thanks to their stickiness should be mentioned to describe their fate in the area. Which one do you think would predominate?

We agree with the reviewer that TEP accumulation in the surface is the result of a complex suite of aggregation/consumption processes. Besides, the reference to the effects of TEP-richness on the fate of POC was a bit misplaced here, where we were discussing the potential reasons why TEP contribution to POC is larger in oligotrophic waters. We will remove the sentence to leave the paragraph:

Lines 367-374: "With our results taken all together, we hypothesize that in oligotrophic conditions TEP–C is the predominant POC fraction, because nutrient limitation favours TEP production by phytoplankton and limits TEP consumption by bacteria. Conversely, in eutrophic conditions, the predominant POC fraction depends on many variables like the community composition, the bloom stage, and sources of TEP different from phytoplankton."

Lines 409-410: Again on aerosol formation, it's a complex process and without any information on the size distribution of TEP in this study I would recommend caution in making such affirmations. Here, it's a bit a "stand alone" sentence without any further explanation, which does not make much sense. It should be expanded and explained better. Also, please see the paper by Quinn et al. 2014 "Contribution of sea surface carbon pool to organic matter enrichment in sea spray aerosol" Nature Geoscience, which actually breaks up the concept of organic aerosols related to phytoplankton blooms (and in this case, TEP).

We agree this sentence was a bit stand alone and too speculative, and have removed it.

Line 444: as mentioned, TEP can also be produced by aggregation of colloids in the absence of phytoplankton, that is, in the presence of polymeric precursors in the dissolved phase. Thus, it would be interesting to see the relationship of TEP to DOC or acidic sugars.

Unfortunately we don't have DOC or acidic sugars data to check this relationship. However, it is worth mentioning that covariation of TEP with DOC or dissolved carbohydrates are not always observed in the field (see for instance Ortega-Retuerta et al. (2009b) in the Southern Ocean). We will add the following information:

"TEP formation could have been enhanced by aggregation of colloids carried by freshwater discharges".

Line 454: UV also inhibits gel aggregation (Orellana and Verdugo 2003, Ultraviolet radiation blocks the organic carbon exchange between the dissolved

phase and the gel phase in the ocean, Limnology and Oceanography). It should be mentioned here.

We will add this comment:

Line 454: "Our results suggest that the roles of UV radiation in breaking up TEP and/or limiting their formation from precursors overcome UV stress–induced TEP production."

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